2015 International Meeting of the Federation of Korean Microbiological Societies

5 (Thu.) ~ 6 (Fri.), November 2015, KINTEX, Korea

Organized by
The Federation of Korean Microbiological Societies (FKMS)

Hosted by
The Korean Society for Microbiology and Biotechnology (KMB)
The Korean Society of Virology (KSV)
The Korean Society of Mycology (KSMy)
The Microbiological Society of Korea (MSK)
The Korean Society for Microbiology (KSMi)

Sponsored by
Gyeonggi Tourism Organization
Initiative for Biological Function & Systems, Yonsei University
Korea National Microorganisms Research Resources Center
Korea Research Institute of Bioscience and Biotechnology
National Research Foundation of Korea
The Korean Federation of Science and Technology Societies

http://www.fkms.kr
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http://www.fkms.kr
2015년
한국미생물학회연합

확장단원의회

<table>
<thead>
<tr>
<th>직위</th>
<th>성명</th>
<th>소속</th>
</tr>
</thead>
<tbody>
<tr>
<td>확장</td>
<td>정건섭</td>
<td>연세대학교</td>
</tr>
<tr>
<td>위 원</td>
<td>금중국</td>
<td>경북대학교</td>
</tr>
<tr>
<td>위 원</td>
<td>노정혜</td>
<td>서울대학교</td>
</tr>
<tr>
<td>위 원</td>
<td>배용수</td>
<td>성균관대학교</td>
</tr>
<tr>
<td>위 원</td>
<td>이상기</td>
<td>순천향대학교</td>
</tr>
<tr>
<td>위 원</td>
<td>이윤수</td>
<td>강원대학교</td>
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<tr>
<td>위 원</td>
<td>이철훈</td>
<td>한양대학교</td>
</tr>
</tbody>
</table>

위 원 | 정상인 | 중앙대학교 |
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Floor Plan
Contents

Schedule .................................................................................................................. 7

Plenary Lectures ...................................................................................................... 69

Symposium

S1 Marine Microbial Genomics and Ecology ............................................................... 74
S2 Ecology of Soil Microorganisms ......................................................................... 79
S3 Host-pathogen Interaction .................................................................................... 84
S4 Fungal Diversity and Ecology .............................................................................. 89
S5 Infection, Inflammation, and Immunity ................................................................. 95
S6 Physiologies and Applications of Industrial Microorganisms ............................. 100
S8 Fungal Morphogenesis and Pathogenesis ............................................................ 105
S9 Antibiotic Resistome: from the Environment to the Clinic ............................... 110
S10 Host Responses against Infectious Agents ........................................................ 118
S11 Pathogenesis of Bacterial Infection .................................................................. 123
S12 Industrial Application of Fungi ......................................................................... 128
S13 Current Topics in Pathogens ............................................................................. 135
S14 Norovirus Detection and Field Applications ..................................................... 142
S15 Emerging Viruses ............................................................................................... 147
S16 Microbiome and Diseases ................................................................................. 152
S17 Cell Signaling in Host-Microbe Interactions ...................................................... 157
S18 Host Defense against Viral Infection .................................................................. 162
S19 Yeast System for Comparative Functional Genomics ....................................... 167
S21 Viral Pathogenesis .............................................................................................. 172
S22 KNMRRC Session I ............................................................................................ 177
S23 KNMRRC Session II .......................................................................................... 183
S24 Research and Business Development of Biomedicinal Material, Poly-γ-glutamic Acid ................................................................. 187
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015 NRF, Division of Life Sciences</td>
<td>195</td>
</tr>
<tr>
<td><strong>Young Scientist Session</strong></td>
<td></td>
</tr>
<tr>
<td>YS1 Young Scientist Session 1</td>
<td>197</td>
</tr>
<tr>
<td>YS2 Young Scientist Session 2</td>
<td>204</td>
</tr>
<tr>
<td>YS3 Young Scientist Session 3</td>
<td>210</td>
</tr>
<tr>
<td>YS4 Young Scientist Session 4</td>
<td>217</td>
</tr>
<tr>
<td>Luncheon Seminar</td>
<td>223</td>
</tr>
<tr>
<td>Poster Session</td>
<td>226</td>
</tr>
<tr>
<td>2015 Bio-exhibition</td>
<td>360</td>
</tr>
<tr>
<td>Author Index</td>
<td>367</td>
</tr>
<tr>
<td>Keyword Index</td>
<td>385</td>
</tr>
</tbody>
</table>
# Schedule

## November 5 (Thursday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00-08:50</td>
<td>Grand Ballroom A</td>
<td>Opening Ceremony [Grand Ballroom A]</td>
</tr>
<tr>
<td>08:50-09:00</td>
<td>Room 306, 307</td>
<td>S1 Marine Microbial Genomics and Ecology</td>
</tr>
<tr>
<td>09:00-11:00</td>
<td></td>
<td>S2 Ecology of Soil Microorganisms</td>
</tr>
<tr>
<td>11:00-11:45</td>
<td></td>
<td>S3 Host-pathogen Interaction</td>
</tr>
<tr>
<td>11:45-12:15</td>
<td></td>
<td>S4 Fungal Diversity and Ecology</td>
</tr>
<tr>
<td>12:15-13:00</td>
<td></td>
<td>S5 Young Scientist Session I</td>
</tr>
<tr>
<td>13:00-13:45</td>
<td></td>
<td>Plenary Lecture 1 [Grand Ballroom A]</td>
</tr>
<tr>
<td>13:45-15:45</td>
<td>Poster Session 2 &amp; Exhibition</td>
<td>S5 Infection, Inflammation, and Immunity</td>
</tr>
<tr>
<td>15:45-17:45</td>
<td></td>
<td>S6 Physiologies and Applications of Industrial Microorganisms</td>
</tr>
<tr>
<td>17:45-18:30</td>
<td></td>
<td>S7 Retirement Session: My Life as a Microbiologist</td>
</tr>
<tr>
<td>18:30-21:00</td>
<td></td>
<td>S8 Fungal Morphogenesis and Pathogenesis</td>
</tr>
</tbody>
</table>

## November 6 (Friday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00-09:00</td>
<td>Grand Ballroom A</td>
<td>S13 Current Topics in Pathogens</td>
</tr>
<tr>
<td>09:00-11:00</td>
<td>Room 306, 307</td>
<td>S14 Norovirus Detection and Field Applications</td>
</tr>
<tr>
<td>11:00-11:15</td>
<td></td>
<td>S15 Emerging Viruses</td>
</tr>
<tr>
<td>11:15-13:00</td>
<td></td>
<td>S16 Young Scientist Session II</td>
</tr>
<tr>
<td>13:00-15:00</td>
<td></td>
<td>Hanteo Prize Awards</td>
</tr>
<tr>
<td>15:00-15:45</td>
<td></td>
<td>Poster Session 2 &amp; Lunch</td>
</tr>
<tr>
<td>15:45-17:45</td>
<td></td>
<td>Plenary Lecture 4 [Grand Ballroom A]</td>
</tr>
<tr>
<td>17:45-18:00</td>
<td></td>
<td>S19 Yeast System for Comparative Functional Genomics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S20 Trials &amp; Research LMD Safety Management</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S21 Viral Pathogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S22 Young Scientist Session IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S23 KMRRC Session I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S24 Research and Business Development of Biomedical Material, Poly-γ-Glutamic Acid</td>
</tr>
</tbody>
</table>
Plenary Lectures

PL1  Sho Yamasaki (Kyushu University, Japan)
Recognition of Mycobacterial Adjuvants through C-type Lectin Receptors ........................................... 70
Chair: Jeong Kyu Park (Chungnam National University)
Sponsored by Infection Signaling Network Research Center

PL2  G. Balakrish Nair (Translational Health Science and Technology Institute, India)
Undernourished Children and the Gut Microbiome ...................................................................................... 71
Chair: Kun-Soo Kim (Sogang University)

PL3  Kwangseog Ahn (Seoul National University, Korea)
Emerging Roles for Noncoding RNAs in Virus-Host Interactions ................................................................. 72
Chair: Yeong-Jae Seok (Seoul National University)

PL4  Ren Sun (UCLA, USA)
Quantitative Viral Genetics at Single Nucleotide Resolution ........................................................................ 73
Chair: Jin-Hyun Ahn (Sungkyunkwan University)
Sponsored by Bacteriophage Bank of Korea
S1

Marine Microbial Genomics and Ecology

Chair: Song-gun Kim (KIRBB)

November 5 (Thu.)
09:00-11:00 Grand Ballroom A

09:00-09:30
Chung Yeon Hwang (Korea Polar Research Institute)
Metagenomic Analysis of Marine RNA Virioplankton in the Vicinity of the Antarctic Peninsula

09:30-10:00
Kae Kyoung Kwon (Korea Institute of Ocean Science and Technology)
Metabolic Collaboration of Thermophilic Microorganisms, Evidence by Metabolic and Genomic Analysis

10:00-10:30
Sung-Keun Rhee (Chungbuk National University)
Meta-Omics Analysis of Bacterioplankton Community Involved in Carbon Remineralization in a Polynya of Amundsen Sea, Western Antarctica

10:30-11:00
Che Ok Jeon (Chung-Ang University)
Genome-Wide Transcriptional Responses of Alteromonas sp. SN2 to Contaminated Seawater and Marine Tidal Flat Sediment

S2

Ecology of Soil Microorganisms

Chair: Jong-Chan Chae (Chunbuk National University)
Jung-Sook Lee (KIRBB)

November 5 (Thu.)
09:00-11:00 Rm 306, 307

09:00-09:30
Seon-Woo Lee (Dong-A University)
Microbiome Analysis of Tomato Rhizosphere to Enhance Plant Growth and Health

09:30-10:00
Woo Jun Sul (Chung-Ang University)
Volcanic Jeju Island: The Hidden Jewel of Soil Microbial Ecology

10:00-10:30
Jong-Shik Kim (Gyeongbuk Institute for Marine Bio-industry)
Microbial Community Function and Structure of Gotjawal Forest Soil, Jeju

10:30-11:00
Ok-Sun Kim (Korea Polar Research Institute)
Comprehensive Study on Soil Bacterial Community Composition on Barton Peninsular and Around Terra Nova Bay, Antarctica
# Symposium

## S3 Host-pathogen Interaction

*Chair*: Yoon-Won Kim (Hallym University)

**09:00-09:30**

**Eui-Cheol Shin** (KAIST)  
Roles of Unphosphorylated ISGF3 in HCV Infection  
---

**09:30-10:00**

**Deog-Yong Lee** (Korea National Institute of Health)  
Evasion of Host Immune System Through the Mutation of Enteric Virus  
---

**10:00-10:30**

**Min-Kyoung Shin** (Gyeongsang National University)  
Transcriptional Profiling of Host Responses to Mycobacterium avium subsp. paratuberculosis Infection and Identifying Biomarker Candidates  
---

**10:30-11:00**

**Beom Seok Park** (Eulji University)  
The Structural Basis of Lipopolysaccharide Recognition by the TLR4-MD-2 Complex  
---

## S4 Fungal Diversity and Ecology

*Chair*: Young Woon Lim (Seoul National University)

**09:00-09:30**

**Jeong Ah Seo** (Soongsil University)  
Microbial Diversity in Korean Traditional Fermenting Starter, Nuruk, Collected in 2013 and 2014  
---

**09:30-10:00**

**Ki Woo Kim** (Kyungpook National University)  
Ultrastructure of the Epiphytic Sooty Mold Capnodium on Walnut Leaves  
---

**10:00-10:30**

**Kang-Hyeon Ka** (Korea Forest Research Institute)  
Cultural Characteristics of Ectomycorrhizal Mushrooms  
---

**10:30-11:00**

**Sang Woo Kim** (Kangwon National University)  
Pathogenesis of Oak Wilt Disease Caused by Raffaelea Species  
---
**International Meeting of the Federation of Korean Microbiological Societies**

### Symposium

**S5**  
**Infection, Inflammation, and Immunity**

*Chair*: Yeonseok Chung (Seoul National University)  
Hyoung-Pyo Kim (Yonsei University)

13:45-15:45 Grand Ballroom A

**S5-1**  
13:45-14:15  
**Hyoung-Pyo Kim** (Yonsei University)  
CCCTC-Binding Factor Controls the Homeostatic Maintenance and Migration of Langerhans Cells in the Skin ......................................................... 96

**S5-2**  
14:15-14:45  
**Eun Jung Park** (National Cancer Center)  
The HIF-1/Glial TIM-3 Axis Controls Inflammation-Associated Brain Damage Under Hypoxia .... 97

**S5-3**  
14:45-15:15  
**Su-Hyung Park** (KAIST)  
New Insights into Antiviral Immunity by Repeated Low-Dose Viral Exposures ....................... 98

**S5-4**  
15:15-15:45  
**Yeonseok Chung** (Seoul National University)  
Antagonism Among IL-12 Family Cytokines in Host Defense Against *Listeria monocytogenes* and Cancer ............................................................................................................. 99

---

**S6**  
**Physiologies and Applications of Industrial Microorganisms**

*Chair*: Pil Kim (The Catholic University of Korea)  
Sang Jun Lee (KRIIBB)

13:45-15:45 Rm 306, 307

**S6-1**  
13:45-14:15  
**Ki Jun Jeong** (KAIST)  
Engineering of *Corynebacterium glutamicum* for the Production of Value-added Biochemicals from Biomass ........................................................................................................ 101

**S6-2**  
14:15-14:45  
**Soo-Keun Choi** (KRIIBB)  
Marker-free Genome Engineering in *Bacillus subtilis* .............................................................. 102

**S6-3**  
14:45-15:15  
**Hyeon-Su Ro** (Gyeongsang National University)  
Higher Fungi and Their Enzyme Systems in Bioremediation ..................................................... 103

**S6-4**  
15:15-15:45  
**Jung-Hoon Sohn** (KRIIBB)  
Industrial Application of Recombinant Yeast with Improved Substrate Spectrum and Stress Resistance ........................................................................................................ 104
Symposium

**S7** Retirement Session: My Life as a Microbiologist
*Chair*: In-Hong Choi (Yonsei University)

*S7-1* 13:45-14:20
*Kwang-Ho Rhee* (Gyeongsang National University)
My Life and Medical Microbiology in Korea

*S7-2* 14:20-14:55
*Luck Ju Baek* (Korea University)
Be the First, Try Something New

*S7-3* 14:55-15:30
*Dong Taek Cho* (Kyungpook National University)
20/20 in Medical Microbiology; Hindsight / Foresight

**S8** Fungal Morphogenesis and Pathogenesis
*Chair*: Seong Hwan Kim (Dankook University)

*S8-1* 13:45-14:15
*Sun-Tae Kim* (Pusan National University)
A Rice Blast Fungus Alpha-N-Arabinofuranosidase B Elicits Host Defense in Rice

*S8-2* 14:15-14:45
*Sang-Keun Oh* (Chungnam National University)
Rpi-blb2 Gene-Mediated Late Blight Resistance in Plants

*S8-3* 14:45-15:15
*Kap-Hoon Han* (Woosuk University)
Comparative Analysis of a Putative HLH Transcription Factor Responsible for Conidiation in Aspergillus Species

*S8-4* 15:15-15:45
*Dil Raj Yadav* (Kangwon National University)
Diversity of Fungi Isolated from Soil of Jeollabuk-do and Chungcheongbuk-do, Korea
International Meeting of the Federation of Korean Microbiological Societies

Symposium

**S9**

**Antibiotic Resistome: from the Environment to the Clinic**

*Chair*: Hor-Gil Hur (Gwangju Institute of Science and Technology)  
Chang-Jun Cha (Chung-Ang University)

November 5 (Thu.)  
15:45-17:40 Grand Ballroom A

**S9-1** 15:45-16:10

**Chang-Jun Cha** (Chung-Ang University)

Phylogenetic, Metagenomic, and Quantitative PCR-Based Analysis of Antibiotic Resistome in Han River

**S9-2** 16:10-16:30

**Jong-Chan Chae** (Chunbuk National University)

Prevalence of Antibiotic Resistant Bacteria in Stream

**S9-3** 16:30-16:50

**Hor-Gil Hur** (Gwangju Institute of Science and Technology)

E. coli in the Yeongsan River Basin: Antibiotic Resistance and Virulence Factors

**S9-4** 16:50-17:15

**Suk-Kyung Lim** (Animal and Plant Quarantine Agency)

Current Status of Antimicrobial Resistance in Veterinary Medicine

**S9-5** 17:15-17:40

**Kwan Soo Ko** (Sungkyunkwan University)

International and Local Antimicrobial Resistant Clones in Korea

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**S10**

**Host Responses against Infectious Agents**

*Chair*: Taegun Seo (Dongguk University)

November 5 (Thu.)  
15:45-17:45 Rm 306, 307

**S10-1** 15:45-16:15

**You-Hee Cho** (CHA University)

*Drosophila* Infection Models to Study Bacterial Pathogens

**S10-2** 16:15-16:45

**Seung Hyun Han** (Seoul National University)

Role of Toll-Like Receptor 2 Ligands Existing in the Cell Wall of *Staphylococcus aureus* in the Infection and Immunity

**S10-3** 16:45-17:15

**Chul-Su Yang** (Hanyang University)

*Toxoplasma Gondii* GRA7-Induced TRAF6 Activation Contributes to Host Protective Immunity in Macrophages

**S10-4** 17:15-17:45

**Li Sun** (Chinese Academy of Sciences, China)

Innate Immune Response of Teleost to Pathogens: The Role of Toll-like Receptors and Antimicrobial Effectors

---

Sponsored by Center for Food Safety and Toxicology, Seoul National University
Symposium

S11  Pathogenesis of Bacterial Infection
Chair: Sang In Chung (Chung-Ang University)

S11-1 15:45-16:15
Joon Haeng Rhee (Chonnam National University)
Unexpected Correlation Between Iron-Assimilating TonB Systems and Invasiveness in Vibrio vulnificus Pathogenesis .......................................................... 124

S11-2 16:15-16:45
Hwa Jung Kim (Chungnam National University)
Role of Macrophage Apoptosis in Mycobacterial Pathogenesis ........................................... 125

S11-3 16:45-17:15
So-Youn Woo (Ewha Womans University)
PD-L1 Mediated Immune Modulation in Mesenchymal Stromal Cells .................................. 126

S11-4 17:15-17:45
Sang Sun Yoon (Yonsei University)
A Single Microbiome Gene Determinant that Affects Host Susceptibility to Enteric Infection .......................................................... 127

S12  Industrial Application of Fungi
Chair: Jong Soo Lee (Pai Chai University)

S12-1 15:45-16:15
Jae San Ryu (Gyeongsangnam-do Agricultural Research & Extension Services)
Identification and Functional Analysis of Mating Type Loci in the Pleurotus eryngii ............... 129

S12-2 16:15-16:45
Jung-Mi Kim (Wonkwang University)
Study of Viral Effects of the Mycovirus (LeV) and Virus-Free Commercial Line in the Edible Mushroom Lentinula Edodes .......................................................... 130

S12-3 16:45-17:15
Jang Eun Lee (Korea Food Research Institute)
Recreation of Korean Traditional Nuruk and the Analysis of Metabolomic Characteristics .......... 132

S12-4 17:15-17:45
Young-Joon Choi (Kunsan National University)
Usability of DNA Sequence Data: from Taxonomy over Barcoding to Field Detection. A Case Study of Oomycete Pathogens .......................................................... 133
### Symposium

**S13**  
**Current Topics in Pathogens**  
Chair: You-Hee Cho (CHA University)  
Moo-seung Lee (KRIIBB)

**November 6 (Fri.)**  
09:00-11:05 Grand Ballroom A

#### S13-1  
09:00-09:25  
**Moo-Seung Lee** (KRIIBB)  
Enterohemorrhagic *Escherichia coli* Shiga Toxins as Multi-functional Proteins are not just Cytotoxins  
136

#### S13-2  
09:25-09:50  
**Gee W. Lau** (University of Illinois at Urbana-Champaign, USA)  
Induction of Airway Mucus Hypersecretion and Glycosylation by *Pseudomonas aeruginosa* Virulence  
137

#### S13-3  
09:50-10:15  
**Eric Déziel** (Institut Armand-Frappier INRS, Canada)  
Exportation of the *Pseudomonas* Quinolone Signal (PQS) Synthase PqsH: a New Puzzle Piece Supporting That Extracellular 4-Hydroxy-2-Heptylquinoline (HHQ) is a Sentinel Signal Precursor  
139

#### S13-4  
10:15-10:40  
**Sang-Wook Han** (Chung-Ang University)  
Functional, Biochemical, and Proteomic Analyses of a Prokaryotic Tyrosine Sulfotranferase  
140

#### S13-5  
10:40-11:05  
**Jihwan Hwang** (Pusan National University)  
Investigation of the Role of *Mycobacterium tuberculosis* Rv2019-Rv2018 Toxin-antitoxin System in *E. coli*  
141

---

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### S14  
**Norovirus Detection and Field Applications**  
Chair: Jong-Soon Choi (Korea Basic Science Institute)

**November 6 (Fri.)**  
09:00-11:00 Rm 306, 307

#### S14-1  
09:00-09:30  
**Geun Woo Park** (Centers for Disease Control and Prevention, USA)  
Application of Macrofoam Swab Based Sampling Method for the Investigation and Control of Human Norovirus Infections  
143

#### S14-2  
09:30-10:00  
**Chi-Yong Eom** (Korea Basic Science Institute)  
Super Resolution Microscopy for Studying Virus Infection  
144

#### S14-3  
10:00-10:30  
**Duwoon Kim** (Chonnam National University)  
Development of Foodborne Virus Concentration and Detection Methods  
145

---

www.fkms.kr | 15
Symposium

S14-4 10:30-11:00
Jae-Hyung You (SolGent Co., Ltd.)
Speeding up Norovirus Diagnostics, SolGent Norovirus Detection System ......................... 146

S15 Emerging Viruses
Chair: Nam-Hyuk Cho (Seoul National University)

S15-1 09:00-09:30
In-Kyu Yoon (International Vaccine Institute, USA)
The Status of Dengue Vaccine Development ............................................................................ 148

S15-2 09:30-10:00
Man-Seong Park (Korea University)
H3N2 Viruses Circulating during 2008-2015 Seasons ................................................................. 149

S15-3 10:00-10:30
Daesub Song (Korea University)
World-wide Threat of Coronaviruses (PEDV and MERS-CoV) ................................................. 150

S15-4 10:30-11:00
Jeong Rae Yoo (Jeju National University)
Therapeutic Effects of Plasmapheresis on Severe Fever with Thrombocytopenia Syndrome · · 151

S16 Microbiome and Diseases
Chair: Sung-Joon Lee (Korea University)

S16-1 13:00-13:30
Jae Bum Kim (Seoul National University)
Regulation of Insulin Sensitivity by Gut Microbiota Alteration via GLP-1 .............................. 153

S16-2 13:30-14:00
Ju-Hoon Lee (Kyung Hee University)
Genome Study of Bifidobacteria and Metagenome Study of Initial Intestinal Microbiota in Newborn Infants Show Their Correlation for Human Intestinal Health ................................................... 154

S16-3 14:00-14:30
Dae Bang Seo (Amorepacific Corp.)
Post-fermented Green Tea Extract Reduces Body Weight, Alleviates Glucose Intolerance and Fatty Liver, and Alters Gut Microbiota Composition in Diet-induced Obese Mice ......................... 155

S16-4 14:30-15:00
Heenam Kim (Korea University)
Strain-Level Dysbiosis in the Human Microbiome and Modern Diseases ........................... 156
Symposium

S17  Cell Signaling in Host-Microbe Interactions
Chair: Seung Hyun Han (Seoul National University)

S17-1  13:00-13:30
Sun-Yang Park (Yale School of Medicine, USA)
Signal-specific Temporal Response by the Salmonella PhoP/PhoQ System: Regulatory Mechanism and a Distinct Type of Behavior ................................................................. 158

S17-2  13:30-14:00
Young Ran Kim (Chonnam National University)
Characterization of Prohibitin 1 as a Host Partner of Vibrio Vulnificus RtxA1 Toxin ................. 159

S17-3  14:00-14:30
Jong-Soo Lee (Chungnam National University)
Roles of Innate Immune Regulators against Viral Infection .................................................. 160

S17-4  14:30-15:00
Myung Hee Kim (KRIIB)
Defensive Function of Aminoacyl-tRNA Synthetases Against Viral Infection ........................ 161

S18  Host Defense against Viral Infection
Chair: Jun Chang (Ewha Womans University)

S18-1  13:00-13:30
Sang-Jun Ha (Yonsei University)
IL-4-Induced Innate CD8+ T Cells Control Persistent Viral Infection ..................................... 163

S18-2  13:30-14:00
Hyun Mu Shin (Seoul National University)
ZBTB32 and Blimp-1 Cooperate in the Epigenetic Programming of Both CD8+ Effector and Memory T Cells During LCMV Infection. ........................................................................ 164

S18-3  14:00-14:30
Yeu-Chun Kim (KAIST)
Microneedle Patches for Improved Influenza Vaccination ...................................................... 165

S18-4  14:30-15:00
Young Bong Kim (Konkuk University)
Baculovirus-based VLP Forming DNA Vaccine for Influenza pdmH1N1 ............................... 166
**Symposium**

**S19**
**Yeast System for Comparative Functional Genomics**
*Chair*: Keun Pil Kim (Chung-Ang University)

**S19-1**
15:45-16:15
**Young Jun Im** (Chonnam National University)
Structural Mechanism of Ergosterol Regulation and Antifungal Resistance by Fungal Sterol Transcription Factor Upc2

16:15-16:45
**Kiwon Song** (Yonsei University)
Direct Interaction of Ste11 and Mkk1/2 through Nst1 Integrates High Osmolarity Glycerol to the Cell Wall Integrity MAPK Pathway in Budding Yeast *S. cerevisiae*

**S19-3**
16:45-17:15
**Sung-Keun Lee** (Inha University)
Lifespan Regulation by Actin Dynamics Regulation and Amino Acids Metabolism

**S19-4**
17:15-17:45
**Jung-Shin Lee** (Kangwon National University)
The Regulation for Histone Modifications During Transcription Elongation

---

**S20**
**Trials & Research LMO Safety Management**
*Chair*: Jong-Won Oh (Yonsei University)

**S20-1**
15:45 -16:35
**In-Ja Song** (KRIBB)
The Laws of Living Modified Organism(LMO)

**S20-2**
16:45-17:35
**Kyung Hwa Choi** (KRIBB)
Safety Management of LMO Facilities

---

**S21**
**Viral Pathogenesis**
*Chair*: Jae Myun Lee (Yonsei University)

**S21-1**
15:45-16:15
**Wang-Shick Ryu** (Yonsei University)
New Insights into the Biology of Hepatitis B Virus: Viral Entry and Viral Oncogenesis
International Meeting of the Federation of Korean Microbiological Societies

**Symposium**

**S21-2 16:15-16:45**
*Suk Kyeong Lee* (The Catholic University of Korea)
MIR-BART 20-5p Targets Both Viral and Cellular Genes in Epstein-Barr Virus-associated Tumors ............................................................... 174

**S21-3 16:45-17:15**
*Kyung Lib Jang* (Pusan National University)
Hepatitis B Virus, DNA Methylolation, and Hepatocellular Carcinoma ............................................................... 175

**S21-4 17:15-17:45**
*Moon Jung Song* (Korea University)
Viral Modulation of Poly (ADP-ribose) Polymerase 1 to Facilitate Lytic Replication of Gammaherpesviruses ............................................................... 176

**S22  KNMRRC Session I**
*Chair*: Sang Seob Lee (Kyonggi University)
November 6 (Fri.)
09:00-11:00 Rm 303

**S22-1 09:00-09:30**
*Young Woon Lim* (Seoul National University)
Korea Mushroom Resource Bank for Wider Application ............................................................... 178

**S22-2 09:30-10:00**
*Seunho Jung* (Konkuk University)
Microbial Carbohydrate-directed Molecular Complexation for Biotechnological Applications ............................................................... 179

**S22-3 10:00-10:30**
*Joong-Ki Kook* (Chosun University)
Antimicrobial Mechanisms of Oleanolic Acid and Ursolic Acid against *Streptococcus mutans* UA159 ............................................................... 180

**S22-4 10:30-11:00**
*Jae-Seoun Hur* (Sunchon National University)
Anti-Desertification Using Artificial Induction of Biological Soil Crust ............................................................... 182

*Sponsored by Korea National Microorganisms Research Resources Center*

**S23  KNMRRC Session II**
*Chair*: Heejoon Myung (Hankuk University of Foreign Studies)
November 6 (Fri.)
13:00-14:30 Rm 303

**S23-1 13:00-13:30**
*John J. Bang* (North Carolina Central University, USA)
Impacts of Climate Change on Microbial Pathogenic Characteristics and Behavior: Inevitable Paradigm Shift ............................................................... 184
Symposium

**S23-2** 13:30-14:00
*Hana Yi* (Korea University)
Microbiological Assessment of Indoor Air Quality .......................................................... 185

**S23-3** 14:00-14:30
*Subhash Yadav* (University of Hyderabad, India)
Improved Cultivation of Bacteria from Coastal Saline Habitats and Their Mining for Novel Carotenoids .......................................................... 186

* Sponsored by Korea National Microorganisms Research Resources Center

**S24**
Research and Business Development of Biomedicinal Material, Poly-γ-Glutamic Acid
*Chair*: Kyung-Soo Hahm (BioLeaders Corp.)

**S24-1** 15:45-16:15
*Moon-Hee Sung* (Kookmin University)
Medical and Pharmaceutical Applications of Poly-γ-glutamic Acid .................................. 188

**S24-2** 16:15-16:45
*Haryoung Poo* (KRIBB)
Immunology of Poly-γ-Glutamic Acid ................................................................................ 190

**S24-3** 16:45-17:15
*Chul-Joong Kim* (Chungnam National University)
The Natural Substance, Poly-γ-glutamic Acid, Induces Antiviral Effects ............................ 192

**S24-4** 17:15-17:45
*Kyung-Jin Min* (Korea University)
Clinical Application of A Natural Polypeptide, Poly-γ-glutamic Acid Which Has Immunotherapeutic Efficacy ........................................................... 194

* Sponsored by BioLeaders Corp.

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**2015 NRF, Division of Life Sciences**

**S24-1** 13:45-14:15
*Joon Kim* (Korea University)
The Role of NRF for the Development of Microbiology and Biotechnology of Korea in 2016 .................................................................................................................. 195

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Symposium

YS1  Young Scientist Session 1
Chair: Bong Hyun Sung (KRIBB)

YS1-1  09:00-09:20
Boram Jang (Sogang University)
sRNA Regulates the Anaerobic Induction of FrsA Expression .................................................. 198

YS1-2  09:20-09:40
Hyunjoon Park (Handong Global University)
Autoinducer-2 Quorum Sensing of Probiotic Lactobacillus spp. .................................................. 199

YS1-3  09:40-10:00
Young Taek Oh (Yonsei University)
Glucose Metabolism Affects Trimethylamine N-Oxide Stimulated Cholera Toxin Production ... 200

YS1-4  10:00-10:20
Jin Hwan Park (Seoul National University)
Characterization of Vibrio vulnificus CabA Essential for Biofilm Development on Abiotic Surfaces and Oysters ......................................................................................................................... 201

YS1-5  10:20-10:40
Hansol Im (Ulsan National Institute of Science and Technology)
Staphylococcus aureus Uses Its Membrane Vesicles to Inhibit Biofilm Formation by Other Bacterial Species ........................................................................................................................................ 202

YS1-6  10:40-11:00
Hyuk Woo Kwon (Dankook University)
First Report of Brown Rot Caused by Cryptococcus pseudolongus on Fruit Body of Lentinula edodes (shiitake) in Korea ......................................................................................................................... 203

YS2  Young Scientist Session 2
Chair: Sang Sun Yoon (Yonsei University)

YS2-1  09:00-09:20
Chang-Kyu Yoon (Seoul National University)
Investigation of the Interaction Partner of HPr in Vibrio cholerae .................................................. 205

YS2-2  09:20-09:40
Jae-Woo Lee (Seoul National University)
Rsd Stimulates ppGpp Hydrolase Activity of SpoT upon Glucose Starvation in Escherichia coli ............................................................ 206

YS2-3  09:40-10:00
Hyun Gi Kong (Dong-A University)
Characterization of the Viable but Nonculturable State in Ralstonia solanacearum .................... 207
Symposium

YS2-4 10:00-10:20
Dong-Hyeon Kim (Konkuk University)
Growth Inhibition of Cronobacter sakazakii in Experimentally Contaminated Powdered Infant Formula by Kefir Supernatant ........................................................... 208

YS2-5 10:20-10:40
Soo-Kyoung Kim (Pusan National University)
Roles of Ornithine Lipid in Pseudomonas aeruginosa Infection to Host Cells ...................... 209

YS3 Young Scientist Session 3
Chair: Yong-Sun Bahn (Yonsei University)

YS3-1 13:00-13:20
Sejeong Kim (Sookmyung Women’s University)
The Role of DesB on Virulence Traits in Pseudomonas aeruginosa ................................ 211

YS3-2 13:20-13:40
Jun-Sun Park (Korea National Institute of Health)
Development of Infectious Clones of a Wild-Type Korean Rabies Virus and Evaluation of Their Pathogenic Potential .................................................................................. 212

YS3-3 13:40-14:00
Young-Eui Kim (Sungkyunkwan University)
Positive Role of Promyelocytic Leukemia Protein in Type I Interferon Response and its Regulation by Human Cytomegalovirus .................................................................................. 213

YS3-4 14:00-14:20
Kwangjoon Jeong (Chonnam National University)
Metabolic Reprogramming after Infection: Knowledges from Systems Biological Analysis of in vivo Transcriptome and Protem of Vibrio vulnificus .................................................. 214

YS3-5 14:20-14:40
Youn-Hee Kim (Seoul National University)
A Novel Natural Compound Alleviates Severe Sepsis .......................................................... 215

YS3-6 14:40-15:00
Sooyoung Lee (Yonsei University)
Hepatitis B Virus X protein Stabilizes Myc Oncoprotein by Inhibiting Ubiquitination of Myc via Binding to Myc and Contributes to Oncogenesis ...................................................... 216
YS4  Young Scientist Session 4  
Chair: Jong-Chan Chae (Chonbuk National University)  
November 6 (Fri.)  
15:45-17:25 Rm 304

YS4-1 15:45-16:05  
Chan Woo Song (KAIST)  
Metabolic Engineering of *Escherichia coli* for the Production of Industrial Platform Chemicals  
.................................................................................................................................................................................. 218

YS4-2 16:05-16:25  
Minho Roh (KAIST)  
Technique of Fine-Tuned Knockdown Using Small Regulatory RNA and Its Application in Metabolic Engineering for Production of Putrescine from *Escherichia coli*  
.................................................................................................................................................................................. 219

YS4-3 16:25-16:45  
Kyuyeon Lee (Handong Global University)  
Autoinducer-2 Mediated Quorum Sensing Influences on Bacterial Growth under Stress Conditions  
.................................................................................................................................................................................. 220

YS4-4 16:45-17:05  
SeongYeol Choi (Ulsan National Institute of Science and Technology)  
Antibiotic Effect of Violacein and Purpose of its Production in Nature  
.................................................................................................................................................................................. 221

YS4-5 17:05-17:25  
Yong Jae Lee (KAIST)  
Development of Novel SRP Machinery-Engineered *E. coli* Mutant for the Secretary Production of Antibodies and Therapeutic Proteins  
.................................................................................................................................................................................. 222

Luncheon Seminar

Research and Publication Ethics in Science and Scientific Writing  
November 5 (Thu)  
11:45-13:00 Rm 303

LS 1 11:45-12:20  
Yong-Sun Bahn (Yonsei University)  
How To Publish a Scientific Paper  
.................................................................................................................................................................................. 224

LS 2 12:20-13:00  
Eun Hee Cho (Chosun University)  
Ethics in Writing a Research Paper  
.................................................................................................................................................................................. 225

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## Poster Session

### Poster Sessions / 포스터세션

<table>
<thead>
<tr>
<th>Session</th>
<th>Date</th>
<th>Topics</th>
<th>Display Time</th>
<th>Presentation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poster Session 1</td>
<td>Nov. 5</td>
<td>A, B, F, I</td>
<td>08:00–17:00</td>
<td>11:45–13:00</td>
</tr>
<tr>
<td>Poster Session 2</td>
<td>Nov. 6</td>
<td>C, D, E, G, H, J</td>
<td>08:00–17:00</td>
<td>11:00–13:00</td>
</tr>
</tbody>
</table>

### Poster Topics

- **A** Systematics and Evolution
- **B** Environment and Ecology
- **C** Physiology and Biochemistry
- **D** Fermentation and Metabolites
- **E** Genetics and Genome
- **F** Infection and Pathogenesis
- **G** Immunology and Signal Transduction
- **H** Biotechnology
- **I** Food Microbiology
- **J** Others

### Poster Zone

[Diagram of the poster zone with specific areas marked for Nov. 5 (Thu) and Nov. 6 (Fri)]

- **Nov. 5 (Thu)**
  - A01–A72, B01–B68, F01–F86, I01–I37
- **Nov. 6 (Fri)**
  - C01–C31, D01–D29, E01–E33, G01–G23, H01–H50, J01–J85
**A-1** Some Unrecorded Species *Psathyrella ammophilla*, *Marasmiellus mesosporus* and *Tulostoma fimbriatum* var. *campestris* from Taean Coastal(Taenahaeanaen) National Park in Korea

Taecheon Kim*, Yangsup Kim1 and Yongwoo Park2
1National Park Research Institute, Korea National Park Service, 2Department of Forest Science, Chungbuk University

**A-2** New Records of Three *Sarcoscypha* Species (Sarcoscyphaceae, Ascomycota) in South Korea

Youngju Min and Changru Kim*
Biological and Genetic Resources Assessment Division, National Institute of Biological Resources

**A-3** *Cohnella collisoli* sp. nov., Isolated from Lava Forest Soil

Keun Chul Lee1, Kwang Kyu Kim1, Jong-Shik Kim2, Dae-Shin Kim3, Suk-Hyung Ko1, Seung-Hoon Yang1, and Jung-Sook Lee1,4*
1KRIBB, 2GIMB, 3World Heritage and Mt. Hallasan Research Institute, 4LST

**A-4** Revision of the Genus *Arthonia* (Arthoniaceae) in Korea

Beeyoung Gun Lee and Jae-Seoun Hur*
Korean Lichen Research Institute (KoLRI), Sunchon National University

**A-5** Bio-control Efficacy of Bacteria against *Fusarium Oxysporum* f. sp. *Conglutinans*

Youngju Min and Changmu Kim*
Biological and Genetic Resources Assessment Division, National Institute of Biological Resources

**A-6** A Bacterium Representing Novel Species in the Genus *Pontibacter*, Isolated from Seawater of Gwangyang Bay

Daein Kim, Jihee Lee, Hyunseok Kang, Miri Lim, and Chinam Seong*
Department of Biology, Sunchon National University

**A-7** Taxonomic Description of *Humibacter soil* sp. nov., Isolated from Soil

Jisan Park1, Yu Ri Kim1, Min-Kyeong Kim1, Ji-Hye Han1, Yochan Joung2, and Seong Bum Kim1
1Department of Microbiology and Molecular Biology, Chungnam National University, 2Department of Biology, Inha University

**A-8** Genome-wide SNP Analysis Associated with Origin and Fruitbody Color in *Flammulina velutipes*

Sung-I Woo1, Eun-Sun Kim1, Jae-Gu Han1, Kab Yeul Jang1, Pyung-Gyun Shin1, Youn-Lee Oh1, Min Ji Oh1, Kyung-Soo Kim2, Sung-Hwan Jo1, and Won-Sik Kang1*
1Mushroom Research Division, National Institute of Horticultural & Herbal Science, Rural Development Administration Chungbuk Eumsung 369-873, 2Department of Applied Biology Graduate School, Kangwon National University, Chuncheon 200-701, 3SEEDERS, Daeduk Industry Academic Corporation Building, Gwanpyeong-dong Yuseong-gu, Daejeon

**A-9** *Micromonospora fulva* sp. nov., Isolated from Forest Soil

Seungyeol Shin1, Hyojin Lee2 and Kyungsook Whang1,2*
1Department of Microbial & Nano Materials, Mokwon University, 2Institute of Microbial Ecology & Resources, Mokwon University

**A-10** Advanced Gene Typing Analysis of *Xanthomonas* Isolated from Korean Pepper

KiHo Park1, Byung-Soo Kim2 and Sa-youl Ghim3*
1School of Life Sciences and Biotechnology, Kyungpook National University, 2College of Agriculture and Life Science, Kyungpook National University, 3School of Life Science and Biotechnology, Kyungpook National University

**A-11** Effects of Coffee and a Traditional Chinese Herbal Medicine on the Growth of Two Gut Lactobacilli from Koreans and Europeans

Seokcheon Song1, Jihyun Lee2, Kyuwon Jeun1, Eungyeoung Heo1, Jineung Kim1, Jaegu Seo3, Myungjoon Jung1, and Eungbin Kim1*
1Department of Systems Biology, Yonsei University, 2Department of Cell and Systems Biology, University of Toronto, Canada, 3R&D Center, Cellbiotech
**Poster Session**

A-12 *Rhodanobacter paracrisesis* sp. nov., isolated from Ginseng Rhizosphere  
Boleumi Lee, Geonyeong Cho and Song-Ih Han*  
Department of Microbial & Nano Materials, Mokwon University

A-13 *Endoradicella ginsengi* gen. nov., *sp. nov., a Slow Growing Bacterium Isolated from Inside of Ginseng Root  
Jun-Su Song1, Seung-Yeol Shin1, In-Hwa Jeon1, and Kyung-Sook Whang1,2*  
1Department of Microbial & Nano materials, Mokwon University, 2Institute of Microbial Ecology and Resources, Mokwon University

A-14 *Sphingobium polysaccharaeus* sp. nov., an Exopolysaccharide-producing Bacterium Isolated from the Rhizosphere of *Angelica sinensis*  
Cho-Hee Hwang, Ye-Rim Lee and Sang-Ho Chung*  
Department of Microbial & Nano Materials, College of Science & Technology, Mokwon University, 88 Doanbuk-ro, Seo-gu, Daejeon 302-318, 2Department of Microbial & Nano Materials, Mokwon University

A-15 *Flavobacterium halomollis* sp. nov., halo-sensitive bacterium isolated from rhizosphere soil of *Angelica sinensis*  
Hye-Won Park, Ju-Ok Kim and Song-Ih Han*  
Department of Microbial & Nano materials, Mokwon university

A-16 *Burkholderia humisilvae* sp. nov., *Burkholderia solisilvae* sp. nov. and *Burkholderia rhizospherae* sp. nov., Isolated from Forest Soil and Rhizosphere Soil  
Jae-Chan Lee1 and Kyung-Sook Whang2,3*  
1Institute of Microbial Ecology and Resources, Mokwon University, 88 Doanbuk-ro, Seo-gu, Daejeon 302-318, 2Department of Microbial & Nano Materials, College of Science & Technology, Mokwon University, 88 Doanbuk-ro, Seo-gu, Daejeon 302-318

A-17 *Halomonas* sp. nov., a Halophilic Bacterium Isolated from the Sediment of a Solar Saltern Pond  
Su-Jin Kim1, Song-Ih Han1, Jae-Chan Lee2, and Kyung-Sook Whang1,2*  
1Department of Microbial & Nano materials, Mokwon University, 2Institute of Microbial Ecology and Resources, Mokwon University

A-18 *Chryseobacterium stabulisuum* sp. nov. isolated from Air in a Pig Farm  
Siwon Lee, Byedol Choe, Youn-Lee Joo, Oh Sang Kwon, Eung-Roh Park*, and Hyen-Mi Chung*  
Environmental Infrastructure Research Department, National Institute of Environmental Research, Incheon 22689

A-19 New Lichen-Forming and Lichenicolous Fungi from South Korea  
Sergii Kondratyuk1*, Laszlo Lokos2, Soon-Ok Oh1, and Jae-Seoun Hur3  
1M. H. Kholodny Institute of Botany, Ukraine, 2Department of Botany, Hungarian Natural History Museum, Hungary, 3Korean Lichen Research Institute, Sunchon National University

A-20 Whole Genome Analysis of Hantaan Virus from Rodents Captured in Gangwon Province, Republic of Korea  
Jeong-Ah Kim1, Won-keun Kim1, Jin Sun No1, Seung-Ho Lee1, Soo-Kyoung Lee1, Ji Hye Kim1, Jeong Hoon Kho1, Daesang Lee1, Dong Hyun Song2, Seong Tae Jeong2, Heung Chul Kim3, Terry A Klein4, and Jin-Won Song1*  
1Department of Microbiology, College of Medicine, Korea University, 2The 5th R&D Institute, 35th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, 4Force Health Protection and Preventive Medicine, 65th Medical Brigade/US Army MEDDAC-Korea

A-21 *Bacillus paralicheniformis* sp. nov., Isolated from Fermented Soybean Paste  
Christopher A. Dunlap1, Soon-Wo Kwon2, Alejandro P. Rooney1, and Soo-Jin Kim*  
1Crop Bioprotection Research Units, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, IL, USA, 2Korean Agriculture Culture Collection (KACC), Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration

A-22 *Friedmanniella aerolata* sp. nov., Isolated from Air  
Soo-Jin Kim, Jae-Hyang Ahn, Hang-Yeon Weon, and Soon-Wo Kwon*  
Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration

A-23 *Flavitallea soli* sp. nov. Isolated from Soil  
Soo-Jin Kim, Jae-Hyang Ahn, Hang-Yeon Weon, Seung-Beom Hong, Soon-Ja Seok, Jeong-Seon Kim, and Soon-Wo Kwon*  
Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration
**International Meeting of the Federation of Korean Microbiological Societies**

**Poster Session**

**A-24** Molecular Diversity of Imjin Virus, A Shrew-borne Hantavirus, in Gangwon Province, Republic of Korea ................................................................. 232
Seung-Ho Lee1, Won-keun Kim2, Daesang Lee2, Dong Hyun Song3, Soo-Young Lee1, Jeong-Ah Kim1, Jin Sun No1, Ji Hye Kim1, Jeong Hoon Kho1, Heung-Chul Kim3, Terry A Klein1, and Jin-Won Song1* 1Department of Microbiology, College of Medicine, Korea University, 2The 5th R&D Institute, Agency for Defense Development, 3Force Health Protection and Preventive Medicine, 65th Medical Brigade/US Army MEDDAC-Korea

**A-25** Previously Uncultured Marine Bacteria Linked to Novel Alkaloid Production ..................................................................................................................... 233
Grace Eun Ju Choi1,2, Sang-Jip Nam2,3, Lauren Paul2, Deanna Beatty2, Christopher A. Kauffman2, Paul R. Jensen2, and William Fenical2 1National Marine Biodiversity Institute of Korea, Seocheon-gun, Chungcheongnam-do, 325-902, 2Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0204, 3Natural Product and Medicine Research Laboratory, Department of Chemistry and Nano Science, Ewha Womans University, Science Hall D-311, Seoul, 120-750

**A-26** In vitro and In vivo Activities of LCB10-0200 a Novel Cephalosporin against Clinical Isolate Bacteria ................................................................. 233
Jinhwan Kwak*, Sanghun Oh, Soonho Lee, Hyeshin Kim and Junhyung Lee Handong global university

**A-27** Isolation and Identification of Wild Yeasts from Soil of Chungcheongnam-do in Korea ........................................................................................................ 233
Sang-Min Han, Sang-Min Bae, Jae-Won Han, and Jong-Soo Lee* Department of Biomedical Science and Biotechnology, Paichai University, Daegu 703-701, Republic of Korea

**A-28** Identification and Classification of Falsiaeromonas sp. Strain AR1 Isolated from a Fresh Water Stream ................................................................. 233
Shalem Raj Padakandla and Jong-Chan Chae* Division of Biotechnology, Chonbuk National University

**A-29** Current-generation by Genus Bowmanella and Bowmanella dokdonensis sp. nov., Isolated from the Seawater of Dokdo, Korea ............................................................................................................................. 234
Ye-Ji Hwang1, Marie Kim2, Hyunwoong Park3, and Sa-Youl Ghim1* 1School of Life Sciences, Research Institute for Ulleungdo & Dokdo Islands and KNU Creative BioResearch Group (BK21 plus project), Kyungpook National University, 80 Daehakro, Bukgu, Daegu 702-701, Republic of Korea, 2Department of Biomedical Laboratory Science, Kyungpook University, 730 Gwangdong-ro, Sinjeong-myeon, Gumi, Gyeongbuk 730-739, Republic of Korea, 3School of Energy Engineering, Kyungpook National University, 80 Daehakro, Bukgu, Daegu 702-701, Republic of Korea

**A-30** The Species Identity of Phellinus linteus (Sanghuang) Extensively Used in Korea ........................................................................................................ 234
Min-Woo Hyun1, Jae-Han Cho1, Kang-Hyo Lee1, Won-Sik Kong1, Gi-Ho Song2, and Jae-Gu Han1* 1Mushroom Research Division, National Institute of Horticultural and Herbal Science, Rural Development Administration, 2Catholic Kwandong University International St. Mary’s Hospital

**A-31** Community Assessment of Endophytic Fungi Isolated from Abies koreana, an Endemic Species of Korea ................................................................. 234
Hyan Jung Lee1, Myounghui Kwak1, Woo Young Bang1, Joo-Hong Ye1, and Soonok Kim1* 1Biological and Genetic Resources Assessment Division, National Institute of Biological Resources, 2Plant Resources Division, National Institute of Biological Resources

**A-32** Identification and Characterization of Antifungal Metabolites Produced by Bacillus sp. Against Phytopathogens ................................................................. 234
Aravind Sundararaman1 and Sang Seob Lee2* 1Department of Biological engineering, Kyonggi University, 2Department of Life Science, Kyonggi University

**A-33** Evaluation of Membrane Permeabilizers’ Effects on Responses of a Genotoxin- and Oxidative Stress-sensitive Escherichia coli Bioreporter ..................................................................................................................... 235
Soh Mabekou Sandrine1 and Robert James Mitchell2* 1Department of Biological science, Ulsan national institute of Science and Technology, 2Department of Biological Science, Ulsan National Institute of Science and Technology

**A-34** Contribution to the Lichen Flora of Vietnam, with Ten New Record Species ........................................................................................................ 235
Liu Dong, Soon Ok Oh, Jung-Shin Park, Beeyoung-Gun Lee, Jung-Jai Woo, and Jae-Seoun Hur* Korean Lichen Research Institute, Sunchon National University
**Poster Session**

**A-35** Lichen Taxonomy Study of Pertusaria (Pertusariaceae, Ascomycota) in South Korea

Jung-Shin Park and Jae-Seoun Hur*

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**A-36** Sediminibacter aquarii sp. nov. Isolated from Sediment of a Fishbowl

Yonghoon Kim¹, Young Nam Kim¹, Bo Bac Kim³, Siwon Lee⁴, and Tae-Young Ahn²*

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**A-37** Mycelial Growth and Microscopic Morphological Characteristics of Poria Cocos

Woo-Sik Jo*, Ju-Ri Park, Min-Gu Kang, and Woo-Hyun Kim

Gyeongbuk Province Agricultural Technology Administration

**A-38** Pelocola oligotropha gen. nov., sp. nov., Isolated from a Shallow Stream Sediment

Dohak Kim, Hansol Kim, Yongseok Ko, and Tae-Young Ahn*

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**A-39** A Noble Agar-hydrolyzing Bacterium Vibrio sp. S4 Isolated from Seawater of Jeju Island, Republic of Korea

Vijayalakshmi D¹, Chang-Ro Lee², Jae-Seon Park², and Soon-Kwang Hong²*

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**A-40** Dankookia rubra gen. nov., sp. no., Isolated from Sediment of a Shallow Stream, Korea

Wan-Hoe Kim, Keunsoo Kang and Tae-Young Ahn*

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**A-41** Dermabacter vaginalis sp. nov. Isolated from the Korean Vaginal Sample

Dong-Ho Chang¹,², Sooyeon Lim³, Sharon Ahn¹, Moon-Soo Rhee¹ and Byoung-Chan Kim¹

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**A-42** Agarolytic Novel Bacterial Strain KA8, Isolated from Seawater of Gwangyang Bay

Jiseo Lee, Misun Kim, Areum Kim, and Chinam Seong*

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**A-43** Allererythrobacter wooponensis sp. nov., Isolated from Freshwater of Woopo Wetland in Korea

Joowon Kang¹, Keunsook Baik², Seon Choi², and Chinam Seong¹*

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**A-44** Plantibacterium curvus gen. nov., sp. nov. Isolated from Surface Sterilized Root of Artemisia princeps

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**A-45** Arbovirus Surveillance in Mosquitoes Collected from Airports, Sea Ports and Animal Quarantine Stations during 2014 in Republic of Korea

Hyeong-Hwa Ha¹, Su-Bong Ha¹, Hyun-Ji Seo¹, Ji-Hye Lee¹, Sung-Hee Kim¹, Hye-Young Jeong¹, Yong-Joo Kim¹, Byoung-Han Kim¹, Heung-Chul Kim¹, and Jee-Yong Park¹*

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**A-46** Isolation and Molecular Identification of Endophytic Fungi from Arctic Plants

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**A-47** More New Records of Pyrenocarpous Lichens from South Korea

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**Poster Session**

**A-48** Phylogenetic Analysis of *Russula* Section Foetentinae (Russulales, Basidiomycota) in Korea ............................................. 238
Hyon Lee1, Myung Soo Park1, Paul Eumil Jung2, Jonathan J. Fong3, Soon-Ja Seok4, and Young Woon Lim1*  
1School of Biological Sciences, Seoul National University, Seoul, 2Korea National Research Resources Center, Seoul, 3Science Unit, Lingnan University, Tuen Mun, New Territories, Hong Kong, 4National Institute of Agricultural Science and Technology, Rural Development Administration, Wanjoo-gu, Jeollabuk-do

**A-49** Two new *Lycopodium* Species Collected from Korea .................................................................................. 239
Chang Sun Kim, Jong Won Jo, Young-Nam Kwag, Soon-Ok Oh, and Sang-Kuk Han*  
Forest Biodiversity Division, Korea National Arboretum

**A-50** Morphological, Physiological and Molecular Characters of Brown Yeast-like Fungus Producing Melanin from the Antarctic ................................................................. 239
T. O. Kondratyuk1*, Jung A Kim2, Kondratyuk A. S.1, and Jae-Seoun Hur2  
1Scientific Educational Centre, Taras Shevchenko National University of Kiev, Ukraine, 2Korean Lichen Research Institute, Sunchon National University

**A-51** Three Gene Phylogeny of the Teloschistaceae (Lecanoromycetes, Ascomycota): Current Status ............................................. 239
Sergii Kondratyuk1*, Jung A Kim2, Anna Kondratyuk3, and Jae-Seoun Hur2  
1M. H. Khodolny Institute of Botany, Ukraine, 2Korean Lichen Research Institute, Sunchon National University, 3Scientific Educational Centre, Taras Shevchenko National University of Kiev, Ukraine

**A-52** *Aquimarinum salinum* gen. nov., sp. nov., and *Aquimarinum pelagicum* sp. nov., a Novel Marine Bacterium of the Phylum *Bacteroidetes* Isolated from Seawater ......................................................... 239
Seung-Jo Yang1,2, Junsun Yu1, YoCHAN Jong1, and Jang-Cheon Cho1*  
1Department of Biological Sciences, Inha University, 2National Marine Biodiversity Institute of Korea

**A-53** Comparative Characterization of Amylolytic Yeast Saccharomycopsis fibuligera and its Sister Species in Genus Saccharomycopsis .................................................................................. 240
Dong Wook Lee, Jin Ho Choo and Hyun Ah Kang*  
Dept. of Life Science, Chung-Ang University, Seoul, 156-756

**A-54** Candidate of New *Entoloma* Species (Entolomataceae, Basidiomycota) in Korea ............................................. 240
Jong Won Jo, Chang Sun Kim, Young-Nam Kwag, Soon-Ok Oh and Sang-Kuk Han*  
Division of Forest Biodiversity, Korea National Arboretum

**A-55** Characterization of Two Bacteriocins Produced by *Staphylococcus* Species to Study Potential Applications for Food Safety .................................................................................. 240
Jonguk Kim, JiSoo Hong, Moran Lee, Jeong-A Lim, Kyu Suk Jung, Sanghyun Han, Jinwoo Park, and Eunjung Roh*  
Microbial Safety Team, National Institute of Agricultural Sciences, Rural Development Administration

**A-56** A New Species of *Aspergillus* Section Cremei Isolated from a Forest Soil in Korea ............................................. 240
Tham Thi Duong, Thuong Thong Nguyen and Hyang Burm Lee*  
Division of Food Technology, Biotechnology & Agrochemistry, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 61186

**A-57** Multi-gene Analysis Reveals New Species within the *Ilyonectria radicicola* Species Complex from Korean Panax ginseng .................................................................................. 241
Man Won Soo1, Jeong Young Song1, Sun Ick Kim2, and Hong Gi Kim1*  
1Department of Applied Biology, Chungnam National University, 2Ginseng Research & Development Institute

**A-58** *Paucibacter planktonicus* sp. nov., a Novel Bacterium Isolated from Daechung Reservoir, Korea ..................................................... 241
Ji-Hun Yun1,2, Dong-Hoon Shin1,2, JunHyuk Lee1,2, and Song-Gun Kim1*  
1Microbial Resource Center/KCTC, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, 2Department of Biomedical Science & Biotechnology, Paj Chai University 155-40 Baejae-ro, Seo-gu, Daejeon, 3Microbial Resource Center/KCTC, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, 4Dept. Biosystem, Hannam University, Daejeon 306-791

**A-59** *Dominibacillus ruralis* sp. nov. Isolated from Air Near the Garlic Field ..................................................... 241
Byool Cho1,2, Hyen-Mi Chang1, Siwon Lee1, Yoon-Leu Joo1, Ji Hye Kim1, Bo-Ram Lee1, Su Jeong Park1, Jang-Cheon Cho1, and Eung-Oh Park1*  
1Water Supply & Suwerage Research Division, National Institute of Environmental Research, Incheon, 2Department of Biological Sciences, Inha University, Incheon
A-60 Mycological Characteristics and Artificial Cultivation and of a Novel *Phellinus linteus* KACC 93057P strain ... 241
Gyeongjin Min1, Amin Kwaak2, Minjae Son3, Sunja Seok4, and Heewon Kang5*
1Graduate School of Bio. & Information Technology, Hankyong National University, Ansan 17579, 2Graduate School of Bio. & Information Technology, Hankyong National University, Ansan 17579, 3Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Wanju 53365

A-61 Possibility of Race Identification for Korean *Plasmodiophora brassicae* Isolates through RAPD Analysis ... 242
Kwang Hoon Kang, Ju Young Park, Mun Won Seo, Jeong Young Song, and Hong Gi Kim*
Department of Applied Biology, Chungnam National University

A-62 *Leucothyrix arctica* sp. nov. Isolated from Arctic Ocean ... 242
Kwoon Baek1,2, Ahyoung Choi1,3, Eu Jin Chung4, Hyangmi Kim5, and Jung-Cheon Cho6*
1Department of Biological Sciences, Inha University, 2Freshwater Bioresources Research Division, Nakdonggang National Institute of Biological Resources, 3Freshwater Bioresources Research Division, Nakdonggang National Institute of Biological Resources

A-63 A New Endophytic Species from *Abies firma* Leaf in Korea ... 242
Thi Thuong Thuong Nguyen and Hyang Burm Lee*
Division of Food Technology, Biotechnology and Agrochemistry, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 61186

A-64 *Syzygites* sp. nov. from Contaminated Mushroom in Korea ... 242
Thi Thuong Thuong Nguyen and Hyang Burm Lee*
Division of Food Technology, Biotechnology and Agrochemistry, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 61186

A-65 Characterization and Identification of Methane Oxidizing Bacteria Isolated in Yellow Loesses from Jeollanam-do, Korea ... 243
Sung Min Park1, Lavanya Madhavara1, and Si Wouk Kim2,3*
1Department of Energy Convergence, Chosun University, Gwangju, 2Department of Environmental Engineering, Chosun University, Gwangju, 3Department of Environmental Engineering, Chosun University, Gwangju

A-66 *Planktotalea arctica* sp. nov., Isolated from Arctic ... 243
Kwoon Baek1,2, Mi-Hwa Lee3, Ji-Hye Han4, Jaeduk Goh5, Sang Cheol Kim6, and Jung-Cheon Cho7*
1Department of Biological Sciences, Inha University, 2Bacterial Resources Research Team, Freshwater Bioresources Research Division, Nakdonggang National Institute of Biological Resources, 3Freshwater Bioresources Research Division, Nakdonggang National Institute of Biological Resources

A-67 *Runella palustris* sp. nov., Isolated from Wetland Freshwater ... 243
Hanul Kim1, Heeyoung Kang2, Tae Yong Jang3, Yochan Joung4, and Kiseong Joh5*
1Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, 2Department of Biological Sciences, Hankuk University of Foreign Studies, 3Department of Environmental Engineering, Chosun University, Gwangju

A-68 *Emticicia paludis* sp. nov., Isolated from Wetland Freshwater ... 243
Heeyoung Kang1, Yochan Joung2 and Kiseong Joh3*
1Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, 2Department of Biological Sciences, Hankuk University of Foreign Studies

A-69 Evaluation of Water Quality of Hot Spring Water for Agricultural Use by *Legionella*-Incidence Risk ... 244
Young-Gun Zo4*, Chang-Yeol Yang2 and Kwang-Ho Kim1*
1Department of Biology, Kyungsung University, 2Crop Protection Division, National Academy of Agricultural Science, 3Department of Biological Sciences, Inha University

A-70 *Musciilaginibacter ginsengisoli* sp. nov. Isolated from a Ginseng-cultivated Soil ... 244
Shin Ae Lee, Jae-Hyung Ahn, Soo-Jin Kim, Jackyeong Song, Soon-Wo Kwon, and Hang-Yeon Weon*
Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Wanju 565-851

A-71 *Hydrogenophaga* sp. LPB000724, a Novel Species Candidate within the Family *Comamonadaceae* ... 244
Eunji Kim1, Si-Kyong Shin2, Sungmi Choi3, and Hana Yi4*
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**International Meeting of the Federation of Korean Microbiological Societies**

**Poster Session**

### Environment and Ecology

**A-72** A Different Viewpoint of the Anamorph Stage of *Mycosphaerella nawae* Based on Phylogenetic and Morphological Characteristics

Yang-Sook Lim1, Seung-Yeol Lee2, Jin-Ho Keum3, Sangkyu Park2, and Hee-Young Jung2*

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**B-1** Detection of *Pseudomonas otitidis* E42 Gene for Polyethylene Degradation

Mi Sun Lim and Mal Nam Kim*

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**B-2** A Single ORF Unique to Pathogenic Strains and ORFs Necessary but Not Sufficient for the Virulence of *Vibrio vulnificus* as Determined by Comparative ORFeome Subtraction

Hye-Jin Kim and Jae-Chang Cho*

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**B-3** A Meta Analysis of Ruminal Archaeal Diversity

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**B-4** Genotypic Diversity and Population Structure of *Vibrio vulnificus* Strains Isolated in Taiwan and Korea as Determined by Multilocus Sequence Typing

Hye-Jin Kim and Jae-Chang Cho*

Hankuk University of Foreign Studies

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**B-5** Bacterial Community Changes during Enrichment Cultivation of Oil-Polluted Soil Loaded with Low-Molecular-Weight Polyethylene

Choong Eun Jin and Mal Nam Kim*

Department of Life Science, Sangmyung University

---

**B-6** Comparisons of ATP Analytic Methods for Measuring Total Active Biomass in Granular Activated Carbon Filters in Water Treatment Plants

Yujin Lee, Min-jeong Kim, Seonjoo Kim, and Gyu-Cheol Lee*

K-water

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**B-7** Microbial Treatment of Food Waste

Soohyun Kim, Youngju Kim and Gi Eun Kim

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---

**B-8** Comparison of Rhizobacterial Communities in Pepper Greenhouse under Different Cropping Systems

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**B-9** Occurrence of the Novel Human Norovirus GI.17 in Coastal Stream Water of South Korea, 2015

Eung Seo Koo1, Man Su Kim1, Yong Seon Choi1, Tae-Ok Kim2, Kwon-Sam Park2, and Yong Soek Jeong1*

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**B-10** Investigation of Diversity of Extremely Halophilic Archaea from Solar Salts

Seo Joon Roh1, Joseph Kwon, Jin-Kyu Rhee2, and Jong-Soon Choi1*

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Poster Session

B-11 Development of Algicidal Reactor with Bio/ceramics Membrane for Controlling of Harmful Algal Blooms (HABs) ........................................... 247
Sae-Byeol Moon¹ and Sang-Seob Lee*²
¹Department of Bioengineering, ²Department of Life Science, Kyonggi University

B-12 Effect of Three Crops Cultivation on DHA (dehydrogenase) Activity in Highland Areas ................................................................. 247
Gyeryeong Bak, Min Kwon, Gyejun Lee, Donglim You, Jongsu Ryu, Juil Kim, Sunnyeoj Ji, and Janggyu Choi
Highland Agriculture Research Institute, National Institute of Crop Science, Rural Development Administration

B-13 Combined Application of Cyanobacteria with Soil Fixing Chemicals for Rapid Induction of Biological Soil Crust Formation ........................................ 248
Chun-Ho Park and Jae-Seoun Hur*
Korean Lichen Research Institute, Sunchon National University

B-14 Combination of Conventional RT-PCR and Quantitative PCR for Human Norovirus Source Tracking in Environmental Waters ........................................... 248
Yong Seon Choi¹, Eung Seo Koo¹, Man Su Kim¹, Hyun Jin Yoon¹, Hyun Bae Choi², Jong Deok Choi², Yong Sik Sin¹, and Yong Seok Jeong¹*
¹Department of Biology, College of Sciences, Kyung Hee University, ²Department of Seafood Science and Technology, Gyeongsang National University

B-15 Monitoring of Culicoides in Korea in 2014 ................................................................................................................................. 248
Su-Bong Ha¹, Hyun-Ji Seo¹, Hyeong-Hwa Ha¹, Sung-Hee Kim¹, Hye-Young Jeoung¹, Yong-Joo Kim¹, Heung-Chul Kim², Byoung-Han Kim¹, and Jee-Yong Park¹*
¹Animal and Plant Quarantine Agency, ²5th Medical Detachment, 18th Medical Command, U.S. Army

B-16 Purification and Detection of Alternaria Mycotoxins Using HPLC and LC-MS/MS ................................................................. 248
Sun Jeong Jeon¹, Jueun Kim², Hye Won Lee¹, Chul Won Lee², and Hyang Burm Lee¹
¹Division of Food Technology, Biotechnology & Agrochemistry, College of Agriculture & Life Sciences, ²Department of Chemistry, Chonnam National University

B-17 A Proenvironmental Removal and Reuse Process of Nitrogen and Phosphate .................................................................................................................. 249
Jaehoon Jo and Gieun Kim
Department of Biotechnology, Seokyeong University

B-18 Morphological Observation of Saccharomyce scerevisiae KCTC 7296 under Sub-MIC with Amphotreicin-B Antibiotics ........................................... 249
Kyungmin Lee, Kwanhyeong Lee and Kyujoong Kim*
Department of Biology, Gangneung-Wonju National University

B-19 Plant Growth-Promoting Bacteria Bacillus sp. CeR16-2, Possessing Drought Stress Tolerance in Kale Plant ................................................................. 249
Dayeon Kim, Ji Hee Han, Jeong Jun Kim, and Sang Yeob Lee*
Agricultural Microbiology Division, National Academy of Agricultural Science, RDA

B-20 A Novel Zinc Dependent Metalloprotease from Pseudoalteromonas sp. Strain SIA1 ...................................................................................... 249
Kyuwon Jeun¹, Sia Kang², Dockyu Kim¹, Hanwoo Kim¹, Haju Park³, Jounghan Yim¹, and Eungbin Kim¹*
¹Department of Systems Biology, Yonsei University, ²Animal and Plant Quarantine Agency, ³Division of Life science, Korea Polar Research Institute

B-21 Developing an Eco-Friendly Quality Compost Using Spent Coffee Grounds, Biochar and Beneficial Microorganisms .......................................................... 250
Jangyeon Yoo¹, Jiyung Song², Sinyung Ha¹, In Soo Kim¹, Jaesoo Chang², and Sung-Cheol Koh¹*
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International Meeting of the Federation of Korean Microbiological Societies

Poster Session

B-22 Eco-Friendly Odor Control and Remediation of a Contaminated Urban Stream Using Beneficial Microorganism (BM)
Jikyung Song1, Jangyeon Yoo2, Eunyoung Jang1, Insoo Kim1, Jaesoo Chang1, and Sungcheol Koh1*
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B-23 Analysis of Gut Bacterial Diversity and Exploration of Cellulose-Degrading Bacteria in Xylophagous Insects
Min-Young Choi1, Jae-Hyung Ahn1, Jaekyung Song1, Seong-Hyun Kim2, Jin-Woo Bae3, and Hang-Yeon Weon1*
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B-24 A Study on the Detective Method and Distribution of Aeromonas Genera in Water Supplies
Jikyung Song1, Jangyeon Yoo2, Eunyoung Jang1, Insoo Kim1, Jaesoo Chang1, and Sungcheol Koh1*
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B-25 Eco-Friendly Marine Sediment as Material with Nitrogen and Phosphorus Removal Efficiency Bacteria Activated Sludge SBR Waste Seawater Treatment System
Seonghyeon Cho
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B-26 Cultural and Molecular Analysis of Bacterial Community Structures in Soils for Environmental Risk Assessment with Genetically Modified Soybean and Red Pepper
Hyun Kim, Dong-Uk Kim, Hyosun Lee, Jungpyo Yun, and Jong-Ok Ka*
Department of Agricultural Biotechnology, Seoul National University

B-27 Survey of Macrofungal Diversity in Forest Genetic Resources Reserve of Mt. Gariwang for Recent 5 Years (2011-2015)
Sung-Min Jeon, Min-Soo Kim and Kang-Hyeon Ka*
Division of Wood Chemistry and Microbiology, Korea Forest Research Institute

B-28 Bio-Resource Values of Some Macrofungi Found in Forest Genetic Resources Reserve of Mt. Gariwang
Sung-Min Jeon and Kang-Hyeon Ka*
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B-29 Comparison of Sampling Methods for Norovirus Recovery on Surface
Kyuseon Cho1, Cheonghoon Lee2, SungJun Park1, and GwangPyo Ko1,3*
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B-30 Evaluation of the Coliphages as Fecal Indicator and Markers to Investigate Fecal Contamination in Shellfish Culturing Areas of South Korea
Kyuseon Cho1, Cheonghoon Lee2, SungJun Park1, and GwangPyo Ko1,3*
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B-31 Invasion of Phytolacca americana Affects to Arbuscular Mycorrhizal Fungal Community
Yu-Jin Jeon1, Yoo-Jeong Lim1, Woo-Ju Park1, Sang-Jun Jeon1, Hyeok Park2, Hang-Seok Choi1, and Ahn-Heum Eom2*
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B-32 Evaluation of Population Changes through Analyzing the Fungal ITS on Soil of the Treatment of Red Clay Processed Nano Material
Se-Hui Kang, Song-Yi Baek and Jung-Suk Sung*
Department of Life Science, Dongguk University

B-33 Study of Genotyping of Wild Mushroom Lentinula edodes in Mt. Jungwang and Mt. Gariwang in Korea.
Yean Sug Jeong, Min Soo Kim and Kang-Hyeon Ka*
Division of Wood Chemistry and Microbiology, Korea Forest Research Institute
**Poster Session**

**B-34** Janthinobacterium lividum Violacein Production and Vesicle Which Contain Violacein to Dissolve It in Aqueous Phase to Counter Their Rivals

SeongYeol Choi, JeaIm Choi and Robert Mitchell*

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**B-35** Struvite Formation and Immobilization of Microbial Cells

JaeHwan Lee and Gi Eun Kim*

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**B-36** Effect of Copper-Resistant Rhizobacteria on Tomato Plant under Copper Stress and Expression Pattern of Metal Stress-Related Genes in Tomato

Min-Ju Kim and Hong-Gyu Song*

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**B-37** Antifungal Activity of Streptomyces vellosus HR29 and Its Multiple Mechanisms against Several Plant-Pathogenic Fungi

Hae-Ryoung Kim and Hong-Gyu Song*

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**B-38** PAH (Polycyclic Aromatic Hydrocarbon) Degrading Endolichenic Bacteria Isolated from Chinese Desert Lichens

Xiaoning Ma and Jae-Seoun Hur*

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**B-39** A Proenvironmental Removal and Reuse Process of Nitrogen and Phosphate

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**B-40** Nitrogen Fixation Abilities Test for Fertilizer

SunHwan Jeong and Sang-Seob Lee*

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**B-41** Study of Changes in the Microbial Community Associated with Climate Change in the Laboratory Scale

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**B-42** Characterization of the Viable but Nonculturable State in Ralstonia solanacearum

Hyeon Gi Kong, Shabir Ahmad, Seung Yeup Lee, Jinhee Choi, and Seon-Woo Lee*

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**B-43** Rhizosphere Analysis of Field-cultivated and Mountain-cultivated Ginsengs

Seung Il Ahn, Byung Wook Yang and Young Tae Hahm*

Department of Systems Biotechnology, Chung-Ang University

**B-44** Optimization of the Culture Conditions of Halobacteria and Its Effect in Growing Crops in High Salinity Areas

Yunji Seo and YongChan Hong*

Cheongshim International Academy

**B-45** Production of 2-Hydroxyalkanoates from Endolichenic Bacteria Using Xenobiotic Compounds as Sole Carbon Source

Shamsun Nahar and Jae-Seoun Hur*

Korean Lichen Research Institute, Sunchon National University

**B-46** Effects of Photosynthetic Bacteria on Odor Reduction and Pollutants Degradation of Swine Waste Water

In-Cheol Park*, Jae-Hyung Ahn and Jae-hong Yoo

Agricultural Microbiology Division, National Academy of Agricultural Science, RDA
International Meeting of the Federation of Korean Microbiological Societies

Poster Session

**B-47** Biocontrol of Sclerotia by Plant Growth Promoting *Bacillus thuringiensis* C25
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**B-48** Arbuscular Mycorrhizal Fungal Diversity in Post-mining Area and Natural Forest Area in Je-cheon, Korea
Hyeok Park1, Kang-Hyeon Ka2 and Ahn-Heum Eom1,*
1Department of Biology Education, Korea National University of Education, 2Division of Wood Chemistry & Microbiology, Korea Forest Research Institute

**B-49** Diversity of Endophytic Fungi Isolated from Leaves of *Camellia japonica* in Jeju, Korea
Yu-Ra Bae and Ahn-Heum Eom*
Department of Biology Education, Korea National University of Education

**B-50** Enrichment and Isolation of Microorganisms Degrading Triazole Fungicides from Agricultural Soils
Jae-Hyung Ahn, Gwan-Hyeong Lee, Ji-Young Kim, Yu-Mi Ro, Jaehong Yoo and Incheol Park
Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration

**B-51** The Transmembrane Hybrid Histidine Kinase BmsA Acts Independently of the Gac Regulon But Both Are Required for Surface Spreading and Biofilm Formation in *Pseudomonas alkyphenolica*
Gwang Su Ha, Hyeong Min Kim, Ji Hyun Ha, and Kyoung Lee*
Department of Bio Health Science, Changwon National University

**B-52** Anaerobic-Anoxic-Oxic (A2/O) Process by Nitrogen and Phosphorus Removal Bacteria with Sediment for Protect Red Tides
Kunho Kim and Sang-Soob Lee*
Department of Bioengineering, Kyonggi University

**B-53** Newly Isolated Bacteriophages Targeting *Pectobacterium carotovorum* subsp. *carotovorum* to Control Soft-Rot Disease
Jeong-A Lim, Jonguk Kim, Jisoo Hong, Eunjung Roh, Kyu Seok Jung, Sanghyun Han, and Jin-Woo Park*
Microbial Safety Team, National Institute of Agricultural Sciences, Rural Development Administration

**B-54** Plant Growth Promotion and Bioactive Compounds Production by Endophyte Isolated from Halophyte in Coastal Wetland
Young-Hyun You*, Pyoungho Yi and Jong-Han Park
Horticultural & Herbal Crop Environment Division, National Institute of Horticultural & Herbal Science, RDA

**B-55** Investigation on Removal Effect of Attached Algae in Spillway of Sedimentation Basin Depending on Materials
Eunyoung Jung1, Churljong Yun2, DongJin Cha1, Minam Lee3, Honggi Park1, and Jintack Choi1
1Water Quality Institute, Busan Water Authority, 2Deoksan Water Treatment Plant, 3Research center for biominealization Disorders, Chonnam National University

**B-56** Inhibition of Norovirus Replication by Plant Extracts in Replicon-Bearing Cell
Gil Jae Lee1, Hyun Ju You2 and Gwang Pyo Ko2,*
1Department of Environmental Health, Graduate School of Public Health, Seoul National University, 2Department of Environmental Health, Graduate School of Public Health, Seoul National University

**B-57** Screening of 18 Phytochemicals with Anti-Noroviral Effects in Replicon-Bearing Cells
Gil Jae Lee1, Hyun Ju You2 and Gwang Pyo Ko2,*
1Department of Environmental Health, Graduate School of Public Health, Seoul National University, 2Department of Environmental Health, Graduate School of Public Health, Seoul National University

**B-58** Molecular Phylogenetic Analysis of Endophytic Fungi Community Isolated from *Oreorchis patens* in Mt. Hambaek
BongHyung Lee and Ahn-Heum Eom*
Department of Biology Education, Korea National University of Education

www.fkms.kr | 35
**Poster Session**

**B-59** Study on AMF Diversity that Live in Extreme Environment of the City

Sang-Joon Kim and Ahn-Heum Eom*
Department of Biology Education, Korea National University of Education

**B-60** Improved PCR Assay for the Species-Specific Identification and Quantitation of *Legionella pneumophila*

Min Seok Cho and Dong Suk Park*
National Academy of Agricultural Science, Rural Development Administration

**B-61** Biodegradation of Octadecane, Eicosane and Docosane Hydrocarbons by Different Strains Isolated from the Oil-Contaminated Soil of South Korea

Dhiraj Kumar Chaudhary and Jaisoo Kim*
Department of Life Science, Kyonggi University

**B-62** Comparison in Structures of Microbial Communities between Mangrove Rhizosphere and Intestine of Mangrove Crabs on a Coral Beach

Kristin Widyasari, Jin-Nam Kim and Young-Gun Zo*
Department of Biology, Kyungsung University

**B-63** Control of Harmful Algal Blooms by Using a Bio-Ceramic Biofilm System

Sylvia Kristyanto and Jaisoo Kim*
Department of Life Science, Kyonggi University

**B-64** Prediction of Potential Invasion of Crops Fungal Pathogen Species in Tropical and Subtropical Regions Due to Climate Change

Jiyoung Park, Bora Chu, Jae-Woo Kim, Min-Young Choi, Jaekyeong Song, and Hang-Yeon Weon*
Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA)

**B-65** Bacterial Diversity in the Tomato Root Environment: a Culture-Dependent Approach Using Various Media and Antibiotics

Jiyoung Park, Born Chu, Jae-Woo Kim, Min-Young Choi, Jaekyeong Song, and Hang-Yeon Weon*
Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA)

**B-66** Diversity and Community Structure of Soil Bacteria Across Four Land-Use Types in Korea

Hang-Yeon Weon*, An-Sung Roh2, Seung-Chul Choi2, Won-Il Choi2, Moon-Tae Choi2, Byung-Koo Ahn2, Hee-Won Kim2, Young-Han Lee2, Min-Young Choi2, Jae-Hyung Ahn2, and Jaekyeong Song2
1National Academy of Agricultural Science, Rural Development Administration (RDA), 2Gyeonggi-do Agricultural Research and Extension Service (ARES)

**B-67** Anaerobic Crude Oil Degradation in Oil-Polluted Subtidal Sediment

Kyungwha Baek1,2*, Yoon-Tong Yang1, and Che-Ok Jeon1
1Team of Marine Microorganisms, MABIK, 2Department of Life Science, Chung-Ang University

**B-68** Selective Flushing of Pyrosequencing Reads of 16S rRNA Genes Predominant in Soil by CaO Treatment

Min-Ji Kim, Young-Gun Zo* and Jin-Nam Kim
Department of Biology, Kyungsung University

**C-1** The Effect of Multiple Phosphorylation on Activity of *Schizosaccharomyces pombe* CDK Inhibitor Rum1

Soo Jeong Kwon, Jae Ho Song and Hee-Moon Park*
Department of Microbiology & Molecular Biology, College of Bioscience & Biotechnology, Changnam National University
C-2 Analysis of a Laccase Promoter Expressed at Low pH in Coprinellus congregates: Induction or Repression
Trieu Dieu Linh Nguyen1,2, Su Yeon Kim1 and Hyoun Tae Choi1
1Molecular Microbiology Lab, Department of Biochemistry, Kangwon National University, 2Vietnam

C-3 Roles of a Novel VeA-Dependent Protein, VdpA, in Aspergillus nidulans Development
Joo Yeon Lim, Eun-Hye Kang and Hee-Moon Park*
Department of Microbiology & Molecular Biology, Chungnam National University

C-4 Establishment of the Protocol for Recombinant PrP Mass Production
Young-Jin Son1, Dae-Hwan Kim1, Keunhye K1, Hae-Gwang Hwang1, Se-Hoon Lee1, JiHyun Lee2, Muhammad Waqas3, Hye-Mi Lee4, Jeongmin Lee5, Su Yeon Kim1, Yeong Seon Lee6, and Chongsuk Ryoo2,4*
1Department of Pharmacy, Sunchon National University, 2Institute of Pharmaceutical Science and Technology, 3Department of Pharmacy, Hanyang University, 4Division of Zoonoses, Center for Immunology & Pathology, National Institute of Health, Korea Centers for Diseases Control & Prevention

C-5 Inhibition of the SenX3-RegX3 Two-Component System by pknB Overexpression in Mycobacterium smegmatis
Eun-Jin Park and Jeong-II Oh*
Department of Microbiology, Pusan National University

C-6 Transcriptional Factors Regulating the ahpC Gene Encoding Alkyl Hydroperoxide Reductase in Mycobacterium smegmatis
Ha-Na Lee and Jeong-II Oh*
Department of Microbiology, Pusan National University

C-7 Protein Secretion by Asexual Spores of Aspergillus nidulans in the Presence of Water before Isotropic Growth
Gwang-Hui Jeong, Do Won Kang, Jung Hun Jeon, Chang-Won Lee, and Jae Won Kim
Division of Applied Life Science(BK21 research) and Research Institute of Life Sciences, Gyeongsang National University

C-8 Different Role of N-terminal Domain and Middle-Domain on Chaperone Activity and Hexamer Stability of Cipl
Sang-Sang Park and Dong-Kwon Rhee*
School of Pharmacy, Sungkyunkwan University

C-9 Comparison of Wood Decomposition Abilities (Quercus spp.) among Lentinula edodes (Shiitake) Cultivars
Youngae Park, Wonchull Bak*, Yeongsoon Jung, Kwangsoock Kim, and Sehan Kim
Division of Wood Chemistry & Microbiology, Forest Research Institute

C-10 Genomic Mutations Shortened the Lag Phase and Accelerated the Growth Rate of Escherichia coli BL21 (DE3) in Succinate Minimal Medium
Hyun Ju Kim1,2, Haeyoung Jeong1,2,3, Dong-Woo Lee4, and Sang Jun Lee1,2,5*
1Biostystems and Bioengineering Program, University of Science and Technology (UST), 2Infection and Immunity Research Center, 3Super-Bacteria Research Center, Korea Research Institute of Bioscience & Biotechnology (KIRIBB), 4School of Applied Biosciences, Kyungpook National University

C-11 Anti-Bacterial Abilities of Essential Oil on Pathogenic Bacteria
Jae Eun Lee*, Jung In Park, Su Mi Yu, and Kyu Ri Park
Mokwon University

C-12 Morphological Characteristics of Sarcodon aspratus Mycelial Colonies Grown on Different Culture Media
Sung-Min Jeon and Kang-Hyeon Ka*
Division of Wood Chemistry and Microbiology, Korea Forest Research Institute

C-13 Comparison of Morphological Characteristics of Amanita spp. Mycelial Colonies Grown on Two Different Solid Media
Sung-Min Jeon and Kang-Hyeon Ka*
Division of Wood Chemistry and Microbiology, Korea Forest Research Institute
<table>
<thead>
<tr>
<th>Poster Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-14 <strong>Effect of Low Temperature for Mycelial Growth of Ectomycorrhizal Mushrooms</strong></td>
</tr>
<tr>
<td>Yeun Sug Jeong¹, Jong-Hwan Lim² and Kang-Hyeon Ka¹*</td>
</tr>
<tr>
<td>¹Division of Wood Chemistry and Microbiology, Korea Forest Research Institute, ²Center for Forest and Climate Change, Korea Forest Research Institute</td>
</tr>
<tr>
<td>C-15 <strong>Biochemical Characterization of a Novel SGNH Hydrolase (Nm21) from Neisseria meningitidis 053442</strong></td>
</tr>
<tr>
<td>Wanki Yoo, Bum Han Ryu, Due Rae An, and T. Dohun Kim*</td>
</tr>
<tr>
<td>Department of Chemistry, College of Natural Science, Sookmyung Women’s University</td>
</tr>
<tr>
<td>C-16 <strong>Hyphal Growth Condition and Cellulase Activity of Agaricus spp.</strong></td>
</tr>
<tr>
<td>Eun-Jin Wang, Jung-A Kang and Kang-Hyeon Ka*</td>
</tr>
<tr>
<td>Division of Wood Chemistry and Microbiology, Korea Forest Research Institute</td>
</tr>
<tr>
<td>C-17 <strong>Optimal Condition of Mycelial Growth and Laccase Activity of Coprinopsis spp.</strong></td>
</tr>
<tr>
<td>Eun-Jin Wang, Yeun Sug Jeong and Kang-Hyeon Ka*</td>
</tr>
<tr>
<td>Division of Wood Chemistry and Microbiology, Korea Forest Research Institute</td>
</tr>
<tr>
<td>C-18 <strong>Enhancement of the Level of FK506 Production by Manipulating Regulatory Genes</strong></td>
</tr>
<tr>
<td>Jin A Jung, Hea Luyng Shin, HEQUING Cui, Ji Yoon Beom, Ji Young Lee, and Yeo Joon Yoon*</td>
</tr>
<tr>
<td>Department of Chemistry and Nano Science, Ewha Womans University</td>
</tr>
<tr>
<td>C-19 <strong>Analysis of Non-Ribosomal Peptide Synthetase Gene Cluster for Tolaasin Biosynthesis in Mushroom Pathogen Pseudomonas tolaasii KACC10082.</strong></td>
</tr>
<tr>
<td>Eun-jin Ju, Dowon Kang, Chang-won Lee, and Jae Won Kim</td>
</tr>
<tr>
<td>Division of Applied Life Science(BK21 research) and Research Institute of Life Sciences, Gyeongsang National University</td>
</tr>
<tr>
<td>C-20 <strong>A Novel Mechanism of Reduction of Dimethyl Sulfoxide in Thermococcus onnurineus NA1</strong></td>
</tr>
<tr>
<td>Ae Ran Choi¹, Min-Sik Kim¹, Sung Gyun Kang², and Hyun Sook Lee¹,²*</td>
</tr>
<tr>
<td>¹Marine Biotechnology Research Center, Korea Institute of Ocean Science and Technology (KIOST), ²Department of Marine Biotechnology, Korea University of Science and Technology (UST)</td>
</tr>
<tr>
<td>C-21 <strong>Cellulolytic Enzyme Activity in Basidiomycetes after Low Temperature Storage</strong></td>
</tr>
<tr>
<td>Yean Sug Jeong and Kang-Hyeon Ka*</td>
</tr>
<tr>
<td>Division of Wood Chemistry and Microbiology, Korea Forest Research Institute</td>
</tr>
<tr>
<td>C-22 <strong>Fluorometric Assay of α-factor Affinity for GPCR Receptor of S. cerevisiae</strong></td>
</tr>
<tr>
<td>Hee Jun Ahn, Hyeong Jin Kim, Jin Woo Hong, Yeon Hui Kim, Seung Yeol Lim, Yu Jin Kang, Min Jeong Lee, Min Seob Seo, Ju Hyeon Kim, and Nam Joo Hong*</td>
</tr>
<tr>
<td>Dept. of applied Microbiology and biotechnology, Yeungnam University</td>
</tr>
<tr>
<td>C-23 <strong>Detection of Protein Interaction with 22kDa-Peptidyl Prolyl cis/trans Isomerase and 70kDa-Heat Shock Protein DnaK of Vibrio anguillarum</strong></td>
</tr>
<tr>
<td>Dong Seop Kang, Jong Min Lee, Mahbubul Pratik Siddique, Soo Young Moon, Gyunyou Noh, and In-Soo Kong*</td>
</tr>
<tr>
<td>Department of biotechnology, Pukyong National University</td>
</tr>
<tr>
<td>C-24 <strong>Evaluation of the Nutritive Value from Korean Pollens Treated with Fungi</strong></td>
</tr>
<tr>
<td>Inpyo Hong, Soonok Woo and Sangmi Han</td>
</tr>
<tr>
<td>National Academy of Agricultural Science, RDA</td>
</tr>
<tr>
<td>C-25 <strong>Characterization of Human Skin Bacterial Strains to Develop Vegetable Antibacterial Substances</strong></td>
</tr>
<tr>
<td>Yunkyoung Lee, Hyuna Jeong, Youngju Jeong, and Youngdo Park*</td>
</tr>
<tr>
<td>Division of biomedical and health sciences, Mokwon University</td>
</tr>
<tr>
<td>C-26 <strong>Molecular and Functional Characterization of Hansenula polymorpha SAT1 Encoding Serine O-Acetyltransferase Involved in de novo Cysteine Biosynthetic Pathway</strong></td>
</tr>
<tr>
<td>Su Jin Yoo¹, Ji Yoon Yeon¹, Hiroshi Takagi², and Hyun Ah Kang*</td>
</tr>
<tr>
<td>¹Lab. of Molecular Systems Biology, Dept. of Life Science, Chung-Ang University, Seoul, 156-756, ²Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan</td>
</tr>
</tbody>
</table>
Poster Session

C-27 Sugar Signal Transduction by N-acetylglucosamine-specific Enzyme II in Vibrio vulnificus
Ji Hee Yoon1, So-Young Park1, Young-Ha Park2, and Yeong-Jae Seok1,a
1Department of Biophysics and Chemical Biology, Seoul National University, 2Department of Biological Sciences and Institute of Microbiology, Seoul National University

C-28 Lifespan Extension of Caenorhabditis elegans by Oxyresveratrol and Its Mechanism
Jiyan Lee1, So-Young Park1, Young-Ha Park2, and Yeong-Jae Seok1,2,a
1Department of Public Health Science (Brain Korea 21 PLUS program), Graduate School, Korea University, 2Department of Chemical Engineering and Biotechnology, Korea Polytechnic University, Shihang-si, Gyeonggi-do 429-793, 3School of Biosystem and Biomedical Science, College of Health Science, Korea University

C-29 Molecular Determination of the Interaction Surface of the RNA Pyrophosphohydrolase RppH and the Diaminopimelate Epimerase DapF
Subin Jung1, Yeong-Jae Seok2,a, and Chang-Ro Lee1,a
1Department of Biological Sciences, Myongji University, 2Department of Biological Sciences and Institute of Microbiology, Seoul National University

C-30 Characterization of the Possible Intermediates Involved in the Post-PKS Modification in FKS06 Parallel Biosynthetic Pathways
Xu Zhao, Eun Ji Kim, Jae Yeon Hwang, Myoun Su Kim, and Shi Ying Jin
Department of Nanochemistry, Ewha Womens University

C-31 Distinct Characteristics of the Substrate Binding Mode in Thermophilic L-Arabinose Isomerase
Jin Myung Choi1, Yong-Jik Lee2, Dong-Woo Lee2, and Sung Haeng Lee1,a
1Department of Cellular and Molecular Medicine, Chosun University School of Medicine, 2School of Applied Biosciences, Kyungpook National University

D Fermentation and Metabolites

D-1 Microbial Phenol Production in Metabolically Engineered E. coli Using Synthetic sRNA Technology
ChanWoo Song, ByoungJin Kim, HyeGwon Park, DoKyun Na, and Sang Yup Lee1,a
Dept. of Chemical and Biomolecular Engineering, KAIST

D-2 Production of 1,3-Diaminopropane from Microbial Cell Factory Developed by Metabolical Engineering
Minho Roh1, Tong Un Chae2, Won Jun Kim1, Sol Choi1, Si Jae Park2, and Sang Yup Lee1,a
1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Plus program), Center for Systems and Synthetic Biotechnology, Institute for the BioCentury, KAIST; 2Department of Environmental Engineering and Energy, Myongji University, 3Bioinformatics Research Center, KAIST

D-3 Development of L-Arginine Producing Microbial Cell Factory through Metabolic Engineering Based on Corynebacterium glutamicum
Minho Roh1, Soek Hyun Park1, Hyun Uk Kim1,a, Tae Yong Kim1,a, Jun Seok Park2, Suok-su Kim3, and Sang Yup Lee1,a
1Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Plus Program), Center for Systems and Synthetic Biotechnology, KAIST; 2Bioinformatics Research Center, KAIST; 3Daesang Corporation Research Center

D-4 Deoxyviolacein Mass Production with High Cell Density Culture and Aggregation of Deoxyviolacein in the Culture Using Escherichia coli
HeeUn Kwon, SeongYool Choi and Robert Mitchell1,a
Department of Biological Sciences, Ulsan National Institute of Science and Technology

D-5 Extraction of Biosurfactant from Streptomyces blastomyceticus
Ho-Yoon Ryu, Jun-Hee Hwang, Nan-Young Kim, Eun-Jin Kim, Jae-young Rho and Jae-heon Kim1,a
Department of Microbiology, Dankook University
D-6  Comparison of Methyl Orsellinate and Sparassol Concentrations via Submerged Culture of Sparassis latifolia

MinSoo Kim1, Kyoung-Tae Lee2, Yong-Bae Park1, and Kang-Hyeon Kal*
1Division of Wood Chemistry & Microbiology, 2Southern Forest Resources Research Center, Korea Forest Research Institute

D-7  Expression Analysis of Genes Related in Two Pathway of Sesquiterpene Biosynthesis in Wood Rot Fungus, Polyporus brumalis

Korea Forest Research Institute

D-8  Proteomic Analysis of Differences in Korean Traditional Wheat-Based Nuruk

Ha-Yeon Song1, Hansaen Jeong1, Dahiye Choi1, Soo-Hwan Yeo2, Dae-Hyuk Kim*, and Jung-Mi Kim1*
1Department of Bio-Environmental Chemistry, 2Department of Agrofood Resource, NAAS, RDA

D-9  Study on the Large Scale Fermentation of Recombinant E. coli for the Production of N-SPERSE

Yeon Ju Kim1, Jun Hak Lee 1, Min Jeong Jang 2, Jung Oh Ahn 2, Jong Gwan Kim1, and Seong Wook Kong1
1INWOO CORPORATION, 2Korea Research Institute of Bioscience and Biotechnology

D-10  454 Pyrosequencing Reveals Mycoflorval Community Dynamics in Traditional Wheat-Based Nuruk Based on Varying Moisture Content

Jyotiranjan Bal1, Suk-Hyun Yun1, Myeong Jin Jo 1, Soo-Hwan Yeo2, Jung-Mi Kim3, and Dae-Hyuk Kim1*
1Department of Molecular Biology, 2Fermented Food Science Division, 3Department of Bio-Environmental Chemistry, Institute for Molecular Biology and Genetics, Chonbuk National University

D-11  Characteristics of Ethanol Fermentation with Low Temperature Adaptation Yeast for Yakju Brewed

Dong-Jun Seo, Ji-Young Mun, Soo-Hwan Yeo, Ji-Seon Kim, A-Ra Jo, and Seong Yeol Baek*
Fermented Food Division, Department of Agro-food Resource, NAAS, RDA

D-12  Reconstruction of Genetic Circuit for 1-Deoxynojirimycin (DNJ) Biosynthesis in Escherichia coli

Sang-Yoon Kim1, Sawarot Maibunkaew1,2, Doo-Byoung Oh1,2, and Ohsuk Kwon1,2*
1Synthetic Biology and Bioengineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 2Biosystems and Bioengineering Program, University of Science and Technology (UST)

D-13  Effect of Metal Ions on Production of Fusaricidin A and B of Paenibacillus kribbensis CU01 Isolated from Yellow Loess

Jaewon Ryu1, Moonjong Kim1 and Si Wouk Kim 1,2*
1Department of Energy Convergence, 2Department of Environmental Engineering, Chosun University

D-14  Herbicidin A Production in Submerged Culture of Streptomyces scopoliiudis M40 Strain

Sanghyun Ha1, Keon Jin Lee1, Sang-II Lee1, Hyun Jung Gwak1, Jong-Hee Lee1, Tae-Woon Kim1, Hak-Jong Choi1, Ja Young Jang1, Jung-Suh Choi2, and Hae Woong Park*
1World Institute of Kimchi, 2Korea Research Institute of Chemical Technology

D-15  Multi Drug Resistant Staphylococcus aureus Growth Inhibition by Violacein from Natural Isolated Strain NII28

SooYeon Kim, SeongYeo Choi and Robert Mitchell*
Department of Biological Sciences, Ulsan National Institute of Science and Technology

D-16  Improvement of the Antioxidant Activity of Safflower Seed (Carthamus tinctorius L.) by Fermentation with β-glucuronidase Producing Lactic Acid Bacteria

Kalam Lee and Sang-Seob Lee*
Department of Life Science, Kyonggi University

D-17  A New Potential UV Filter, 5-Hydroxymellein Isolated from Endolichenic Fungus

Lu Zhao1 and Jae-Seour Hur*
1Korean Lichen Research Institute, Sunchon National University, 2Korean Lichen Research Institute (KoLRI), Sunchon National University
<table>
<thead>
<tr>
<th>Poster Session</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D-18</strong> Characterization and Identification of MJM7007 as Novel Producer of Gentamicin B</td>
</tr>
<tr>
<td>Longbin Qi1, Ji-su Han1, Yingyu Jin1, and Joo-woon Suk1</td>
</tr>
<tr>
<td>1Department of Biomedulation, Myongji University, 2China, 3Center for Nurtaceutical and Pharmaceutical Materials, Myongji University, 4Division of Bioscience and Bioinformatics, Myongji University</td>
</tr>
<tr>
<td><strong>D-19</strong> Mutual Antagonism between Anthranilate and Indole in Biofilm Formation of Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Xi-Hui Li, Soo-Kyoung Kim, Jungmin Oh, and Joo-Hee Lee*</td>
</tr>
<tr>
<td>Lab of Microbiology, Department of Pharmacy, College of Pharmacy, Pusan National University</td>
</tr>
<tr>
<td><strong>D-20</strong> Bacterial Diversity in Traditional Wheat-Based Nuruk Based on Varying Moisture Content</td>
</tr>
<tr>
<td>Jyotiranjan Bal1, Suk-Hyun Yun1, Myeong Jin Jo1, Soo-Hwan Yeo2, and Dae-Hyuk Kim1*</td>
</tr>
<tr>
<td>1Department of Molecular Biology, Department of Bioactive material sciences, Institute for Molecular Biology and Genetics, Chonbuk National University, 2Fermented Food Science Division, Department of Agrofood Resource, NAAS, RDA</td>
</tr>
<tr>
<td><strong>D-21</strong> New Sesquiterpenes from a Culture Broth of Coprinus echinosporus and Their Antioxidant Activity</td>
</tr>
<tr>
<td>Dae-Won Kim1, In-Kyoung Lee1, Soon-Ja Seok2, and Bong-Sik Yun1*</td>
</tr>
<tr>
<td>Division of Biotechnology, Chonbuk National University, 2Rural Development Administration</td>
</tr>
<tr>
<td><strong>D-22</strong> Chemical Constituents of the Fruiting Body of Glaziella splendens</td>
</tr>
<tr>
<td>Ji-Yul Kim, Byung Soon Hwang, Dae-Won Kim, E-Eum Woo, Yoon-Ju Lee, Van Minh Nguyen, In-Kyoung Lee, and Bong-Sik Yun*</td>
</tr>
<tr>
<td>Division of Biotechnology, Chonbuk National University</td>
</tr>
<tr>
<td><strong>D-23</strong> A Comparison of Carotenoid Production by Rhodobacter capsulatus PS-2 under Photoheterotrophic and Chemoheterotrophic Culture Conditions</td>
</tr>
<tr>
<td>Heon-Woo Gu1, Ki Moon Bong1, Jong Min Kim1, In-Cheol Park2, and Pyoung Il Kim1*</td>
</tr>
<tr>
<td>1Jeonnam Bioindustry Foundation, Bio Control Research Center, 2Agriculture Microbiology Division, National Academy of Agricultural Science</td>
</tr>
<tr>
<td><strong>D-24</strong> Chemical Constituents of the Culture Broth of Coprinus cinereus</td>
</tr>
<tr>
<td>Van Minh Nguyen1, E-Eum Woo1, In-Kyoung Lee1, Soon-Ja Seok2, and Bong-Sik Yun1*</td>
</tr>
<tr>
<td>Division of Biotechnology, Chonbuk National University, 2Rural Development Administration</td>
</tr>
<tr>
<td><strong>D-25</strong> Diversity and Multifunctional Properties of Actinomycetes</td>
</tr>
<tr>
<td>Jee-min Lim, Yoonjung Ju, Yunjung Oh, Dong-Jin Park, and Chang-Jin Kim*</td>
</tr>
<tr>
<td>Industrial Bio-materials Research Center, KRIBB</td>
</tr>
<tr>
<td><strong>D-26</strong> Medium Optimization of a Potential Herbicide from Strepomyces scopoliridis N29</td>
</tr>
<tr>
<td>Yoonjung Ju1, Dong-Jin Park1, Jung Sup Choi2, and Chang-Jin Kim1*</td>
</tr>
<tr>
<td>1Industrial Bio-material Research Center, Korea Research Institute of Bioscience and Biotechnology, 2Eco - friendly and New Materials Research Group, Korea Research Institute of Chemical Technology</td>
</tr>
<tr>
<td><strong>D-27</strong> Adaptive Laboratory Evolution of Starch-utilizing Yeast Saccharomycopsis fibuligera for an Improved Stress Tolerance</td>
</tr>
<tr>
<td>Boung-Hyuk Yoo1, Soo-Hwan Yeo2 and Myoung-Dong Kim1*</td>
</tr>
<tr>
<td>1Department of Food Science and Biotechnology, Kangwon National University, 2Fermented Food Science Division, Department of Agro-Food Resource, NAAS, RDA</td>
</tr>
<tr>
<td><strong>D-28</strong> Quality Comparison of Detoxified Rhus Verniciflua Vinegars Produced by Various Acetobacter Bacteria</td>
</tr>
<tr>
<td>Ji-Soon Kim, A-Ra Jo, Ji-Young Mun, Soo-Hwan Yeo, Dong-Jun Seo, SeongYeol Baek*</td>
</tr>
<tr>
<td>Fermented Food Science Division, Department of Agro-food Resource, NAAS, RDA, Wanju-gun, Jeollabuk-do 53365, Republic of Korea</td>
</tr>
<tr>
<td><strong>D-29</strong> Screening and Selection of Bacillus Strains as Potential Starter Cultures for the Fermentation of Low-salted Doenjang</td>
</tr>
<tr>
<td>Hye Hee Jeon, Ji Young Jung and Che Ok Jeon</td>
</tr>
<tr>
<td>Department of Life Science, Chung-Ang University</td>
</tr>
</tbody>
</table>
**Poster Session**

**E-1** Complete Genome Sequence of *Mycobacterium tuberculosis* K from a Korean High School Outbreak, Belonging to the Beijing Family

Seung Jung Han1, Yong-Joon Cho2, Taeksun Song3, Jong-Seok Kim1, Soo Young Choi1, Jong sik Chun2, Sang-Nae Cho1,3, and Sang Jae Shin1*

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**E-2** Metagenome-based Microbial Community Structure of the Arctic Soil from Midtre Lovénbreen Glacier Foreland, Svalbard

Yoon Ji Seok1, Hee Seon Lim2, In-Tae Cha3, Ji Young Jung4, Yoo Kyung Lee4, and Myung-Ji Seo3*

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**E-3** Completion of the Circular Mitochondrial Genome of Pepper Anthracnose Caused by Colletotrichum acutatum

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**E-4** Useful Gene Identification of Bacillus methylotrophicus CBMB205 Isolated from Rhizosphere Soil of Rice (*Oryza sativa* L.)

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**E-5** Genome Characterization of Methylobacterium phylosphaerae CBMB27 Isolated from Leaf Tissues of Rice

Kyeong Hwangbo1, Hee Chung1, Jemin Yoo1, Ki Yoon Kim2, Tong Min Sa2, and Yi Lee1*

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**E-6** Genome Characterization of Pandoraeathiooxydans ATSB16 Isolated from Rhizosphere Soil of Sesamum indicum L.

Kyeong Hwangbo1, Hee Chung1, Jemin Yoo1, Ki Yoon Kim2, Tong Min Sa2, and Yi Lee1*

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**E-7** Genome Analysis of Psychrophilic Arthrobacter sp. PAMC 25486 Isolated from the Arctic Region Reveals Evolutionary Implications of the Genus *Arthrobacter*

Jong-Hyun Jung1, Dong-Ho Kim1, Jong-il Choi1, and Sangyong Lim1*

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**E-8** A simple Screening Method Using Lignocellulose Biodegradation for Effective Breeding in *Agaricus bisporus*

Youn-Lee Oh1,2, Kab-Yeul Jang1, Youn-Keol Nam1, Eun-Sun Kim1, Min Ji Oh1, Pyung-Gyun Shin1, Won-Sik Kong1, and In-Geol Choi2*

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**E-9** Comparative Genome Analysis of Three Genera Belonging to the Family Streptomycetaceae

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---

278-280
Poster Session

E-10 Complete Genome Analysis of Dyella thiooxydan ATSB10 isolated from Rhizosphere Soil of Sunflower (Helianthus annuus L.)

Kyong Hwangbo1, Hee Chung1, Jemin Yoo2, Ki Yoon Kim3, Tong Min Sa 3, and Yi Lee4
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E-11 Biochemical Characterization of Laccase Isozymes in Pleurotus ostreatus

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E-12 Genetic Heterogeneity of Varicella-Zoster Virus

Jeong Seon Jeon1, In Kyo Kim1, Ji Seon Park1, Ok Sarah Shin2, and Chan Hee Lee1
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E-13 Characterization of Copper-Inducible Fungal Laccase Promoter in Pichia pastoris expression system

Minseok Kim and Hyeon-Su Ro
Division of Applied Life Science(BK21) and Research Institute of Life Sciences, Gyeongsang National University

E-14 Rapid Gene Knockout Method Using Single Helper Plasmid Expressing Red and Cre Recombinases

ChanWoo Song and SangYup Lee
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E-15 Water Extracts from Spent Mushroom Substrate of Hericium erinaceus induce Expression of Defense Genes in Tomato Plants

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E-16 The MpkB MAPK is Not Essential for Sterigmatocystin Production in Aspergillus nidulans

Sang-Cheol Jun1, Jong-Hwa Kim2, Kap-Hoon Han2, and Kwang-Yeop Jahng1
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E-17 Functional Analysis of Transcription Factors Containing CCAAT-DNA Binding Domain

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E-18 The whcD Gene of Corynebacterium glutamicum is Important for Cell Growth and Affects Responses to Hydrogen Peroxide-mediated Oxidative Stress

Dong-Seok Lee1, Yoonhee Kim2, and Heung-Shick Lee3
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E-19 sRNA Regulates the Anaerobic Induction of FrsA Expression

Boram Jang, Kyung-Jo Lee and Kyo-Ho Lee
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E-20 Comparative Genome Analysis of Five Lichen-Forming Fungi and Two Symbiotic Algae

Soo-Yeong Park1, Hyan jung Song2, Jae-young Choi2, Yong-Hwan Lee3, and Jae-Seoung Han1
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E-21 Genome Project for Hypsizigus marmoreus at Rural Development Administration (RDA), Korea

Seunghwan Kim1, Jinho Jeong1, Eun-Sung Song1, Hee-Jun Cho1, Youn-Lee Oh2, Won-Sik Kong2, In-Geol Choi1, Byoung-Moo Lee1, and Jeong-Gu Kim1
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www.fkms.kr | 43
Poster Session

E-22  RDS1 Involved in Yeast-To-Mycelium Transition in Lichen-Forming Fungus Umbilicaria muehlenbergii  ........................................... 283
Min-Hye Jeong¹, Sook-Young Park¹, Jung A Kim¹, Yong Hwa Cheong¹, and Jae-Seon Hur*¹
¹Korean Lichen Research Institute, Sunchon National University, ²Dept. of Bio-Environmental Science, Sunchon National University

E-23  Draft Genome of the Streptomyces rubrolavendulae MJM4426, a Strain Producing Staurosporine  ...................................................... 283
Seunghwan Kim¹, Jinho Jeong¹, Eun-Sung Song¹, Hee-Jung Cho¹, Byoung-Moo Lee¹, Jinhua Cheng¹, Joo-Won Suh¹, and Jeong-Cu Kim¹*
¹Genomics Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Jeonju 54874, ²Division of Bioscience and Bioinformatics, Myongji University, Yongin 17058

E-24  Analysis of Complete Genome Sequence and Virulence Factors of Bacillus cereus FORC_010, Food-borne Pathogen Isolated from Fresh Kimchi in Korea  ............................................................ 283
Hongjun Na¹,², Seunghee Jeong¹,², Hyun Soo Seo¹,², Ju-Hoon Lee³, and Sangryeol Ryu¹³*
¹Department of Agricultural Biotechnology, Research Institute for Agriculture and Life Sciences, Seoul National University, ²Foodborne-pathogens Omics Research Center, Seoul National University, ³Division of Food Science and Biotechnology, Graduate School of Biotechnology, Kyung Hee University, Yongin 446-701

E-25  Whole Genome Sequencing of Faecalibaculum rodentium ALO17, Isolated from C57Bl/6J laboratory Mouse Feces  .................................................. 284
Sooyeon Lim, Dong-Ho Chang and Byoung-Chan Kim*
Korean Research Institute of Bioscience and Biotechnology

E-26  Development of Open Source Genome Annotation Pipeline and Interactive Visualization Server  .................................................... 284
Byeonghyeok Park, Min-Jeong Baek, Byoungnam Min, and In-Geol Choi*
Department of Biotechnology, Korea University

E-27  Developing Fungal Genome Annotation Pipeline: From Gene Prediction to Functional Annotation  .................................................... 284
Byoungnam Min, Jongjae Park, Byounghyeok Park, Min-Jeong Baek and In-Geol Choi*
Department of Biotechnology, Korea University

E-28  A Study on Biological Diversity with Bioinformatics  ............................................................ 284
Dong Su Yu¹* and Seung Hwa Yoo
Department of Ecological Assessment, National Institute of Ecology

E-29  Analysis of Mutational Events of Varicella-Zoster Virus Passaged in vitro Cell Culture  .......................................................... 285
Youn Hee Won¹, Jeong Hwa Yoon¹, Min Ho Kim¹, Jin Hyun Ahn¹, and Chan Hee Lee¹*
¹Department of Microbiology, Chungbuk National University, Cheongju, ²Department of Molecular Cell Biology, Sanghyunkeun University School of Medicine

E-30  Complete Genome Sequence of Multidrug-Resistant Staphylococcus haemolyticus S167 Strain Which Forms ica-Independent Biofilm  ............................................. 285
Jisoo Hong, Jonguk Kim, Moran Lee, Jeong-A Lim, Kyu Suk Jung, Sanghyun Han, Jinwoo Park, and Eunjung Roh*
Microbial Safety Team, National Institute of Agricultural Sciences, Rural Development Administration

E-31  Production and Role of Nitric Oxide During Development in Neurospora crassa  .......................................................... 285
Anchalee Pengkit¹, Seong-Sil Jeon¹, Suo-Ji Son¹, Ki-Bum Kim¹, Jae-Ho Shin¹, Eun-Ha Choi¹, and Gyungsoo Park¹²*
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E-32  Development of Highly Sensitive Cadmium and Lead Microbial Biosensors Using Synthetic CadC-T7 Genetic Circuits  ........................................ 285
Hyun Ju Kim¹,², Haeyoung Jeong¹,², Dong-Woo Lee¹, and Sang Jun Lee¹²*
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E-33  Functional Analysis of Putative Peroxidases in Fusarium graminearum  .......................................................... 286
Yoonji Lee¹, Hokyoun Son¹ and Yin-Won Lee¹*
¹Department of Agricultural Biotechnology, Seoul National University, ²Center for Food and Bioconvergence, Seoul National University
**Poster Session**

**F-1** Comparison of Acid-Fast Staining Procedure for *Mycobacterium tuberculosis*

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**F-2** Formation of PrP Aggregates and their Morphological Analysis

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**F-3** Development of Infectious Clones of a Wild-type Korean Rabies Virus and Evaluation of their Pathogenic Potential

Jun-Sun Park1, Chi-Kyeong Kim1, Ji-Hye Um1, Young Ran Ju1, Yeong Seon Lee1, Young-Ki Choi2, and Su Yeon Kim1*

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**F-4** Identification of Interacting Partners for Viperin in Human Cytomegalovirus Infection

Hyejin Jeon and Jun-Young Seo*

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**F-5** First Description of CTX-M-3 Extended-Spectrum β-lactamase in *Shiga toxin-producing Escherichia coli* O103:H2

Jin Seok Kim, Soo Jin Kim, Eunkyung Shin, Kyung-Hwan Oh, Gyung Tae Chung, Cheon-Kwon Yoo, and Junyoung Kim*

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**F-6** Actinomycin D Inhibits the Virulences of *Staphylococcus aureus*

Jin-Hyung Lee, Yong-Guy Kim and Jintae Lee*

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**F-7** Cinnamaldehyde and Eugenol Inhibit Biofilm Formation and Toxin Production against *Pseudomonas aeruginosa*

Yong-Guy Kim, Jin-Hyung Lee and Jintae Lee*

School of Chemical Engineering, Yeungnam University

**F-8** The Innate Immune Response in *P. aeruginosa*-fed Infected *Drosophila melanogaster* by Low-dose Radiation

Sun Hwa Lee, Doo-Hyeon Kang, Cha Soon Kim, Kwang-Hwan Oh, Gyung Tae Chung, Cheon-Kwon Yoo, and Junyoung Kim*

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**F-9** Metabolic Reprogramming after Infection: Knowledges from Systems Biological Analysis of *in vivo* Transcriptome and Proteome of *Vibrio vulnificus*

Kwangjoon Jeong1,2, Shae Eun Lee2,3, Che-Hun Jung2,4,5 and Joon Haeng Rhed1,2,5*

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**F-10** Predatory Bacterium, *Bdellovibrio bacteriovorus* Affected by Diffusible Signal Factor

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**F-11** Gingipains-Mediated Enhancement by *Porphyromonas gingivalis* of *Tannerella forsythia* Phagocytosis

Young-Jung Jung1, Hye-Kyoung Jun1, Eun-Ju Ryu1, Seok-Joo Lee1, and Bong-Kyu Choi1,2*

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F-12 Activation of ER Stress and Apoptosis in M. fortuitum Infected Macrophages

Sung-Man Oh1,2, Yun-Ji Lim1,2, Ji-Ae Choi1,2, Jung-Hwan Lee1,2, Ji-Ye Han1,2, Sang-Hee Jo1,2, and Chang-Hwa Song1,2,3,*

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F-13 Production of Pseudoviruses for Ebola Virus and Marburg Virus Using Lentiviral Vector System

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F-14 Generation of Ebola Virus-like Particles in Drosophila Expression System

Hyeon-Seo Kim, Sun-Whan Park, Won-Ja Lee, and Woo-Young Choi*
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F-15 The Function of Lipocalin 2 Production in Macrophages against Mtb Infection

Ji-Ae Choi1, Yun-Ji Lim1,2, Ji-Ye Han1,2, Sung-Hee Jo1,2, Sung-Man Oh1,2, and Chang-Hwa Song1,2,3,*

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F-16 Lysophosphatidylcholine Controls Mycobacterium tuberculosis Infection by Promoting the Process of Phagosomal Maturation through PI3K-p38 Pathway in Mouse Macrophage

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Department of Biological Sciences, Kangwon National University

F-17

F-18

F-19 Detection of Hantaan Viral RNA from Anti-HTNV IgG Sero-negative Rodents in a Highly Endemic Area, Republic of Korea

Jin Sun No1, Won-Keun Kim1, Jeong-Ah Kim1, Seong-Ho Lee2, Sook-Young Lee1, Ji Hye Kim1, Jeong Hoon Kho1, Duesang Lee2, Dong Hyeon Song2, Seong Tae Jeong2, Heung-Chul Kim3, Terry A. Klein4, and Jin-Won Song1,*

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F-20 Xanthine Oxidase Inhibitory Activity of Extracts Prepared from Aloe vera and Aloe Arborescens in vitro

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F-21 The Effect of Hypoxia-Mimic Agent on Innate Defense against S. Typhimurium Infection in Mouse Macrophages

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F-22 Differences in Biofilm Formation and Expression of Biofilm-Associated Genes between Epidemic and Minor Clones of Carbapenem-Resistant Acinetobacter baumannii

Gati Noble Selasi, Asiimwe Nicholas, Seok Hyun Na, Hyo Il Kwon, Yoo Jeong Kim, So Hyun Jun, Hyejin Jeon, and Je Chul Lee*

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F-23 Outer Membrane Protein A Plays a Role in Antimicrobial Resistance of Acinetobacter nosocomialis

Hyo Il Kwon1, Man Hwan Oh2, So Hyun Jun1, Hyejin Jeon1, Seok Hyeon Na1, Yoo Jeong Kim1, and Je Chul Lee*

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F-24 PrP0 Regulates RhoA-mediated Neurite Outgrowth
Hyeji Kim1, Haejun Kim1, Byungki-Jang1, Hong-Geok Choi3, Jeong-Ho Park1, Jae-Bong Park2, Yong-Sun Kim1, and Eun-Kyoung Choi2,3
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F-25 Neuronal Cell Lines Established from Bank Vole Transgenic Mice
Myoung-Ju Choi1,2, Hong-Seok Choi3, Eun-Kyoung Choi1,2, and Yong-Sun Kim1,2,3
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F-26 Induced Activation of the Innate Immune Response to Pathogens by Low-dose Radiation in Drosophila melanogaster
Doo-Hyeon Kang, Sun Hwa Lee, Cha Soon Kim, Soon Young Nam, Kwang Hee Yang, Ji-Yong Kim, and In Kyung Lee*
Low-dose radiation research Team, Radiation Health Institute, Korea Hydro & Nuclear Power Co., Ltd.

F-27 Five sRNAs, Qrr1-5, are Involved in Fine Regulation of Virulence Genes Depending on Quorum Sensingand Iron in Vibrio vulnificus
Yancheng Wen1, In Hwang Kim1, Na-Young Park1, Kwen-Woo Lee1, So-Yeon Kim1, and Kun-Soo Kim2*
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F-28 Lactobacilli Isolates from Healthy Vaginal Fluids Inhibited Bacterial Vaginosis- and Vulvovaginal Candidiasis-associated Pathogens
Kyeongju Lee1, Hyun Ju You1,2 and GwangPyo Ko1,3*
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F-29 Effect of Incomplete Lipopolysaccharide on Virulence and Immunogenicity in Salmonella enterica Serovar Enteritidis
Soyeon Park*, Youngjae Cho, Quang Lam Truong, Kiju Kim, Bokyung Park and Tae-Wook Hahn*
College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon, 200-701, Republic of Korea

F-30 Comparative Anti-mycobacterial Activity and Cytotoxicity of Deoxypergularinine Derivative 5 and 1st Line Anti-tuberculosis Drugs in vitro
Zerin Tamanna, Lee Minjung, Kim Sukyoung, Jang Woong-Sik, Jiyo Mi Anirban, Seo Hoon Hee, and Song Ho-Yeon*
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F-31 Screening for Anti-quorum Sensing Activity of Streptomyces Extracts Using by Bio-reporter Strains
Seungjin Lee1, JooWon Suh2,3 and SeungChun Park2*
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F-32 Autoinducer-2 Mediated Quorum Sensing Influences on Bacterial Growth under Stress Conditions
Kyuyeon Lee1, Hyunjoon Park1, Hyun-Kil Shin2, and Wilhelm Holzapfel*
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F-33 High Throughput Detection of β-Lactamase Genes Conferring Antibiotic Resistance
Kwang Seung Park, Jung Hun Lee, Asad Mustafa Karim, and Sang Hee Lee*
National Leading Research Laboratory of Drug Resistance Proteomics, Department of Biological Sciences, Myongji University

F-34 Glucose Metabolism Affects Trimethylamine N-oxide Stimulated Cholera Toxin Production
Young Tack Oh and Sang Sun Yoon*
Department of Microbiology and Immunology, Yonsei University College of Medicine

F-35 Nucleotide Composition and Genomic Diversity Pattern in Influenza A Virus
Daew Park*, Byung-Chul Kim1 and Jong Bhak2
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Poster Session

F-36  First Isolation of Severe Fever with Thrombocytopenia Syndrome Virus from Haemaphysalis longicornis Ticks Collected in SFTS Outbreak Areas in the Republic of Korea, 2013
Seok-Min Yun, Dong-In Park, Jong Yool Roh, and Won-Ja Lee
Division of Arboviruses, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongju-si
Division of Medical Entomology, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongju-si

F-37  Molecular Detection of Tick-borne Viruses from Ticks Collected in the Republic of Korea, 2014
Seok-Min Yun, Ye-Ji Lee, Woo-Young Choi, Heung-Chul Kim, Sung-Tae Chong, Terry A. Klein, and Won-Ja Lee
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Public Health Command Region-Pacific, Camp Zama, Japan, Address: 65th Medical Brigade, Unit 15281, APO AP 96205-5281, USA

F-38  Application of Detergents to Plasmin Assay of Biological Samples
Soyoung Park, Jeeyoung Kim, Muhammad Waqas, Jiyun Lee, Hye-Mi Lee, Dae-hwan Kim, and Chongsuk Ryu
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F-39  Increase of Plasmin Activity During the Progress of Prion Disease in Animal Models
Soyoung Park, Muhammad Waqas, Jiyun Lee, Hye-Mi Lee, Dae-hwan Kim, and Chongsuk Ryu
Department of Pharmacy, College of Pharmacy, Hanyang University, Ansan
Institute of Pharmaceutical Science and Technology, Hanyang University, Ansan

F-40  LC3/Atg8 Contributes to HCMV Growth by Facilitating Virion Release in an Autophagy-independent Manner
Kye Ryeeong Park, Young-Eui Kim, and Jin-Hyun Ahn
Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine

F-41  Interaction of HCMV pUL26 with the Cellular ISGylation System
Ye Ji Kim, Young-Eui Kim, Myoung Kyu Lee, Ki Mun Kwon, and Jin-Hyun Ahn
Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine

F-42  Distinct Commensals Induce Interleukin-1β via NLRP3 Inflammasome in Inflammatory Monocytes to Promote Intestinal Inflammation in Response to Injury
Sang-Uk Seo, Nobuhiko Kamada, Donghyun Kim, and Gabriel Nunez
Department of Pathology, University of Michigan, USA
Department of Internal Medicine, University of Michigan, USA

F-43  The Prevalence and Characteristics of Bacillus cereus Isolated in Korea, 2012-2014
Sahyun Hong, Nan-Ok Kim, Su-Mi Jung, Haeyong Na, Gyung Tae Chung, Cheon-Kwon Yoo, and Won Kean Seong
Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health

F-44  Enteric Bacteria Isolated from Diarrheal Disease in Korea, 2012-2014
Nan-Ok Kim, Su-Mi Jung, Haeyong Na, Gyung Tae Chung, Cheon-Kwon Yoo, and Won Kean Seong
Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health

F-45  Characterization and Trans-complementation Study of a Recombinant Virus Harboring Point Mutations in ORF49 of Murine Gammaherpesvirus 68
Woo-Chang Cheong, Byung Chul Kim, Ae-Ra Lee, Junsoo Kim, Hye-Ri Kang, Hyun Jun Jang, Joo-Hee Park, Kwang-Yeon Hwang, and Moon Jung Song
Department of Biosystems and Biotechnology, Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University
Structural Proteomics Laboratory, Department of Biosystems and Biotechnology, Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University
Virus-Host Interactions Laboratory, Department of Biosystems and Biotechnology, Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University

F-46  The Proportion and Characteristics of Salmonella spp. in Korea During the Past Ten Years (2005-2014)
Young-Sun Yun, Su-Jin Chae, Gyung Tae Chung, Cheon-Kwon Yoo, and Deog-Yong Lee
Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health

F-47  Molecular Characterization of Viral Pathogens Causing Sporadic Acute Gastroenteritis in Children <5 Years of Age in Korea, 2013-2014
Bo-Mi Hwang, Sunyoung Jung, Hyun Ju Jeong, Gyung Tae Chung, Cheon-Kwon Yoo, and Deog-Yong Lee
Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health

48 | 2015 International Meeting of FKMS
F-48 Analysis of Immune Responses in Mice Immunized with Brucella abortus Recombinant Proteins .................................................. 298
Young Bin Im, Myungwon Jung and Han Sang Yoo*
Department of Department of Infectious Diseases, College of Veterinary Medicine, Seoul National University

F-49 Mycobacterium massiliense Type II Genotype Leads to Higher Level of Colony Forming Units and TNF-α Secretion from Human Monocytes than Type I Genotype .......................................................... 299
Byoung-Jun Kim, Bo-Ram Kim, So-Young Lee, Jeong-Ryeol Gong, Ga-Na Kim, Yoon-Ho Kook, and Bum-Joon Kim*
Department of Microbiology and Immunology, Seoul National University College of Medicine

F-50 NADH Dehydrogenase of Vibrio vulnificus GMCP6 Contributes to Energy Production by Providing Proton Motive Force to ATP Synthase I .......................................................... 299
Sao Puth1,2, Kwangjoon Jeong1, Wenzhi Tan1,2, Soo Eun Lee1, and Joon Haeng Rhee5,6*
1Department of Microbiology and Clinical Vaccine R&D Center, Chonnam National University Medical School, 2Interdisciplinary Graduate Program of Molecular Medicine, Chonnam National University, 3Department of Microbiology, Chonnam National University Medical School, 4Department of Pharmacology and Dental Therapeutics, Seoul National University College of Dentistry, Chonnam National University, 5Department of Microbiology and Clinical Vaccine R&D Center, Chonnam National University Medical School, 6Interdisciplinary Graduate Program of Molecular Medicine, Chonnam National University

F-51 The Activity of Protease IV, a Major Virulence Factor of Pseudomonas aeruginosa is Affected by Quorum Sensing System .......................................................... 299
Jungmin Oh1,2,3, Soo-Kyoung Kim2,3,4, Xi-Hui Li1,2,3, and Joon-Hee Lee2,3,4*
1Lab of Microbiology, Department of Pharmacy, 2College of Pharmacy, 3Pusan National University, 4Lab of Microbiology, Department of Pharmacy

F-52 A Bacterial NIMBY Response: Staphylococcus aureus Membrane Vesicle Impede Biofilm Formation by Other Bacteria .......................................................... 299
Hansol Im and Robert J. Mitchell*
School of Life Science, Ulsan national institute of Science and Technology

F-53 Molecular Characterization of Hepatitis A Virus Causing an Outbreak at a Residential Facility for the Disabled, Republic of Korea .......................................................... 300
Eun Kyung Shin1, Kyung-Hwan Oh1, Mun Ju Kwon1, Cheon-Kwon Yoo1, and Gyu Young Chung1
1Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health, 2Division of Microbiology, Incheon Institute of Public Health and Environment Research

F-54 Quorum Sensing Inhibitors to Prevent Biofilm Formation of Periodontopathogens .......................................................... 300
Eun-Ju Ryu1, Jachyun Sim1, Yoon-Ki Ko1, Kwang-Soo Jung1, and Bong-Kyu Choi1,2*
1Department of Oral Microbiology and Immunology, School of Dentistry, Seoul National University, 2Dental Research Institute, Seoul National University

F-55 Functional Role of NLRP10 in HOK-16B Cells Infected with Tannerella forsythia and Fusobacterium nucleatum .......................................................... 300
SeokJoo Lee, Yuliang Choi, HyeKyoung Jun, and BongKyu Choi*
Departments of Immunology and Molecular Microbiology, Seoul National University

F-56 Characterization of Mouse-adaptation of Yamagata Lineage B Virus .......................................................... 300
Eun-Ha Kim1, Hyeok-il Kwon1, Su-Jin Park1, Semi Kim3, Young-il Kim1, Eun-Ji Choi2, Young-Jae Si2, and Young-Ki Choi1*
1Microbiology Department, Chungbuk National University, 2Microbiology Department, Chungbuk National University, 3Microbiology Department, Chungbuk National University

F-57 Roles of Ornithine Lipid in Pseudomonas aeruginosa Infection to Host Cells .......................................................... 301
Soo-Kyoung Kim, Xi-hui Li, Jungmin Oh, and Joon-Hee Lee*
Lab of Microbiology, Department of Pharmacy, College of Pharmacy, Pusan National University

F-58 Development of Capture ELISA Using Human Anti-BoNT/B Monoclonal Antibodies .......................................................... 301
Yu-Ri Kim, Eun-Sun Choi, Jae-Eun Park, Gi-Eun Rhie, Yeon-Hee Kim
Division of High-Risk Pathogen Research, Center for Infectious Diseases and Prevention, Korea National Institute of Health, Osong Health Technology Administration Complex, 187, Osonggaengnyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju, Chungcheongbuk-do

International Meeting of the Federation of Korean Microbiological Societies
www.fkms.kr | 49
**Poster Session**

**F-59** Enhanced Antibody Neutralization Against Botulism by Apoptotic Cell Targeting Peptide Conjugates

Eun-Sun Choi¹, Yu-Ri Kim¹, Gi-En Rhie¹, Kiweon Cha², and Yeon-Hee Kim¹*  
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**F-60** Novel Mutation in an 8-bp Deletion of the Hepatitis B Virus X Gene Leads to Occult Infection in Korean Vaccinated Individuals

Hong Kim, Jeong-Ryeol Gong, Seoung-Ae Lee, Ji-Eun Kim, Ji-Seon Jeon, and Bum-Joon Kim*  
Department of Microbiology and Immunology, Seoul National University

**F-61** Gender Disparity Induction by Male-Specific Hepatitis B Virus Large Surface Protein Variant W4P in Hepatocarcinogenesis

Seoung-Ae Lee, Hong Kim, Ji-Eun Kim, Ji-Seon Jeon, and Bum-Joon Kim*  
Department of Microbiology and Immunology, Seoul National University

**F-62** Anti-sepsis Therapeutic Effects of HY-209 on LPS Induced Mouse Liver by Mass Spectrometry-based Label Free Quantitative Analysis

Sungyoon Moon¹, Youngju Lee¹, Youn-Hee Kim², and Bum-Joon Kim*  
¹Department of Microbiology, Dankook University

**F-63** Construction of an EGFP-expressing Replication Competent Porcine Circovirus Type 2

Sungyoon Moon¹, Youngju Lee¹, Youn-Hee Kim², and Bum-Joon Kim*  
¹Department of Microbiology, Dankook University

**F-64** Cell-type Specific Regulation of KSHV Replication by OX40-OX40L Interaction

Jinjong Myoung*  
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**F-65** Interferon-γ Inhibits KSHV Replication in Endothelial Cells but Not in B Cells

Jinjong Myoung*  
Korea Zoonosis Research Institute, Chonbuk National University

**F-66** KSHV Replication is Modulated by Jak-STAT pathways

Jinjong Myoung*  
Korea Zoonosis Research Institute, Chonbuk National University

**F-67** Investigation of Roles of Cytokines on KSHV Reactivation

Jinjong Myoung*  
Korea Zoonosis Research Institute, Chonbuk National University

**F-68** KSHV Infection in Tonsillar B Cells is Spontaneously Lytic

Jinjong Myoung*  
Korea Zoonosis Research Institute, Chonbuk National University

**F-69** Mycobacteria-dependent ROS Production is Mutually Dependent on the Dectin-1 Expression in Human Airway Epithelial Cells

Hye-Mi Lee¹,², Chang-Hwa Song¹,², Hwa-Jun Ko¹,², Jeong-Kyu Park¹,², and Eun-Kyoong Jo³,⁴  
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**F-70** Expression of the Global Regulator LeuO is Self-Regulated by Coordinated Binding of H-NS, ToxR, and LeuO on Cognate cis-Acting Elements

Na-Young Park, In-Hwag Kim, Yancheng Wen, Keun-Woo Lee, Jung-A Kim, Kwang-Hwan Jung*, Kyu-Ok Lee*, and Kun-Soo Kim*  
Department of Life Science and Interdisciplinary Program of Integrated Biotechnology, Sogang University

**F-71** Cyclo(L-Phe-L-Pro) Facilitates the Survival of Vibrio vulnificus under Oxidative Stress

So-Yeon Kim, In-Hwag Kim and Kun-Soo Kim*  
Department of Life Science, Interdisciplinary Program of Integrated Biotechnology, Sogang University
**Poster Session**

**F-72** Host Restriction Mechanisms Against HIV-1 Replication and AIDS Pathogenesis .................................................. 304
Yu-Jin Park1, Cheol-Hee Yoon2, Jung-Hyup Hong2, Minji Rim1, and Yong-Soo Bae1,*
1Department of Biological Science, Sungkyunkwan University, 2Korean National Institute of Health, 3Dept. of Biological Science, Sungkyunkwan University

**F-73** Irradiated Group B Streptococcal Whole Cell Vaccine Induces Protective Cellular as Well as Humoral Immune Responses ........................................................................................................ 305
A Yeung Jang, Sangyong Lim, Dongho Kim, and Ho Seong Soo* Radiation Biotechnology Research Division, Korea Atomic Energy Research Institute

**F-74** A Synthetic Latch Domain Peptide Vaccine Confers Serotype-independent Protection from Group B Streptococcus Challenge ........................................................................................................ 305
Shunmei Lim, Zhi Yong, Sangyong Lim, Dongho Kim, and Ho Seong Seo* Radiation Biotechnology Research Division, Korea Atomic Energy Research Institute

**F-75** Humanized Virus Suppressing Factor (hzVsf), a humanized Monoclonal Antibody, against Influenza A (H1N1) Virus Infection Suppresses Viral Replication and Inflammatory Response ........................................................................................................ 305
Min Soo Kim1,2, Sungman Park1,2, Young-Jun Kim1,2, Yong-Jun Lee1,2, Sang-Jin Park1,2, Young-Nam Lee1,2, Ji-Hoon Kim1, Min-Woo Kim1, Young Deuk Kim1,2, and Yoon-Won Kim1,* 1Institute of Medical Science, School of Medicine, Hallym University, 2ImmuneMed Inc., 3Department of Microbiology, School of Medicine, Hallym University

**F-76** Virus Suppressing Factor (VSF) Inhibits Hyperglycemia and Pancreatic Islet Inflammation in Diabetes Mellitus Induced by Encephalomyocarditis Virus ........................................................................................................ 305
Min Soo Kim1,2, Young-Jun Kim1,2, Sungman Park1,2, Min-Woo Kim1, Young Deuk Kim1,2, and Yoon-Won Kim1,* 1Institute of Medical Science, School of Medicine, Hallym University, 2ImmuneMed Inc., 3Department of Microbiology, School of Medicine, Hallym University

**F-77** Anti-viral Effects of *Hyrtios* sp. and *Haliclona* sp. on Rotavirus-infected Caco-2 Cells .................................................. 306
Su Im Koh, Joo Yeon Kang and Hea Soon Shin* College of Pharmacy, Doksung Women’s University

**F-78** Post-translational Modification of TANK-binding Protein 1 is Regulated by a Viral Immune Modulator of Gammaherpesvirus ........................................................................................................ 306
Seangho Ryu, Hye-Ri Kang, Woo-Chang Cheong, and Moon Jung Song* Virus-Host Interactions Laboratory, Department of Biosystems and Biotechnology, Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713

**F-79** Introduction of Chonbuk, Gyeongsang, and Kyungpook National University Hospital Branches of National Culture Collection for Pathogens ........................................................................................................ 306
Hye Soo Lee1, Yong Gon Cho 1, Won-Kil Lee2, Yoo-Chul Lee3, Woo-Kon Lee4, and Myung-Je Cho4,* 1Department of Laboratory Medicine, Chonbuk National University School of Medicine, 2Department of Laboratory Medicine, Kyungpook National University School of Medicine, 3Department of Microbiology, Kyungpook National University School of Medicine, 4Department of Microbiology, Gyeongsang National University School of Medicine

**F-80** Host Transcriptional Analysis of *Mycobacterium avium* subsp. *paratuberculosis* Infection in Animal Model .......................... 306
Min-Kyoung Shin1 and Han Sang Yoo2,* 1Department of Microbiology, Gyeongsang National University School of Medicine, 2Department of Infection Diseases, College of Veterinary Medicine, Seoul National University, 3Institute of Green Bio Science and Technology, Seoul National University

**F-81** The Effect of Branched-chain Amino Acids Supplementation on the Replication of *Salmonella Typhimurium* in Nitric Oxide-producing Raw 264.7 Macrophages ........................................................................................................ 307
Yoon Mee Park and Iel Soo Bang* Department of Microbiology and Immunology, Chosun University School of Dentistry

**F-82** The Role of the Spy (spheroplast protein y) Gene in Response of *Salmonella Typhimurium* to Reactive Oxygen/nitrogen Species ........................................................................................................ 307
Hwa Jeong Lee and Iel Soo Bang* Department of Microbiology and Immunology, Chosun University School of Dentistry

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*www.fkms.kr*
F-83 Rapid Detection of blaPC-2 and blaNDM-1 type Carbapenemase-producing Enterobacteriaceae from Clinical Isolates by Loop-mediated Isothermal Amplification
Hye Jin Kim1, Hyung Sun Kim2, Jae Myan Lee1,2, Dongyeon Yong1,3, and Sang Sun Yoon1,3,*
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F-84 The Identification of Putative Drugs Against Salmonella Typhimurium Using Comparative Genomic Analysis of Metabolic Pathways
SeungJin Lee, SooHee Choi and SeungChun Park*
College of veterinary Medicine, Kyungpook National University

F-85 Development of a Functional Diaper by Using Phellodendron Amurense RUPR
Seon Ryu, Jae Hak Im, Mi Jin Kim, Young Eun Choi, Hui Yeon Jeong, and Su-jung Kim*
Department of Biomedical Laboratory Science, Daegu Health College, Daegu 702-722

F-86 In Vitro Studies on the Inhibition of Mycobacterial Cell Wall Mycolic Acid and Promoting Intracellular Killing by DPG-5: Promising Anti-Mycobacterial Activity
Md. Anirban Jyoti1, Woong-Sik Jang1, SuKyung Kim1, Tamanna Zerin1, Kung-Woo Nam2, and Ho-Yeon Song1*
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G Immunology and Signal Transduction

G-1 Apo-9-fucoxanthine, Isolated from Sargassum muticum, Suppress Pro-inflammatory Cytokine Production in CpG-stimulated Immune Cells by Down Regulating Mitogen-activated Protein Kinase Pathway
Irshad Ali and Young-Sang Koh*
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G-2 HCMV Regulation of Receptor-Interacting Protein Kinase 1 (RIPI)-Mediated NF-κB Signaling
Ki Mun Kwon, Se Eun Oh and Jin-Hyun Ahn*
Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine

G-3 Characterization of Surface Binding Protein to Signal Molecules Related to Quorum Sensing of E.coli SE15 Isolated form Indwelling CA-UTI Patients
Sang Rim Kang1 and Sang-Seob Lee2*
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G-4 Enterococcus faecium Isolated from Chicken Cecum Stimulates Immunomodulating Activity and Promotes Longevity in Caenorhabditis elegans
Insuk Sim1, Keun-Tae Park2, Gayeung Kwon3, and Young-Hee Lim1,3,4*
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G-5 Peptide H Suppresses TNFα Expression in Human Breast Cancer MDA-MB-231 Cells
Jamen Park and Hanbok Kim*
Department of Biotechnology, The Research Institute for Basic Sciences, Hoseo University

G-6 Inhibitory Effect of Sargassum micracanthum on Collagenase Activity
Nan-Young Bae1, Won-Min Pak1, Min-Ji Kim1, Ji-Hye Park1, Sun-Hee Park1, Koth-Bong-Wo Ri Kim2, and Dong-Hyun Ahn1,2*
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International Meeting of the Federation of Korean Microbiological Societies

Poster Session

G-7 The Inactive *Vibrio vulnificus* Cysteine Protease Domain as Strong Immunogen .......................................................... 310
Tae Hee Lee and Kyung Min Chang *
Department of Microbiology and Immunology, Chonbuk National University Medical School, Jeonju, Jeonbuk

G-8 Sea Lettuce (*Ulva fasciata*) Extract Inhibits the CpG-induced Inflammatory Response by Attenuating the Mitogen-activated Protein Kinase and NF-κB Pathways .......................................................... 310
Zahid Manzoor and Young-Sang Koh*
Department of Microbiology & Immunology, and Brain Korea 21 PLUS, Jeju National University School of Medicine, Jeju, Jeju-Do

G-9 The Oncolytic Activity of Spore-Forming Bacillus Species in H1650 Human Lung Cancer Cells .......................................................... 311
Miso Yang, In-Taek Jang, Chul-Hee Choi, Chang-Hwa Song, Eun-Kyeong Jo, Hwa-Jung Kim, and Jeong-Kyu Park*
Department of Microbiology, School of Medicine, Chungnam National University

G-10 Anti-inflammatory Activity of *Griphola frondosa* Ethanol Extract in LPS-induced RAW 264.7 Cells and Mouse Models .......................................................... 311
Minji Kim1, Jihye Park1, Nanyoung Bae1, Sunhee Park1, Kothbongwoori Kim2, and Donghyun Ahn1*
1Department of Food Science and Technology, Pukyong National University, Busan 48513, 2Department of Microbiology and Immunology, College of Medicine, Pukyong National University

G-11 Host Immune Responses to *Mycobacterium tuberculosis* Antigen Rv34xx .......................................................... 311
Hye-Soo Park, Han-Gyu Choi, Kang-In Lee, Seunga Choi, and Hwa-Jung Kim*
Department of Microbiology and Infection Signaling Network Research Center, College of Medicine, Chungnam National University, Daedeon

G-12 Crystal Structure of NleB2, an Enteropathogenic *Escherichia coli* Type III Effector Protein .......................................................... 311
Junbap Park, Juyeon Kim and Hyun-Soo Cho*
Department of Systems Biology, Yonsei University

G-13 Lactic Acid Bacteria Polarize Mouse Splenocytes and Bone Marrow Derived Macrophage (BMDM) to a M-2 Macrophages .......................................................... 312
In-Taek Jang, Mi-So Yang, Chul-Hee Choi, Hwa-Jung Kim, and Jeong-Kyu Park*
Department of Microbiology, School of Medicine, Chungnam National University, Daejeon

G-14 Positive Role of Promyelocytic Leukemia Protein in Type I Interferon Response and its Regulation by Human Cytomegalovirus .......................................................... 312
Young-Eun Kim and Jin-Hyun Ahn*
Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine

G-15 The Anti-Influenza Activity of Wogonin Involves Modulation of Interferon (IFN) and AMPK Signaling Pathways .......................................................... 312
Rakkyun Seong1, Jiae Kim1, YounChul Kim2,3, and Ok Sarah Shin4,5*
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G-16 Improvement of Tight Junction Integrity in Caco2 Cell Monolayers by Oxyresveratrol .......................................................... 312
HyunA Jo1, Dahyun Hwang2 and Young-Hee Lim1,3*
1Department of Integrated Biomedical and Life Sciences, Graduate School, Korea University, 2Department of Public Health Science (BK21 PLUS Program), Graduate School, Korea University, 3Department of Public Health Science (BK21 PLUS Program), Graduate School, Korea University

G-17 Crystal Structure of *Toxascaris leonina* Galectin with Two Carbohydrate Recognition Domains .......................................................... 313
Mi Suk Jeong1, Eun Young Hwang1, So Young Park 1,2, and Se Bok Jang 1*
1Department of Molecular Biology, Pusan National University, 2Department of Parasitology, School of Medicine, Pusan National University

G-18 Oral Mucosal Vaccine Against HIV-1 Using Poliovirus-derived CTL Vaccine Vector .......................................................... 313
Myeong-Ho Kang, Jung-Hyub Hong, Minji Rin, and Yong-Soo Bae*
Department of Biological Sciences, Sungkyunkwan University
**Poster Session**

**G-19** Diverse Innate Signaling is Required for the Activation of *Mycobacterium chelonae*-mediated CCL2 and CCL5 Expression in Macrophages

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**G-20** *Mycobacterium tuberculosis* RpFE Promotes Simultaneous Th1- and Th17-type T-cell Immunity via TLR4-dependent Maturation of Dendritic Cells

Han-Gyu Choi, Hye-Soo Park, Kang-In Lee, Seunga Choi, and Hwa-Jung Kim*

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**G-21** Mycobacterium avium Complex Protein MAV200X Induces Macrophage Apoptosis through Toll-Like Receptor 4

Kang-In Lee, Han-Gyu Choi, Hye-Soo Park, Yeo-Jin Son, Chul Hae Choi, and Hwa-Jung Kim*

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**G-22** Vaccine Development by Using Outer Membrane Vesicles Released from a Detoxified Mutant of Enterotoxigenic *Escherichia coli*

Seung-Hak Cho*, Su-Mi Jung, Gyung Tae Chung, Won Keun Seong, and Cheon-Kwon Yoo

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**G-23** The Production of Thymic Stromal Lymphopoietin (TSLP) is Regulated by Synergistic Effects of Toll-like Receptor Agonists in Bone Marrow-derived Dendritic Cells

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**H-1** Establishment of *Escherichia coli* Strain for Production of 5-Aminolevulinic Acid through Metabolic Engineering

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**H-2** Assembly of a Biosynthetic Pathway for Production of Short-chain Alkane in *Escherichia coli*

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**H-3** Establishing a Synthetic Pathway for Production of Gamma-butyrolactone in *Mannheimia succiniciproducens*

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**H-4** Technique of Fine-Tuned Knockdown Using Small Regulatory RNA and Its Application in Metabolic Engineering for Production of Putrescine from *Escherichia coli*

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**Poster Session**

**H-5**  
Recombinant Immunogenic Influenza Hemagglutinin Glycoproteins  
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**H-6**  
Expression and Characterization of Dimeric Single Chain Variable Domain Fragment (ScFv) Fused to *E. coli* Alkaline Phosphatase and Its Application for a Sensitive Direct Immunoassay  
Jeong-Hyo Lee1, Seung-Hee Han1, Jin-Hee Kang1, Beom Ku Han2, and Jin-Kyoo Kim1  
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**H-7**  
Development of High Copy Plasmid for the Enhanced Production of Recombinant Proteins in *Leuconostoc citreum*  
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**H-8**  
Fruit and Citrus Peel Waste as a Valorization Biomass for the Bioethanol Production  
Eun Jin Cho1, In Seong Choi1 and Hyeun-Jong Bae2,3  
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**H-9**  
Conjugated to Streptavidin  
Functional Expression and Characterization of Target-specific Single-Chain Variable Domain Fragment (ScFv) Fused to *E. coli* Alkaline Phosphatase and Its Application for a Sensitive Direct Immunoassay  
Jeong-Hyo Lee1, Seung-Hee Han1, Jin-Hee Kang1, Beom Ku Han2, and Jin-Kyoo Kim1  
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**H-10**  
Development of Production System of Cellulase and Laccase Derived from Brown-Rot Fungus *T. Palustris* in *Pichia pastoris* X-33  
Division of Wood Chemistry & Microbiology, Korea Forest Research Institute (KFRI)

**H-11**  
Antimicrobial Effect of Plant Endophytic Fungi Isolated from *Morus alba* L.  
Yangseon Kim, Hyunjung Lee, Wooyoung Bang, Changmu Kim, Joo-Hong Yeo, and Soonok Kim*  
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**H-12**  
Functional Analysis of Recombinant Human and Putative Fungal α-GlCNac Transferases Expressed in *Saccharomyces cerevisiae*  
Hye Ji Oh1, Hye Yun Moon1, Seon Ah Cheon1, Eun Jung Thak1, Jin Won Cho2, Yoonsoo Hahn1, and Hyun Ah Kang1  
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**H-13**  
Development of 9E10 Single-Domain Nanobody (9E10 VH) Against c-myc Peptide through Camelization of Murine Heavy Chain Variable Domain to Increase Solubility  
Seung-Hee Han1, Jeong-Hyo Lee1, Jin-Hee Kang1, Beom Ku Han2, and Jin-Kyoo Kim1  
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**H-14**  
Funtional Expression and Characterization of Target-specific Single-Chain Variable Domain Fragment (ScFv) Conjugated to Streptavidin  
Seung-Hee Han1, Jeong-Hyo Lee1, Jin-Hee Kang1, Beom Ku Han2, and Jin-Kyoo Kim1  
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**H-15**  
Enhancement of Hexane Tolerance in *Pseudomonas* sp. BCNU 106 by Addition of Trehalose  
Bo Ra Lim, Hye Jung Choi and Woo Hong Joo*  
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**H-16**  
A Novel Psychrophilic Alkaline Lipase from Metagenome of Dokdo Sediment  
Su-Yeon Kim, Sohyeon Seo, Sang-Hong Yoon, Bon-sung Koo, Joon-Soo Sim, Bum-Soo Hahn, and Chang-Muk Lee*  
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International Meeting of the Federation of Korean Microbiological Societies

www.fkms.kr | 55
Poster Session

**H-17** Expression Analysis of Codon-optimized NNV Capsid Protein in the Yeast *Yarrowia lipolytica* for Recombinant Vaccine Development

Van Thinh Luu, Hye Yun Moon, Hong-Jin Kim, Jee Youn Hwang, Min-Gyeong Kwon, Bo-Kyu Kang, and Hyun Ah Kang

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**H-18** Differential Transcriptional Response to Toluene by *Pseudomonas* sp. BCNU 106

Hye Jung Choi, Bo Ram Lim, Bo Ra Lim, Sung Min Ha, and Woo Hong Joo

Department of Biology, Changwon National University 319

**H-19** Oxidative Stress Response in *Pseudomonas* Strains Exposed to Toluene

Hye Jung Choi, Bo Ram Lim, Bo Ra Lim, Sung Min Ha, and Woo Hong Joo

Department of Biology, Changwon National University; Department of Biology, Changwon National University 319

**H-20** Flux Optimization of TCA Cycle for Efficient Production of Fumaric Acid

ChanWoo Song and Sang Yup Lee

Dept. of Chemical and Biomolecular Engineering, KAIST 319

**H-21** Development of a Metabolically Engineered Strain for the Production of Beta Alanine, a Precursor for Nylon-3 Synthesis

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**H-22** Optimal Strain and Knockdown Target Screening Method Using Synthetic Small Regulatory RNA for Enhanced Production of Tyrosine and Cadaverine

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**H-23** Aminovalerate and Glutarate Production Pathway Introduced into *Escherichia coli*

Yoosung Ko, Sijae Park, Eun Young Kim, Won Noh, Hye Min Park, Young Hoon Oh, Seung Hwan Lee, Bong Keun Song, Jonggeun Jegal, and Sang Yup Lee

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**H-24** Optimization of a Sialic Acid-Binding Lectin Purification Procedure from a Mushroom Fruiting Body

Seonghan Kim

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**H-25** Antigen-Specific Chicken Antibody Selection from Non-immunized Antibody Gene Library using β-lactamase – based Protein Fragment Complementation Assay (PCA)

Dong Woon Park, Seung-Hee Han, Jeong-Hyo Lee, Jin-Hec Kang, Beom Ku Han, and Jin-Kyoo Kim

Department of Microbiology, Changwon National University; Optifarm Inc. 321

**H-26** Engineering of Sugar Transport in *Corynebacterium glutamicum* for Enhanced D-psicose Production

Dae-yun Lee, Seong-Hee Jeong, Min-Jin Choi, Rachelle Canete, Sang-Hee Kang, Hyeon-Seo Lee, and Seon-Won Kim

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**H-27** SpiE Interacts with *Corynebacterium glutamicum* WhcE and is Involved in Heat and Oxidative Stress Responses

Jung Chul Park, Joon-Song Park, Younhee Kim, Pil Kim, Eung Soo Kim, and Heung-Shick Lee

Department of Biotechnology and Bioinformatics, Korea University; Department of Oriental medicine, Semyang University; Department of Biotechnology, Catholic University of Korea; Department of Biological Engineering, Inha University 321
**International Meeting of the Federation of Korean Microbiological Societies**

### Poster Session

**H-28** The Fine-tuning of TodST-based Biosensor in *Pseudomonas putida*

Sang-Yoon Kim¹, Doo-Byoung Oh²,3, Seung-Goo Lee¹,², and Ohsuk Kwon¹,²

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**H-29** Enhancement of Cyclohexane Tolerance in *Pseudomonas sp. BCNU 106* by Addition of Trehalose to Culture Media

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**H-30** Enhancement of Solvent Tolerance in *Pseudomonas sp. BCNU 106* with Glycerol

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**H-31** Substrate Specificity Engineering of *Thermoanaerobacter ethanolicus* Secondary Alcohol Dehydrogenase by Rational Design

Chang Sup Kim* and Amos K. Dwamena

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**H-32** An Alkylhydroperoxidase D-like Protein Encoded by the *Corynebacterium glutamicum* Gene Contributes to the Peroxide Stress Response

Eun-Ji Hong¹, Younhee Kim² and Heung-Shick Lee³

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---

**H-33** Intra Species Variation of *Bjerkandera adusta* Concerning Decolorization and Oxidation of Organic Pollutant

Aslan Hwanhwi Lee, Sookyoon Jang, Gyu-Hyeok Kim, and Jae-Jin Kim*

Division of Environmental Science & Ecological Engineering, Korea University

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**H-34** Isolation of *Chlorella vulgaris* Mutants Producing High Lipid and their Characterization

Soo-Jeong Choi and Jae-Hwa Lee*

Department of Pharmengineering, Silla University

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**H-35** Characteristics of a Recombinant FSH and LH of Japanese Eel with and without CTP of Equin Produced in Silkworm Using BmNPV Baculovirus

Sun Mee Hong¹, Ji Hyun Choi², Sun Jung Jo³, Dae Jung Kim³, and Kwan Sik Min³


---

**H-36** Development of Novel SRP Machinery-engineered *E. coli* Mutant for the High-level Secretory Production of Antibodies and Therapeutic Proteins

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**H-37** Nanoparticle Based Anti-Biofilm Surfaces

Ramasamy Mohankandhasamy, Jin-Hyung Lee, Murugan Mohankumar, and Jintae Lee*

School of Chemical Engineering, Yeungnam University

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**H-38** Control of the Pathogenic Bacteria, Bioremediation and Upcycling for the Waste Substances in the Chicken Farm

Won Mun Kim, Kwang-Su Lee and Ki-Sung Lee*

Department of Biology & Medicinal Sciences, Paichai University

---

**H-39** Biofunctionality and Bioconversion Activities of KA6(*Bacillus licheniformis*), KA84(*Enterococcus faecium*), KA89(*Bacillus licheniformis*), KA107(*Bacillus licheniformis*)

Won Mun Kim, Kwang-Su Lee and Ki-Sung Lee*

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**H-40** Bioconversion Efficiency in Accordance with the Substrate(Arg:Arginine) Concentrations

Won Mun Kim, Kwang-Su Lee and Ki-Sung Lee*

Department of Biology & Medicinal Sciences, Paichai University
**Poster Session**

**H-41** Novel Bacteria-Phage Pairs Showing Phage-Antibiotic Synergy (PAS) and Study on Its Mechanism
Minjin Kim and Heejoon Myung*
Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

**H-42** Monoclonal Antibodies Specific for the NS 1 Protein of Japanese Encephalitis Virus Suitable for Diagnostic Purpose
Gowoon Cha, WonJa Lee, MyungGuk Han, and YoungEui Jeong *
Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

**H-43** Microbial Network Analysis in a Pilot-Scale MBR
So-Yeon Jeong1, Taewoo Yi1 and Tae Gwan Kim 2*
1National Institute of Ecology, 2Pusan National University

**H-44** Effect of Commercialized Chlorine Dioxide to Clinical Bacteria
Sukyul Jung*, Ayoung Park, Ayoung Kang, Gewon Choi, Yuri Ko, Jungeun Kim, and Minhee Chang
Department of Biomedical Laboratory Science, Molecular Diagnostics Research Institute, Namsan University

**H-45** Gargling Alternative of Bacterial Inhibition to Chlorhexidine Gluconate
Sukyul Jung*
Department of Biomedical Laboratory Science, Molecular Diagnostics Research Institute, Namsan University

**H-46** Effect of Monosodium Glutamate to a Mammalian Cell and *Escherichia coli*
Sukyul Jung*
Department of Biomedical Laboratory Science, Molecular Diagnostics Research Institute, Namsan University

**H-47** Cloning of the Inositol Phosphoceramide Synthase Gene (*AUR1*) from Stress-Tolerant Yeast *Pichia kudriavzevii*
Boung-Hyuk Yoo1, Soo-Hwan Yeo2 and Myoung-Dong Kim1*
1Department of Food Science and Biotechnology, Kangwon National University, Chuncheon, 2Fermented Food Science Division, Department of Agro-food Resource, NAAS, RDA,Wanju-gun, Jeollabuk-do 565-851

**H-48** Engineering of *Corynebacterium glutamicum* for Production of Tryptophan
Youmi Kim and Jinho Lee*
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**H-49** Engineering of *Corynebacterium glutamicum* for Production of Vanilla Flavors
Hyebin Jung, Ara Ko and Jinho Lee*
School of Food Biotechnology and Nutrition, Kyungsung University

**H-50** Hydrolysis of *Codium fragile* by Mannanase from *Bacillus subtilis* R2AL2A
Mi-Hwa Lee1, Suae Kim2, Juyeon Lee2, Ji-Hye Han1, Kiwoon Baek1, Sang Chul Jeong1, and Young-Do Nam2*
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**Food Microbiology**

**I-1** Characterization of *Leuconostoc mesenteroides* BL-1A Isolated from Baechu Kimchi
Ohah Kwon*
Major in Biological Science, College of Natural Science, Keimyung University

**I-2** Production of a Functional Enzyme Food with *Bacillus licheniformis* and *Lactobacillus casei* by Two-Step Fermentation
Jo-Eun Kang and Gi-Seong Moon*
Department of Biotechnology, Korea National University of Transportation
I-3 Degradation of Fucoidan by Fucoidan-degrading Enzyme from *F*ormosa *sp.*
Ji Hyo Choi and Soobok Lee*
Department of Food and Nutrition, Yonsei University

I-4 Characterization of *Vibrio vulnificus* CadA Essential for Biofilm Development on Abiotic Surfaces and Oysters
Jin Hwan Park1, Song Yee Jang2, Hae Naem Kwon2, Myung Hee Kim3, and Sang Ho Choi1
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I-5 Optimizing Culturing Conditions and Comparative Fibrinolytic Activities of Fibrinolytic Enzymes from *Bacillus subtilis* Strain
Mihyun Kim1, Yungheon Cho2, Eun Jung Cho2, and Seungik Cho2,4
1Department of Oriental Medicine Resources, Mokpo National University, Muan, Jeonnam 534729

I-6 Isolation of γ-Aminobutyric Acid-Producing *Enterococcus faecium* JK29 from Traditional Fermented Foods and Its Culture Optimization for GABA Production
Heeseon Lim1, In-Tae Cha2, and Myung-Ji Seo1,2
1Department of Life Sciences, Graduate School of Incheon National University, Incheon 22012, 2Division of Bioengineering, Incheon National University, Incheon 22012

I-7 Preparation and Quality Characteristics of the Fermentation Product of Gastrodia Elata Blume Powder by Lactic Acid Bacteria
Eunju Kim, Yoonjeong Jung, Soojeong Ji, Jaehyun Kim, Yeun Kim, and Shin young Park*
Dept. of Agrofood Resources, National Academy of Agricultural Science, RDA

I-8 Evaluation of the Efficiency of Compounds Present in Ingredients of Kimchi against Influenza A Virus Infection Using Proteomic Analysis
Se-Young Cho1, Song Hak Kim1, Bipin Vaidya2, Kyung Seo Oh1, Sung Hyoung Kim1, Joseph Kwon4, and Duwoon Kim1,5
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I-9 Enhancement of Conjugated Linoleic Acid and Isoflavone-Aglycone Contents in Different Fermented Soybean Cultivars with Mycelia of *Polyozellus multiplex*
Hee Yul Lee1, Chung Eun Hwang1, Min Ju Ahn1, Byong Won Lee2, Yu Young Lee2, Choonwo Lee2, Byung Joo Kim2, Ji Yong Park2, Eun Young Sim2, and Kye Man Cho1
1Department of Food Science, Gyeongsung National University of Science and Technology, 2Department of Central Area, Crop Science, National Institute of Crop Science (NICS), Rural Development Administration (RDA)

I-10 Production of Ginseng Berry makgeolli and Analysis of its Physicochemical Properties
Sae Kyul Kim, Jae Hee Choi and Young Tae Hanm*
Department of Systems Biotechnology, Chung-Ang University

I-11 Ergothioneine Contents of Shiitake (*Lentinula edodes*) Fruiting Bodies on Sawdust Media with Different Nitrogen Sources
Jiheon Park, Won Chull Bak* and Rhim Ryoo
Korea Forest Research Institute

I-12 Taxonomic Characterization and Safety of Nuruk Molds Used Industrially in Korea
Seung-Beom Hong4, Hyeon-Jeong Kim1, Soon-wo Kwon1, Sun-Ja Seok1, Su-Jin Kim1, Jeong-Seon Kim1, Soo-Hyung Chung2, and Han-Hong Yoon1
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**Poster Session**

**I-13** Breeding of a New Cultivar *Lentinula edodes* (Shiitake) Strain “Sanmaru 2ho” and its Characteristics ——— 331
Woo Chull Bak, Youngae Park* and Rhim Ryoo
Division of Wood Chemistry & Microbiology, Forest Research Institute

**I-14** *Aspergillus* Associated with Traditional Korean *Meju* ——— 331
Seung-Beom Hong*, Dae-Ho Kim¹, Soon-wo Kwon¹, Sun-Ja Seok¹, Soo-Jin Kim¹, Jeong-Seon Kim¹, and Robert Samson²
¹Korean Agricultural Culture Collection, Agricultural Microbiology Div. National Academy of Agricultural Science, ²CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands

**I-15** Biocontrol Activity of Rice-originated Antagonistic Bacterial Strains against *Aspergillus flavus*, *Aspergillus candidus* and *Aspergillus fumigatus* on Stored Rice ——— 331
Mohamed Mannaa and Ki Deok Kim*
College of Life Sciences and Biotechnology, Korea University

**I-16** Antioxidant Activity of Cultivation Extracts from *Lactobacillus* spp. on the Modified-MRS Induced by *Trichoderma harzianum* β-mannanase ——— 332
Younghun Choi, Jinyoung Park, Misun Im, and Gwigun Park
Dept. of Food Science and Biotechnology, Gachon University

**I-17** Occurrence of *Salmonella enterica* and *Listeria monocytogenes* in Indigenous Microbial Community Formed Soil. ——— 331
Sora Kim, Dong Hwan Lee, Jeong-A Lim, Jonguk Kim, Jisoo Hong, Jae-Gee Ryu, Jin-Woo Park, Eun Jung Roh, and Kyu Seok Jung*
Microbial Safety Team, National Institute of Agricultural Science, Rural Development Administration

**I-18** Characterization of a Bacteriocin Produced by *Bacillus amyloliquefaciens* Isolated from Kimchi ——— 333
Ulziituya Batjargal, Ji-Young Lee, Su-bin Oh and Dae-Ook Kang*
Department of Bio Health Science, Changwon National University

**I-19** Purification of the Bacteriocin Produced by *Bacillus tequilensis* 10b Isolate ——— 333
Minjung Lee, Jinjae Lee and Bong-Soo Kim*
Department of Life Sciences, Hallym University

---

**I-20** Microbial Contamination Analysis of Commercial Processed Egg Products in Korea ——— 332
Ji-Kyoung Jung, Hong-Chul Jin, Jin-Hwan Choi, Sang-Yong Lee, Sun-Young Kim, Eun Jung Roh, and Sang-Woo Cho
Dept. of Food Safety, Holdings Technology Office, Pulmuone

**I-21** Biological activity of dietary fiber from *Cladosiphon novae-caledoniae* (Mozuku) ——— 332
Soo-Jeong Choi¹, Ji Min Ha¹, Hyun-Jin Park², and Jae-Hwa Lee¹*
¹Department of Pharmengineering, Silla University, ²Cholokand

**I-22** Antioxidant Activity of Cultivation Extracts from *Bifidobacterium* spp. on the Modified-MRS Medium Induced by *Xylogonae sphaerospora* β-mannanase ——— 333
Giyeoung Gweon, Sangjun Lee, Jiyeong Lee, and Gwigun Park*
Dept. of Food Science Biotechnology, Gachon University

**I-23** Purification of the Bacteriocin Produced by *Bacillus tequilensis* 10b Isolate ——— 333
Ji-Young Lee, Ulziituya Batjargal, Su-bin Oh and Dae-Ook Kang*
Department of Bio Health Science, Changwon National University

**I-24** Analysis of Microbiota on Ear Shells Collected from Different Regions at Summer and Winter ——— 333
Minjung Lee, Jinjae Lee and Bong-Soo Kim*
Department of Life Sciences, Hallym University
**International Meeting of the Federation of Korean Microbiological Societies**

### Poster Session

**I-25** Enhancement of Isoflavone Aglycone Contents and Biological Effects in Different Fermented Soybean Powder Milk with *Lactobacillus plantarum* P1201

Hwang Chung Eun1, Lee Hee Yul1, Ahn Min Ju1, Lee Byong Won2, Lee Yu Young2, Lee Choowo2, Kim Byung Joo2, Park Ji Yong4, Sim Eun Yeong2, and Cho Kye Man1*

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**I-26** Change of Phytoestrogen and Biological Activity during Solid-state Fermentation of Soybean by Mycelia of *Polyozellus multiplex*

Hwang Chung Eun1, Lee Hee Yul1, Ahn Min Ju1, Lee Byong Won2, Lee Yu Young2, Lee Choowo2, Kim Byung Joo2, Park Ji Yong4, Sim Eun Yeong2, and Cho Kye Man1*

1 Department of Food Science, Gyeongnam National University of Science and Technology, 2 Department of Central Area, Crop Science, National Institute of Crop Science (NICS), Rural Development Administration (RDA)

**I-27** Antioxidant Activities and Isoflavone Contents in the Produced *Cheonggukjang* with Different Soybean Cultivars by *Bacillus amyloliquefaciens* 9-3

Hee Yul Lee1, Chung Eun Hwang1, Min Ju Ahn1, Byong Won Lee2, Yu Young Lee2, Choonwo Lee2, Byung Joo Kim2, Ji Yong Park2, Eun Yeong Sim2, and Kye Man Cho1*

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**I-28** Incidence, Antibacterial Resistance, and Molecular Characteristics of Non-typhoidal *Salmonella* Including Extended-spectrum β-lactamase Producers in Chicken Carcasses

Soo-Kyoung Lee, Dasom Choi, Dong-Hyeon Kim, Hong-Seok Kim, Jin-Hyeok Yim, Young-Ji Kim, Il-Byeong Kang, Duna Jeong, Kun-Ho Seo*

KU Center for Food Safety, College of Veterinary Medicine, Konkuk University

**I-29** Enzyme IIαt post-translationally Regulates Propionate Metabolism in *Salmonella enterica* Serovar Typhimurium

Dajeong Kim1, Woongjae Yoo1, Bokyung Son1, Hyunjin Yoon2, and Sangryeol Ryu1*

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**I-30** Survival and their Inactivation Kinetics of *B. cereus* and *E. coli* in Simulated Gastric Fluid

Daekeun Hwang1,2, Seung Min Kim1 and Hyun Jung Kim1,2*

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**I-31** Enzyme Production by Fungal Isolates from the Korean Traditional Starter, Nuruk

Emily Carroll and Jeong-Ah Seo*

School of Systems Biomedical Sciences Soongsil University

**I-32** Progress in the Development of a Transgenic Mouse Model Susceptible to Human Norovirus Infection

Jinjong Myoung*

Korea Zoonosis Research Institute, Chonbuk National University

**I-33** Changes of Microbial Diversity, Free Amino Acids and Flavor Compounds During Fermentation of yeulmu-mulkimchi Made of Bitter Melon Ingredient

Min Ju Ahn, Chung Eun Hwang, Hee Yul Lee, and Kye Man Cho*

Department of Food Science, Gyeongnam National University of Science and Technology

**I-34** Antimicrobial Activities of Coagulase-negative Staphylococci Against *Staphylococcus aureus*

Moran Lee, Jonguk Kim, Jisoo Hong, Kyu Suk Jung, Sanghyun Han, Jinwoo Park, and Eunjung Roh*

Microbial Safety Team, National Institute of Agricultural Science, Rural Development Administration

**I-35** Inactivation of Murine Norovirus by Underwater Arc Discharge through Capsid and Particle Damages

Eun-Jung Lee1, Woosong Lee2, Jong-Won Oh2, and Yun-Ji Kim1*

1 Research Group of Food Safety, Korea Food Research Institute, 2 Department of Biotechnology, Yonsei University

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**www.fkms.kr | 61**
I-36 Fermentation Condition of Black Garlic Vinegar with an Addition of Apple Extract ........................................ 336
Sangman Ha, Hyejung Choi and Woobeng Joo*
Department of Biology and Chemistry, Changwon National University

I-37 Development of an In-frame Reporter System Measuring MARTXV M Production in Vibrio vulnificus ..................... 337
Chang Uk Choi, Su Jin Yum and Hee-Gon Jeeong*
Department of Food Science and Technology, College of Agriculture and Life Sciences, Changnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764

J-1 Characterization of Salmonella spp. Isolated in Patients with Acute Gastroenteritis in North Gyeonggi-do, Korea ................. 338
Bu Geon Lim*, Hae Geun Hong, Jong Seong Son, Min Jung Park, Hyeon Ho Lee, Gang Bum Lee, and Gu Hwan Kim
North Branch, Gyeonggi-do Institute of Health & Environment

J-2 Set1, the Methyltransferase for H3-K4, is a Negative Regulator of Morphogenesis in Candida albicans ..................... 338
Jeane Kim and Jung-Shin Lee*
Molecular Biochemistry Lab, Department of Molecular Bioscience, College of Biomedical Science, Kangwon National University, 1 Kangwondakchugil, Chuncheon-si, Gangwon-do, 200-701

J-3 Partial Purification and Analysis of Antimicrobial Fatty Acids from Allium Hookeri Root ........................................ 338
Nanhee Kwon1, Ducon Park2, Jungen Kim1, Minsuk Bae*, and Seungsik Cho*
1Department of Pharmacy, College of Pharmacy, Mokpo National University, Muan, Jeonnam 534729, 2Department of Environmental Engineering, Mokpo National University, Muan, Jeonnam 534729

J-4 Distribution of Pathogenic Filamentous Fungi Resources for Diagnostic Test and Research ........................................ 338
Mi Hee Kim, Kyeong Min Lee and Kyu Jam Hwang*
Pathogen Resource TF, Center for Infectious Diseases, National Institute of Health

J-5 Expression of Enterovirus71 Virus-like Particles as Vaccine Candidate ................................................................. 339
Hye-Jin Kim, Ho Sun Son, Jung-Ah Lee, Hyeon-Ji In, Heeji Lim, Jung Sik Yoo, Sang Won Lee, and June-Woo Lee*
Division of Vaccine Research, Center for Infectious Diseases, Korea National Institutes of Health, Korea Centers for Disease Control and Prevention

J-6 First Isolation of Taylorrella equigenitalis from Horses in Republic of Korea ............................................................. 339
Hye-Young Jeoung*, Ji-Hye Lee, Sung-Hee Kim, Jee-Yong Park, and Byounghan Kim
Animal and Plant Quarantine Agency

J-7 Fluid Mixing Control on Lab-on-a-Disc Using Coriolis Force .............................................................................. 339
MooJung Seo and JaeChern Yoo*
College of Information and Communication Engineering, Sungkyunkwan University

Suk-Hyun Yun1, Song Hee Lee2, Yo-Han Ko1, Hye-Young Jung1, Jung-Mi Kim2, and Dae-Hyuk Kim1*
1Department of Molecular Biology, Department of Bioactive material sciences, Institute for Molecular Biology and Genetics, Chonbuk National University, 2Department of Bio-Environmental Chemistry, Wonkwang University

J-9 Opuntia ficus-indica var. Saboten Mediates the Antidiabetic Activity in Pancreatic β cell ........................................... 340
Kang-Hyun Leem1, Myung-Gyou Kim*1 and Hye Kyung Kim2
1College of Korean Medicine, Semyung University, 2Dept. of Food & Biotechnology, Haeseo University

J-10 A New Record of Xylogone sphaerospora from Crop Field Soil in Korea .............................................................. 340
Hye-Seung Kim1, Yong Hyun Um1, Sang Woo Kim1, Dil Raj Yadav1,2, Mahesh Adhikari1,2, and Youn Su Lee*
1Division of biological Resources Sciences, Kangwon National University, Chuncheon 200-701, 2Nepal
Poster Session

J-11 Surface Display of Cyclodextrin Glycosyltransferase (CGTase) in Pichia pastoris using Glycosylphosphatidylinositol (GPI)-Anchored Protein as an Anchoring Motif ........................................ 340
Sungbi Kim, Daewoon Kim and Hyunsewhan Lee* Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Protein Research Center for Biotechnology, GRRC

J-12 Opuntia ficus-indica var. Saboten Enhances Glucose Uptake and Balances Adipogenesis and Lipolysis Properties ................................................................. 340
Hye Kyung Kim¹, Myung-Gyou Kim² and Kang-Hyun Leem⁴*
¹Dept. of Food & Biotechnology, Hanieo University, ²College of Korean Medicine, Semyung University

J-13 Korea National Microorganisms Research Resource Center ...................................................... 341
Se Joung Yeom and Sang Soob Lee* Kyonggi University, Research Center #201 San 94-6 Iui, Yeongtong, Suwon, Gyeonggi, 443-760

J-14 Investigation of Distribution Characteristics of Indoor Airborne Bacteria in Greenhouse for Oyster Mushroom Cultivation ......................................................... 341
Hye Soo Kim¹, Chul Hwan Kim², Chan-Jung Lee³, and Soo Jeong Cho⁴*
¹Department of Pharmaceutical Engineering, Gyeongsang National University of Science and Technology, ²Mushroom Research Division, NIHIS, RDA

J-15 Serological Surveillance of AHS, EIA and EVA in Korea in 2014 .................................................... 341
Sung-Hee Kim, Ji-Hye Lee, Jeong-Yong Park, Yong-Soo Kim, Byoung-Han Kim, and Hye-Young Jeoung Animal and Plant Quarantine Agency

J-16 The Effects of Laminarin, a Polysaccharide from Seaweed, on Cecal and Fecal Microbiota of High Fat-fed Mice ........................................................................ 341
Jungman Kim, Son G. Nguyen and Tatsuya Unno*
Faculty of Biotechnology, Jeju National University

J-17 Effect of Different Cultivars and Milling Degrees on Quality Characteristics of Barley Makgeolli ................................................................. 342
Hye-young Park¹*, Seok Tae Jeong², Induck Chol¹, Sei Kwan Oh¹, Koan Sik Woo¹, and Soon Duck Yoon¹
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J-18 Loop-mediated Isothermal Amplification (LAMP) Assays for Rapidly Detection of Pea Enation Mosaic Virus Associated with Leguminous Crop ........................................ 342
Jin-Young Lee¹, Jin-Ho Kim², Ji-Yeon Kim¹, Byeong-Hee Kim¹, Siwon Lee¹, and Jae-Young Rho¹*
¹Department of Microbiology, College of Natural Sciences, Dankook University, ²Department of Chemistry, College of Natural Sciences, Dankook University, ³Environmental Infrastructure Research Department, National Institute of Environmental Research

J-19 First Report of Aspergillus awamori as a Fungal Pathogen of Garlic (Allium sativum) .................. 342
Ji Yeon Oh¹, Gyeong Deok Han¹ and Ki Deok Kim²*
¹College of Life Sciences and Biotechnology, Konkuk University, ²College of Life Sciences and Biotechnology, Korea University

J-20 A Newly Isolated Bacteriophage PBES 02 Infecting Cronobacter sakazaki ................................. 342
Hyung Ju Lee¹, Wan II Kim¹, Young Chan Kwon¹, and Heejoon Myung⁴*
¹Hana High School, ²Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

J-21 Cultivational and Fruiting Characteristics of Monokaryotic Strains of Lentinula edodes ............... 343
Byeong-Suk Ha and Hyeon-Su Ro*
Division of Applied Life Science(BK21 plus) and Research Institute for Life Science, Gyeongsang National University

J-22 Seasonal Difference of Control Efficacy of Entomopathogenic Fungus Isaria javanica Against Sweet Potato Whitefly with in Greenhouse ........................................... 343
Jin Ju Yoon, Jung Haew Hwang, Byung Ju Lee, Ji Hee Han, Sang Yeob Lee, and Jeong Jun Kim*
Agricultural Microbiology Division, National Academy of Agricultural Science, RDA, Jeonju, 565-851

J-23 Bactericidal Effect of Plasma Discharged from Tap Water ....................................................... 343
EE Yoon¹, Se Chul Chun⁴*, Kyung Yong Lee⁴, and Min Yeon Lee⁵
¹College of Life and Environmental Sciences, Konkuk University, ⁴C Gate Inc. 14, Gwan digital 2-ro, Geumcheon-gu, Seoul
Poster Session

J-24 Aphidial Effect of Culture Fraction of Entomopathogenic Fungi Beauveria bassiana .......................... 343
Ga Yung Jeong, Jin Ju Yoon, Ling Xie, Jeong Jun Kim*, Ji Hee Han, and Sang Yeob Lee
Agricultural Microbiology Division, National Academy of Agricultural Science, RDA, Jeonju, 565-851

J-25 Antagonistic Action of Microbes Against Fusarium spp. ................................................................. 344
Dami Park, Hyoenheui Ham, Soohyang Lee, Sung Kee Hong, Jae-Gee Ryu, and Theresa Lee*
Microbial Safety Team, National Academy of Agricultural Science

J-26 Niveibacterium glucosivorans gen. nov., sp. nov., Isolated from Wetland ........................................ 344
Jeesun Chun1, Ji Young Kang1,2, Mi Jung Kim1 and Kwang Yeop Jahng1, 3*
1Department of Life Sciences, Chonbuk National University, 2Applied Microbiology Research Center, Bio-Materials Research Institute, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 3Institute for Molecular Biology and Genetics, Chonbuk National University

J-27

J-28 Autoinducer-2 Quorum Sensing of Probiotic Lactobacillus spp. ....................................................... 344
Hyanjoon Park1, 2*, Kyueon Lee3, Hae-Yun Kil Shin2, and Wilhelm Holzapfel1
1Department of Advanced Green Energy and Environment, Handong Global University, 2Department of Life Science, Handong Global University

J-29 The Relationship Between Commensal Microbes and Gut Homeostasis on Lifespan of Drosophila ........ 345
Hye-Yeon Lee, Shin-Hae Lee, Woong Seo, and Kyung-Jin Min*
Department of Biological Sciences, Inha University

J-30 Effect of Various Protectants on Survivability During Freeze-drying Process of Neisseria gonorrhoeae and Streptococcus pneumoniae Isolates ................................................................. 345
Young Kyoung Park, Kyeong Min Lee and Kyu Jam Hwang*
Pathogen Resource TF, Center for Infectious Diseases, National Institute of Health

J-31 Multiplex PCR Method for Specific Detection of Ralstonia solanacearum Race 3 Biovar 2 Strains ............ 345
Mi An, Yangho Jang, Jeongwoo Kang, Hae Chul Park, and Kwang Jick Lee*
Animal and Plant Quarantine Agency

J-32 Combination Effects of Organic Materials and Bacillus thuringiensis on Spodoptera exigua .................. 345
Ji Hee Han*, Jihye Yoon, Sujin Son, Jeong Jun Kim, and Sang Yeob Lee
Agricultural Microbiology Division, National Academy of Agricultural Science, RDA

J-33 Marine Fungal Resource Bank ........................................................................................................... 346
Jae Young Park, Myung Soo Park, Ji Eun Eom, and Young Woon Lim*
Seoul National University

J-34 First Report of Brown Rot Caused by Cryptococcus pseudolongus on Fruit Body of Lentinula edodes (shiitake) in Korea .......................................................... 346
Hyukwoo Kwon1, Yeongho Yun2, Hangyu Ko2, and Seonghwan Kim3*
1Department of Microbiology, Dankook University, Cheonan, Chungnam, 373-714, 2Department of Microbiology, Dankook University, Cheonan, Chungnam 330-714, 3Mushroom Research Center, National Forestry Cooperative Federation, Yeosu 469-803

J-35 The Role of DesB on Virulence Traits in Pseudomonas aeruginosa ................................................... 346
Sejeong Kim1, Jinmyeong Ha1, Yohan Yoon1, and Kyoung-Hee Choi1*
1Department of Food and Nutrition, Sooskyung Women, 2Department of Oral Microbiology, Wonkwang University

J-36 The Effect of Chemical Disinfectants on Bacillus spp. from Activated Sludge in Slaughterhouse .............. 346
Mi An, Yangho Jung, Jeongwoo Kang, Hae Chul Park, Jaeyoung Song, and Kwang Jick Lee*
Animal and Plant Quarantine Agency
International Meeting of the Federation of Korean Microbiological Societies

Poster Session

**J-37** Evaluation of the Anti-inflammatory Effect of Ginsenosides from Black Ginseng Extract Against *Propionibacterium acnes* .......................... 347

Seonjeong Maeng, Byungwook Yang and Youngtae Hahm*
Department of Systems Biotechnology, Chung-Ang University

**J-38** Biological Control of *Fusarium oxysporum* of Lettuce by *Trichoderma* sp. ......................................................... 347

Myoungjun Jang1, Changho Kim1, Yongkoo Cho1, Seongmin Kim1, Dongil Shin1, Hyunjoo Lee2, Youngsu Lee2, and Taeseok Oh2*
1Department of Plant Resources, Kongju National University, 2Environmental Agriculture Research Division, Gyeonggido Agricultural Research & Extension Services

**J-39** Unreported Fungal Species of *Didymosphaeriaceae* from Soil in Korea .......................................................... 347

Mahesh Adhikari1,2,3, Sang Woo Kim1,2, Dil Raj Yadav1,2,3, Yong Hyun U1,2, Hyun Seung Kim1,2, and Youn Su Lee2,4*
1Division of Biological Resources Sciences, Kangwon National University, Chuncheon 200-701, 2Nepal, 3Division of Biological Resources Sciences, 4Division of Biological Resources Sciences

**J-40** A Serological Surveillance of West Nile and Japanese Encephalitis Virus in Horses from South Korea in 2014 ........................... 347

Jihye Lee, Sunghee Kim, Jeeyong Park, Yongjoo Kim, Byounghan Kim, and Hyeyoung Jeoung*
Animal and Plant Quarantine Agency

**J-41** Optimum Conditions for Hot Water Extraction from *Opuntia ficus-indica* var. Saboten Using Response Surface Methodology .................................................. 348

Byung Wook Yang1, Seon Jeong Maeng1, Seung Il Ahn 1, Sae Kyul Kim 1, Jae Hee Choi1, Heejung Jung2, Yejin Choi2, Byung Yong Kim2, and Young Tae Hahm1*
1Department of Systems Biotechnology, Chung-Ang University, 2Department of Food Science and Biotechnology, Kyung Hee University

**J-42** Optimum Condition of Demucilage Process for *Opuntia ficus-indica* var. Saboten (Pad) with Enzyme Treatment .......................... 348

Byung Wook Yang1, Seon Jeong Maeng1, Seung Il Ahn1, Sae Kyul Kim1, Jae Hee Choi1, Heejung Jung2, Yejin Choi2, Byung Yong Kim2, and Young Tae Hahm1*
1Department of Systems Biotechnology, Chung-Ang University, 2Department of Food Science and Biotechnology, Kyung Hee University

**J-43** Development of Real-time RT-PCR to Differentiate Rift Valley Fever Virus from Clone 13 Vaccine ............................ 348

Hyun-Joo Kim1*, Hye-Rhyoung Lyoo1, Sung-Kyu Kim1, Jeong-Soo Choi1, Yoon-Hee Lee1, and Byoung Han Kim2*
1Foreign Animal Disease Division, Animal and Plants Quarantine Agency, 2Foreign Animal Disease Division, Animal and Plants Quarantine Agency

**J-44** Investigation of Methane Emission Mechanisms from Rice Based on Comparative Paddy Soil Metagenomics ........................ 348

Son G. Nguyen, Jungman Kim and Tatsuya Unno*
Faculty of Biotechnology, Jeju National University

**J-45** Center for Fungal Genetic Resources (CFGR): Housing Plant Pathogenic Fungi for Educational and Research Purposes ............................... 349

Yee Kyoung Yoon and Yong-Hwan Lee*
Center for Fungal Genetic Resources, Seoul National University, Seoul 151-921

**J-46** Korean Metagenome Bank for Exploiting Microbial Diversity .......................................................... 349

Jung-Hoon Yoon*
Department of Food Science and Biotechnology, Sungkyunkwan University

**J-47** Microbial Carbohydrate Resource Bank (MCRB) ......................................................... 349

Seunho Jung*
Department of Bioscience and Biotechnology, Microbial Carbohydrate Resource Bank(MCRB) & Center for Biotechnology Research in UBITA (CBRU), Konkuk University, 1 Hwayangdong, Gwangjin-gu, Seoul 143-701

**J-48** Bacteriophage Bank .......................................................... 349

KyoungEun Cha and Heejoon Myung*
Dept. of Bioscience and Biotechnology, Hankuk University of Foreign Studies

www.fkms.kr | 65
## Poster Session

<table>
<thead>
<tr>
<th>Poster</th>
<th>Title</th>
<th>Authors</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>J-49</td>
<td>Korea Mushroom Resource Bank</td>
<td>Jae Young Park, Nam Kye Kim, Hey Young Choi, Mi Jin So, and Young Woon Lim*</td>
<td>Seoul National University, 1 Gwang-wo, Gwanak-gu, Seoul 08826</td>
</tr>
<tr>
<td>J-50</td>
<td>Korea Bank for Pathogenic Viruses</td>
<td>Ki-Joon Song*</td>
<td>Department of Microbiology College of Medicine, Korea University 126-1, Anam-dong, 5-ga, Seongbuk-gu, Seoul 136-705</td>
</tr>
<tr>
<td>J-51</td>
<td>Plant Virus GenBank</td>
<td>Ki Hyeon Ryu</td>
<td>Dept. of Horticulture, Biotechnology and Landscape Architecture</td>
</tr>
<tr>
<td>J-52</td>
<td>Lichen as a Novel Bioresources in Korea</td>
<td>Jae-Seoun Hur* and Young Jin Koh</td>
<td>Korean Lichen Research Institute, Sunchon National University, sunchon, 540-742</td>
</tr>
<tr>
<td>J-53</td>
<td>Korean Collection for Oral Microbiology</td>
<td>Joong-Ki Kook*, Soon-Nang Park, Eojin Jo</td>
<td>Department of Oral Biochemistry, School of Dentistry, Chosun University, Sunchon-dong, Dong-gu, Gwangju 501-759</td>
</tr>
<tr>
<td>J-54</td>
<td>Culture Collection of Antimicrobial Resistant Microbes</td>
<td>Eunju Shin*, Hyunjin Hong, Hakmi Lee, Minyoung Lee, and Yeonhee Lee</td>
<td>Culture Collection of Antimicrobial Resistant Microbes, Department of Biology, Seoul Women’s University</td>
</tr>
<tr>
<td>J-55</td>
<td>Korea Environmental Microorganisms Bank</td>
<td>Yong Jin Kim and Sang Seob Lee*</td>
<td>#103 Research Center, Kyonggi University, 154-42 Gwanggyo-dong-gu, Sinoon-ci, Gyeonggi-do 443-760</td>
</tr>
<tr>
<td>J-56</td>
<td>Biocontrol of Lettuce Sclerotinia Rot Using <em>Trichoderma virens</em> and <em>Trichoderma harzianum</em></td>
<td>Sang Yeob Lee*, Jeong Jun Kim, and Jie Hee Han</td>
<td>Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Wanju 55365</td>
</tr>
<tr>
<td>J-57</td>
<td>Antifungal Activity of Fungal Pathogens of Ginseng by <em>Bacillus amyloliquefaciens</em></td>
<td>Sang Yeob Lee*, Jeong Jun Kim, and Dayeon Kim</td>
<td>Agricultural Microbiology Division, National Academy of Agricultural Science</td>
</tr>
<tr>
<td>J-58</td>
<td>Control Effect of Tomato Bacterial Wilt Caused by <em>Ralstonia solanacearum</em> Using Water Extract of Spent Mushroom Media of <em>Hericium erinaceus</em></td>
<td>Sang Yeob Lee*, Jeong Jun Kim, and Jie Hee Han</td>
<td>Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Wanju 55365</td>
</tr>
<tr>
<td>J-59</td>
<td>Salicylic Acid Reduces OmpF Expression to Become <em>Salmonella Typhimurium</em> More Resistant against <em>Cephalosporins</em></td>
<td>Mi Hyun Kim, Sukho Park, Yeongjin Hong, and Phil Youl Ryu*</td>
<td>Department of Microbiology, Chonnam National University Medical School, Gwangju</td>
</tr>
<tr>
<td>J-60</td>
<td>Occurrence of <em>Aspergillus/Penicillium</em> Species in Korean Cereals</td>
<td>SoSoo Kim, Ji-Soon Park, Hyeonhui Ham, Soohyung Lee, Sung Kee Hong, Ji-Gee Ryu, and Theresa Lee*</td>
<td>Microbial Safety Team, National Academy of Agricultural Science</td>
</tr>
<tr>
<td>J-61</td>
<td>Quantitative Analysis of Activated Coagulation Factor XI as an Impurity in Human Plasma-derived IVIG Products</td>
<td>Hokyung Oh, Kikyung Jung, Sang-Mi Park, Yong Seok Kang, Ji-Hye Kim, Garam Min, Kiwon Han, Sung Hwan Han, Hoon Song, Jiyoung Lee, Soon Hwa Seong, So Young Han, and Chiyoung Ahn</td>
<td>Blood Products Division, National Institute of Food &amp; Drug Safety Evaluation, Ministry of Food &amp; Drug Safety</td>
</tr>
<tr>
<td>J-62</td>
<td>Different Pathogenicity of Entomopathogenic Fungi Cultured in Different Media to Control Aphid</td>
<td>Ling Xie, Jin Ju Yoon, Jeong Jun Kim*, Ji Hee Han, and Sang Yeob Lee</td>
<td>Agricultural Microbiology Division, National Academy of Agricultural Science, RDA, Jeonju, 565-851</td>
</tr>
</tbody>
</table>
**Poster Session**

**J-63** Development of Peptide Nucleic Acid Multi-probe- real-time PCR Method Targeting hsp65 Gene for Identification Between Mycobacterium abscessus Strains

Byoung-Jun Kim, Seok-Hyun Hong, Bo-Ram Kim, So-Young Lee, Jeong-Ryeol Gong, Ga-Na Kim, Yoon-Hoh Kook, and Bum-Joon Kim*

Department of Microbiology and Immunology, Seoul National University College of Medicine

**J-64** An Analysis and Study of Domestic and Foreign Cases by Institutional Biosafety Committee

Jaeyeong Song, Kyunghwa Choi, Seungchul Shin, Seungpyo Hong, and Younghae Roh*

National Research Safety Headquarters, Korea Research Institute of Bioscience & Biotechnology

**J-65** Antibacterial Activity of Essential Oils against Acidovoraxavenae subsp. citrulli, causing Bacterial Fruit blotch (BFB) of Cucurbit Plants

Sukyung Cho', Okhee Choi', Jaeyeong Cho', Byeoung-Sam Kang', and Jinwoo Kim*1,2

1Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 53282, 2Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 53282

**J-66** Intestinal Microbes Affect the Lifespan Extension of Dietary Restriction in Drosophila melanogaster

Ah Yoon, Shin-Hae Lee, Hye-Yeon Lee, and Kyung-Jin Min*

Department of Biological Sciences, Inha University

**J-67** DNA Delivery and Immunogenicity of VLP Forming Baculoviral Vaccine against Influenza pdmH1N1

Yong-Dae Gwon, Yoonki Heo, Hansam Cho, Yeondong Cho, Ki Hoon Park, Yuyeon Jang, Hanul Choi, Sei Hyun Kim, Jong Kwang Yoon, and Young Bong Kim*

Department of Bio-industrial Technologies, Konkuk University

**J-68** Baculovirus Based DNA Vaccine against Porcine Reproductive and Respiratory Syndrome Virus Vaccine

Yeondong Cho1, Yoonki Heo1, Hee-Jung Lee1, Kang Chang Kim2, Sehyun Kim3, Jaeyeong Song4, Hyuck-Se Kwon5, Mi Kyoun Won6, Jong-Bok Lee7, and Young Bong Kim*8

1Department of Bio-industrial Technologies, Konkuk University, 2KBNP Technology Institute, KBNPhc., Yeosu, 3Department of Infectious Diseases, College of Veterinary Medicine, KonKuk University

**J-69** Bacillus subtilis HS01 Producing Cellulase and Xylanase, Isolated from Spent Mushroom (Pleurotus ostreatus) Substrates

Hye Soo Kim1, Chul Hwan Kim2, Hong Chul Kim3, Chan-Jung Lee2, and Soo Jeong Cho1*

1Department of Pharmaceutical Engineering, Gyeongsan National University of Science and Technology, 2Department of Bioindustrial Technologies, KonKuk University

**J-70** Evaluation of Commercial Probiotics for Animal from Korea Using a Barcoded Pyrosequencing

Hyo Jung Lee, Sang Eun Jeong and Che Ok Jeon*

Department of Life Science, Chung-Jong University, Seoul, 156-756

**J-71** Pedobacter humicola sp. nov., isolated from Soil of Hwaseong in South Korea

Ram Hari Dahal and Jaisoo Kim*

Department of Life Science, Kyonggi University

**J-72** Pedobacter humicola sp. nov., isolated from Soil of Hwaseong in South Korea

Ram Hari Dahal and Jaisoo Kim*

Department of Life Science, Kyonggi University
<table>
<thead>
<tr>
<th>Poster Session</th>
</tr>
</thead>
</table>

| J-74 | Variorax soli sp. nov., Isolated from Forestry Soil Using Modified Uncultured Method | Tuan Manh Nguyen and Jaisoo Kim* | Department of Life Science, Kyunggi University | 356 |

| J-75 | Development of Nested PCR Assay for Detection of Mycosphaerella nawae in Korea | Yang-Sook Lim1, Seung-Yeol Lee2, Sang-Hwa Lee2, Sangkyu Park2, and Hee-Young Jung* | 1Persimmon Experiment Station, Gyeongsangbuk-do Agricultural Research & Extension Services, Sangju 37268, 2College of Agricultural and Life Sciences, Kyungpook National University, Daegu 41566 | 356 |

| J-76 | Diagnosis of Various Types of Symptoms on Apple in Korea | Han-Dong Lee, Jin-Ho Keum, Sangkyu Park, In-Kyu Kang, Seung-Yeol Lee, and Hee-Young Jung* | College of Agricultural and Life Sciences, Kyungpook National University, Daegu 41566 | 356 |

| J-77 | Degradation of Biogenic Amine by Lactobacillus arizonensis in Food | Bo Ram Lim, Hye Jung Choi and Woo Hong Joo* | Department of Biology and Chemistry, Changwon National University, Changwon 51140 | 357 |

| J-78 | Degradation of Biogenic Amines by Staphylococcus equorum Isolate from Jeotgal | Bo Ram Lim, Hye Jung Choi and Woo Hong Joo* | Department of Biology and Chemistry, Changwon National University, Changwon 51140 | 357 |

| J-79 | Bacillus subtilis BCNU 1330 Strain and its Use in Biogenic Amine Reduction | Bo Ram Lim, Hye Jung Choi and Woo Hong Joo* | Department of Biology and Chemistry, Changwon National University, Changwon 51140 | 357 |

| J-80 | Reduction of Biogenic Amine by Ocenobacillus Sojae Isolate from Jeotgal | Bo Ram Lim, Hye Jung Choi and Woo Hong Joo* | Department of Biology and Chemistry, Changwon National University, Changwon 51140 | 357 |

| J-81 | Tyrosinase Inhibitory and Antioxidant Activities of Cryptococcus albidusimilis BCNU3010 Isolated from Persimmon | Sung Min Ha, Hye Jung Choi and Woo Hong Joo* | Department of Biology and Chemistry, Changwon National University, Changwon 51140 | 358 |

| J-82 | Tyrosinase Inhibitory and Antioxidant Activities of Rhodotorula glutinis BCNU3009 Isolated from Strawberry | Sung Min Ha, Hye Jung Choi and Woo Hong Joo* | Department of Biology and Chemistry, Changwon National University, Changwon 51140 | 358 |

| J-83 | Evolution of CTX Phages of Vibrio cholerae | Eun Jin Kim, Hyun Jin Yu, Sungryeol Choi, and Dong Wook Kim* | Department of Pharmacy, College of Pharmacy, Hanyang University, Ansan 426-791, Institute of Pharmacological Research, Hanyang University, Ansan 426-791 | 358 |

| J-84 | Wild Mushroom Diversity in Dongbaekdongsan in Jeju Island | Geum Ran Ahn1, Han Ah Cho1, Hye In Cho1, Yeo Hong Yun1, Soon Ja Seok2, Pyung Yeol Ko3, and Seong Hwan Kim* | 1Department of Microbiology and Institute of Basic Sciences, Dankook University, Cheonan, 31116, 2Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Wanju, 55365, 3Faculty of Bioscience and Industry, Jeju National University, Jeju, 63243 | 358 |

| J-85 | Prevalence, Biochemical Properties, and Toxin Characterization of Clostridium perfringens Isolated from Beef in Meat Retailer Differing in Processing Temperature | Dana Jeong, Dong-Hyeon Kim, Il-Byeong Kang, Hong-Seok Kim, Jin-Hyeok Yim, Soo-Kyoung Lee, Young-Ji Kim, and Kun-Ho Seo* | KU Center for Food Safety, College of Veterinary Medicine, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 143-701 | 359 |
Recognition of Mycobacterial Adjuvants through C-type Lectin Receptors

Sho Yamasaki
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C-type lectin receptors (CLRs) comprise a large family of proteins that share a common structural motif and are involved in various immune responses. Among them, ITAM-coupled CLRs are recently identified as pattern recognition receptors (PRRs) for pathogens. We found that macrophage-inducible C-type lectin (Mincle/Clec4e) is an FcRγ-coupled activating receptor for Mycobacterium tuberculosis. A widely-known mycobacterial adjuvant, cord factor (also called trehalose-6,6′-dimycolate, TDM), was identified as a Mincle ligand. TDM induced potent adjuvant activity in vivo, whereas it was abolished in Mincle-deficient mice. We found that another CLR, MCL (Clec4d), also recognizes mycobacterial cord factor and promotes Mincle expression. Indeed, innate and acquired immune responses induced by TDM were abrogated in both Mincle- and MCL-deficient mice. Furthermore, host immune responses during mycobacterial infection were ameliorated in the absence of Mincle and MCL. We recently found that another CLR Dectin-2 (Clec4n) also recognizes mycobacteria through a hydrophilic lipoglycan, lipoalabinomannan (LAM). This interaction triggered unique adjuvant activities that are distinct from those induced by TDM-Mincle axis. Thus, these three CLRs clustered on the same chromosome are found to be activating receptors for mycobacteria. In addition to mycobacteria, Mincle also recognizes pathogenic fungi and gram-negative bacteria that lack TDM. Instead of TDM, several novel glycolipids were identified as Mincle ligands from these pathogens. These ligands possessed adjuvant activity in mice, as the injection of the purified ligands augmented T cell responses. These findings shed light on CLRs as emerging immune receptor family for wide spectrum of pathogens, and thus CLRs could be potential targets for the development of novel adjuvant.
Undernourished Children and the Gut Microbiome

G. Balakrish Nair

Centre for Human Microbial Ecology, Translational Health Science and Technology Institute, Faridabad, India

Undernutrition is defined as the outcome of inadequate dietary intake. The onset and progression of undernutrition has been attributed to a variety of causal factors, which includes insufficient food, impaired immune responses, repeated enteric infections, dysregulated gut permeability all of which culminate in mal-absorption. Each of the conditions in the vicious cycle of undernutrition worsens and perpetuates each other. The gut microbiota as a link between environmental factors such as diet and the host has been largely ignored. However recent studies involving a large cohort of twin pairs from Malawi, a resource poor country and also studies conducted in Bangladesh support a relationship between diet, gut microbiota and health and/or nutritional status and is also involved in severe acute malnutrition.

A recent study conducted in a rural setting in West Bengal in India examined the metagenomes of 20 children with varying nutritional status. This study represents the first investigation of its kind in India. Across the 20 microbiomes, 72%, 36.2% and 4.2% of the sequences could be assigned at the phylum, genera and species levels, respectively. Eight phyla and a core set of 23 genera belonging to four phyla were identified. Differential abundances were observed across samples with varying nutritional status with positive/negative associations of specific taxonomic and functional group. Unexpectedly, the probiotic genera like Lactobacillus and Bifidobacterium were not observed to have any significant correlation with nutritional indices. Several functional categories associated with nutrient uptake and metabolism was observed to be over-represented in the positively correlated Clusters of Orthologous Groups. The results of the analysis indicated that none of the Carbohydrate active enzyme families were significantly correlated with the cumulative nutritional index. However, when CAZyme families having similar abundance patterns were grouped together, positive correlation with one of the group and nutritional was discerned. Distinct changes in genera co-occurrence networks with progressive decrease in the nutritional status of the children were another key finding.

The Indian study describes patterns wherein the gut microbiome varies in response to nutritional status in a manner that nutritional deprivation leads towards “a disease promoting microbiota.” The biological basis of such changes and the mechanisms governing the occurrence of these patterns remain to be experimentally verified. Further studies are required to formulate a microbial basis of therapy for undernourished children.
Emerging Roles for Noncoding RNAs in Virus-Host Interactions

SungChul Kim, Sanghyun Lee, and Kwangseog Ahn

School of Biological Sciences, Seoul National University, Center for RNA Research, Institute for Basic Science (IBS), Republic of Korea

The discoveries of viral and cellular factors mediating virus-host interactions have allowed us to uncover the key molecular mechanisms of viral infection and escape from host immune responses. The primary focus of intense investigation has been identifying novel protein factors that could provide insights into the virus-host interplay. Most viruses have evolved diverse strategies to maximize their coding capacity in a compact genome size. For example, introns do not exist in viral genome. Many viral genes are transcribed as a polycistron. The open reading frames in a polycistron use a common promoter element or poly-adenylation signal. Human cytomegalovirus (HCMV) is predicted to contain over 250 open reading frames. Surprisingly, almost half of the transcribed RNA represents noncoding RNAs, which include at least 22 miRNAs and 4 long noncoding RNAs (lncRNAs) whose functions are, as yet, largely unknown, raising a question about the functional roles of these noncoding RNAs. Our recent studies revealed that HCMV-encoded miRNAs modulate the expression of various host genes to evade host-immune responses. We also observed that some of HCMV IncRNAs are essential for viral replication. Intriguingly, our data showed that intergenic RNA sequences conserved in all clinical HCMV isolates constitute a microRNA decay element (miRDE), which directs the selective turnover of mature miR-17 and miR-20a within the miR-17-92 cluster in a sequence-specific manner, resulting in accelerated virus production. These data suggest that HCMV miRNAs, lincRNAs, and even intergenic genome can contribute to the repertoire of host-pathogen interactions during viral infection. To better understand the complex virus-host interaction networks, we applied an AGO-CLIPseq-based approach to characterize the global miRNA targetome during productive HCMV infection. We also developed a bioinformatic quantitation method, ACE-scoring, to accurately identify miRNA target sites and calculate the targeting efficacy of miRNA-target interactions. We collectively discovered about 3,900 canonical human mRNA targets of HCMV miRNAs and validated the functionality of miRNA-target interactions and their correlation with calculated ACE-scores. We observed and verified various genes in cellular pathways known to be important for HCMV infection progression. Our quantitative temporal data on miRNA targetomes provide a key foothold for the study of HCMV virology, facilitating the detailed analysis of and further insight into viral pathogenesis along with the development of an effective, durable and nontoxic RNA-based antiviral therapy.
Quantitative Viral Genetics at Single Nucleotide Resolution

Ren Sun

Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, USA

Traditionally, forward genetics selects individual mutants with a particular phenotype, then identify the genetic locus responsible for the phenotype; while reserve genetics. Genetics has been a major driving force of discoveries in biomedical science, however the genetic approach usually identifies the linkage of one phenotype with one mutation, thus limiting the throughput. To improve the output and resolution of genetics, Dr. Sun has developed a quantitative and high-resolution genetics platform that provides functional genomic maps at single nucleotide resolution and at the entire viral genome scale. First a large library of mutant viruses is generated. The phenotype of each mutant in the library is quantitatively measured using error-correction high throughput sequencing. He has developed and demonstrated the method with influenza virus, HIV and HCV. The technology will significantly accelerate virologic studies, including systematic characterization of viral sequences essential for each step of viral replication and virus-host interactions, such as interactions with interferon responses. The information about the function of each amino acid, in combination with the corresponding 3D structures of the viral protein, will provide critical information about potential drug targets and epitopes for vaccine development.
Symposium

S1 Marine Microbial Genomics and Ecology
Metagenomic Analysis of Marine RNA Virioplankton in the Vicinity of the Antarctic Peninsula

Chung Yeon Hwang1*, Jaclyn A. Mueller2, and Grieg F. Steward2

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2Department of Oceanography, University of Hawaii, USA

Viruses play an important role in the biogeochemical cycle and the gene flux in marine plankton ecosystems. Multiple lines of evidence that RNA viruses comprise a significant fraction of virioplankton are emerging. However, oceanic RNA viromes in the Antarctic seas are limited. During the IBRV Araon cruise in 2013, each 50 L of surface seawaters at 4 offshore stations was collected and filtered to concentrate viruses by the FeCl3 flocculation method. Marine viruses were recovered and purified by the CsCl gradient ultracentrifugation. Nucleic acids of viral fractions were extracted and subsequently DNAs were removed by adding DNase. Metagenome libraries were generated from RNAs using the random priming-mediated sequence-independent single-primer amplification. Size ranges of 300-600 bp in the libraries were selected and sequenced by 454 pyrosequencing with the GS-FLX Titanium chemistry, yielding 333 Mb with 764,764 reads as raw data in total. In the presentation, results and its implications will be discussed.
Metabolic Collaboration of Thermophilic Microorganisms,
Evidence by Metabolic and Genomic Analysis

KaeKyoung Kwon1,2*, Kyung Mo Kim3,4, Sang-Heon Lee3,4, Sung-Hyun Yang1, Ji Hye Oh1, and Jung-Hyun Lee1,2

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Marine environments are one of the major sources of diverse microorganisms. Among them, thermophilic microorganisms attracted due by its potential on industrial application. The main sources of thermophilic microorganisms are hot springs, geothermal area, hydrothermal vents, etc. Moderate thermophiles or thermotolerant microorganisms has been reported from near shore or deep sediments. Total 12 strains affiliated with three genera were isolated from enriched culture broth with damaged algae at 50°C. Amongst only 1 strain affiliated with Microbulbifer thermotolerans MEBiC11484 could degrade kelp flakes into powder form, however, only small amounts of sugar was detected. When assayed with DNS method, strain MEBiC11484 could utilize laminarin, cellobiose, and xylan but the rate of cellobiose degradation was very low. Caenispirillum salinarum MEBiC11482, an another isolate, utilize cellobiose and laminarin very well. In case of Bacillus aeolius MEBiC11481 beta-glucosidase activity was very strong. Xylanase activity was boosted when strains were mixed. Optical changes were progressed during cultivation with chitin and agar with strain MEBiC11484 but the sugar production was not observed. Two set of alginate lyase, beta-agarase subunit a and b, a chitinase, beta-glucosidase, and endo-beta-xylanase genes were pedicted from genome of strain MEBiC11484. However, strain 11482 possess different kinds of glycosyl hydrolases. Genomic analysis showed that starch and glycerophospholipid metabolic pathways were completed by combination of 3 strains. Further physiological and genomic analyses will conducted.

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Meta-Omics Analysis of Bacterioplankton Community Involved in Carbon Remineralization in a Polynya of Amundsen Sea, Western Antarctica

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Polynyas, areas of open water surrounded by sea ice, are sites of intense primary production and ecological hotspots in the Antarctic Ocean. During multi-year expeditions, changes in microbial community were observed between peak and late phase in the phytoplankton bloom. Although Gammaproteobacteria, Alphaproteobacteria, and Bacteroidetes were dominant, significant differences were revealed at genus-level. Glaciecola and uncultured Flavobacteriales constituted high proportions in the marine bacterioplankton at the peak of the bloom. In the late phase of the bloom, Polaribacter, gammaproteobacterium Ant4D3, SAR92, and Methylophaga were major constitutions. These bacterioplankton responses to phytoplankton bloom in Amensen Sea were studied using metagenomic and metatranscriptomic analysis. This results provide new insights into the roles of bacterioplankton in biogeochemical cycles in high-latitude polynyas.
Genome-Wide Transcriptional Responses of *Alteromonas* sp. SN2 to Contaminated Seawater and Marine Tidal Flat Sediment

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*Alteromonas* sp. SN2, isolated from crude oil-contaminated marine tidal sediment, is a copiotrophic marine bacterium with the ability to degrade polycyclic aromatic hydrocarbons (PAHs) effectively in its habitats. Here, a comprehensive analysis of genome-wide gene expression was performed to investigate the ecophysiological properties and environmental behavior of strain SN2 in marine tidal flats and seawater. Four environmental mimic conditions, consisting of tidal flat-naphthalene (TF-N), tidal flat-pyruvate (TF-P), seawater-naphthalene (SW-N), and seawater-pyruvate (SW-P) were prepared and the genome-wide transcription of strain SN2 was comprehensively and quantitatively assessed using an Illumina mRNA sequencing approach. The transcriptional profiles clustered by habitat (TF-N/TF-P and SW-N/SW-P), rather than carbon source, suggesting that the former may exert a greater influence on genome-wide expression in strain SN2 than the latter. Metabolic mapping of cDNA reads from strain SN2 based on KEGG pathway showed that metabolic and regulatory genes associated with energy metabolism, translation, and cell motility were highly expressed in all four test conditions, probably highlighting the copiotrophic properties of strain SN2 as an opportunistic marine *r*-strategist. Differential gene expression analysis revealed that strain SN2 effected specific cellular responses to environmental variables (tidal flat, seawater, naphthalene, and pyruvate) and exhibited certain ecological fitness traits — its notable PAH degradation capability in seasonally cold tidal flat might be reflected in elevated expression of stress response and chaperone proteins, while fast growth in nitrogen-deficient and aerobic seawater probably correlated with high expression of glutamine synthetase, enzymes utilizing nitrite/nitrate, and those involved in the removal of reactive oxygen species.
Symposium

S2 Ecology of Soil Microorganisms

International Meeting of the Federation of Korean Microbiological Societies
Microbiome Analysis of Tomato Rhizosphere to Enhance Plant Growth and Health

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Plant disease development is significantly influenced by environmental factors, in addition to the genetic factors of a plant host and a pathogen. Among them, microbiota that inhabits plant rhizosphere may also contribute to disease development and plant resistance. Bacterial wilt caused by \textit{Ralstonia solanacearum} is significantly affected by soil conditions near plant rhizosphere, probably by microbiota. In this study, we investigated the microbial community structure of tomato rhizosphere using two different tomato cultivars, Hawaii 7996 as a bacterial wilt resistant cultivar and Moneymaker as a bacterial wilt susceptible cultivar. Microbial communities of rhizosphere of field-grown tomato cultivars were analyzed using amplified 16S rRNA genes and whole metagenome sequences. Taxonomic comparison of the amplified 16S rRNA sequences or recruited 16S rRNA genes from whole metagenome revealed that relative abundance of the class \textit{Flavobacteriia} was higher in the rhizosphere of Hawaii 7996 than that of Moneymaker. On the other hand, relative abundance of \textit{Bacilli} and \textit{Betaproteobacteria} were higher in the rhizosphere of Moneymaker. Comparison of gene contents, \textit{de novo} assembly and gene prediction were conducted with whole metagenome data. When the assembled scaffolds were sorted out according to the bacterial phyla, scaffolds assigned to \textit{Bacteroidetes} had higher fold-coverage in Hawaii 7996 than Moneymaker. Furthermore, the genome of an unclassified \textit{Flavobacteriaceae} bacterium was discovered with a higher proportion of genes related to the specific sugar metabolism. Microbial communities of tomato rhizosphere revealed that rhizosphere microbiome between Hawaii 7996 and Moneymaker was distinguishable and disruption of microbiota by switching host plants resulted in the altered disease progress in tomato cultivars, suggesting that microbiome function in the plant rhizosphere may play a pivotal role in controlling the incidence or severity of bacterial wilt. In addition, several bacterial isolates of the class \textit{Flavobacteriaceae} were selected from tomato rhizosphere with remarkable activity for plant growth promotion.
Volcanic Jeju Island: The Hidden Jewel of Soil Microbial Ecology

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On the Jeju Island in Korea, the word “Gotjawal” refers to any naturally formed that grows on basalt-flow rocky terrain and presents a virtually impassable mixture of trees and undergrowth. Gotjawal forests are characterized by lava dooms, microclimates and ecological features shaped by volcanic activity. Jeju’s lava forests may be a globally unique area and critical for understanding lava-formed forests. The Gotjawal forest is considered to have three important features such as “rain water penetrating to the groundwater aquifer”, “plants specific to this ecosystem (species shelter)”, and “its formation in rocky areas”. The purpose of this study was to analyze the composition, diversity and function of bacteria in the Gotjawal soils.

We analyzed the bacterial diversity and function (Nitrogen fixation) in soils collected from Gyorae & Sanyang Gotjawal forest, where globally unique topography, geography and ecological features support a forest grown on basalt flows from 110,000 to 120,000 years ago and 40,000 to 50,000 years ago. The soils at the site are fertile with rocky areas and are home to endangered species of plants and animals. Rainwater penetrates to the groundwater aquifer which is composed of 34% organic matter containing rare types of soil and no soil profile. According to a principal component analysis of 454-pyrosequencing and Illumina mi-seq results for the C, GR, SY soils, well-separated clusters were produced by region for all samples during the 4 seasons and the 6 repetitions. Analysis of *nifH* gene showed 510,054(C), 205,191(GR), 200,774(SY) *nifH* gene abundance. Major nitrogen fixing bacteria phylum is *proteobacteria* for all sites: C; 79.6%, GR; 95.2%, SY; 93.2%. *Nitrospirillum* is the most nitrogen fixing bacteria genus for all sites. *Geobacter* and several bacteria were increased in Gotjawal sites. The characteristics of Gotjawal soil which are determined by lava morphology, vegetation and groundwater penetration might be reflected in the bacterial & nitrogen fixing microbial community composition. The results of our study will provide important insights into the understanding of the soil microbial community in Gotjawal forest soils.

In addition, we intended also to discover novel or related genes of lignocellulose degradation and its degrader, indigenous microorganisms. In this study, the bacterial community and metagenome data from surface deposits of Torreya Nucifera which located at Gotjawal in Jeju were analyzed. PacBio RS platform was conducted to obtain environmental metagenome data that was for comparing reference genes, ORF and sequence related with lignocellulose degradation. The novel gene sequence encoding lignocellulose degradable enzymes such as peroxidase, laccase, cellulase, xylase etc., were found.
Microbial Community Function and Structure of Gotjawal Forest Soil, Jeju


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We analyzed the structure and function of the microbial community of Gotjawal forest soils, in the Jeju Island, Korea, which have grown on volcanic soils. The unique basalt morphology, diverse vegetation associated with microclimates, and ecological features shaped by volcanic activity of this region have supported the growth of the forest on lava flows. These forests also function as a roof over pristine aquifers, as well as porous rocks that purify and recharge groundwater. Overall, the Gotjawal forest represents a species-rich ecosystem, but these forests have been gradually disappearing in recent decades. Approximately 50% of these forests have been destroyed by unregulated construction and urbanization. The term “Gotjawal soils” specifically refers to soils derived from these forests, and these soils are characterized by high organic matter content and fertility and low rocky soil content.

We report the study of the functions and structure of the microbial community in Gotjawal soils using GeoChip. Twenty soil samples were collected in November 2010 from four large regions representing the entire Gotjawal forest: Hankyung-Andeok (HA) Gotjawal Terrain in the west, Aewol Gotjawal (AW) Terrain in the northwest, Jocheon-Hamduck (JH) Gotjawal Terrain in the north-east, and Gujwa-Seongsan (GS) Gotjawal Terrain in the east. Two representative areas of the JH Gotjawal terrain, Gyorae and Dongbackdongsan, were regrouped into the AW and GS Gotjawal Terrains, and the Jeoji (JJ) area of HA Gotjawal Terrain was reclustered into the AW Terrain using hierarchical clustering of GeoChip microarray data. Furthermore, we present the potentials of biosurfactant-producer and carbon fixation and nitrogen fixation genes of Gotjawal soil DNA by cloning and pyrosequencing.

Our study represents the first analysis of function and structure of the microbial community using GeoChip and through pyrosequencing of Gotjawal soils. Future studies will address whether the characteristics of Gotjawal soils, which are influenced by lava morphology, and vegetation, are reflected in the composition and key function of the microbial community.

Keywords: Gotjawal forest soil, Microarray (GeoChip), Microbial community structure

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Comprehensive Study on Soil Bacterial Community Composition on Barton Peninsular and Around Terra Nova Bay, Antarctica

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Antarctica is the coldest and driest continent in our planet. Over the last few decades, the terrestrial ecosystems in Antarctica had been recognized as an aseptic place because of the extreme habitat conditions for life. With the dedicated terrestrial biological research, we begin to understand the uniqueness and complexity of these fragile ecosystems. Through the application of molecular techniques and further development of next generation sequencing technology in microbiology era, we realized that the microorganisms are highly diverse and their community structure is much more complex. In ecological function in these harsh habitats containing simple diversity of flora and fauna, microorganisms play a more significant role. Therefore, it is important and essential to understand what the dominant bacterial taxa are and what environmental variables predict bacterial community structure. Here in this presentation, the study on soil bacterial community structures and the correlation with physicochemical properties on Barton Peninsular in King George Islands of Antarctic Peninsular and around Terra Nova Bay in Victoria Land will be presented and discussed.

Keywords: Antarctica, Barton Peninsular: Terra Nova Bay, 16S rRNA gene, Bacteria, physicochemical properties
Symposium

S3 Host-pathogen Interaction
Roles of Unphosphorylated ISGF3 in HCV Infection

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Hepatitis C virus (HCV) is a positive-stranded RNA virus in the family Flaviviridae, and approximately 170 million people are infected with HCV worldwide. Acute HCV infection is spontaneously cleared in 20-30% of patients, but the majority of patients fail to clear the virus and develop chronic persistent infection. In HCV-infected liver, it has been known that upregulation of interferon-stimulated genes (ISGs) is sustained. Here, we investigated the mechanism of prolonged ISG expression and its implication in interferon (IFN) responsiveness during HCV infection in relation to unphosphorylated ISGF3 (U-ISGF3), recently identified as a tripartite transcription factor formed by high levels of IRF9, STAT1, and STAT2 without tyrosine phosphorylation of STATs. In liver tissues of HCV-infected patients and HCV-infected cells, the expression of IRF9, STAT1, and STAT2 was elevated without tyrosine-phosphorylated STAT1, and U-ISGF3-downstream ISGs were upregulated. The U-ISGF3 components were detected in the nucleus of HCV-infected cells and bound to the promoter regions of a set of ISGs. Induction of U-ISGF3 by forced expression of IRF9, STAT2, and phosphorylation-defective STAT1 was sufficient to upregulate ISGs. Moreover, we found that U-ISGF3 induction depended on IFN-α and -β produced by HCV-infected cells. Importantly, ISG15, one of the U-ISGF3-downstream ISGs, was critical for IFN-α resistance in cells with U-ISGF3 induction by IFN-α pretreatment. ISG15 conferred IFN-α resistance on cells by regulating the abundance of USP18 protein. Our data demonstrate that U-ISGF3 induced by IFN-α drives prolonged expression of a set of ISGs, leading to chronic activation of innate responses and conferring refractoriness to IFN-α in HCV-infected liver.
Evasion of Host Immune System Through the Mutation of Enteric Virus

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Viral gastroenteritis was generally induced in a child less than 5 years old by type A rotavirus, enteric adenovirus, astrovirus, sapovirus, except for norovirus. These were designated as surveillance required pathogens legally and this was performed by KNIH (Korea National Institute of Health) as a name of EnterNet-Korea. In this point, we analyzed genotype of enteric virus in Korea to investigate evasion of host immune system.

Norovirus is typical single stranded (+) RNA virus and genogroup I and II generally infect human. Norovirus GII.4 is main genotype in the world and continuously mutated their genome. Several GII.4 variants were reported and, in recent, Sydney variant induced outbreak in the world. However, GII.17 is emerging genotype in south-east Asia and induced several outbreaks last winter seasons. In recent, it occur the huge antigenic change of norovirus in south-east Asia and it may spread to other area. The antigenicity of type A rotavirus is determined by the combination of VP4 and VP7. The proportion of epidemic genotypes was usually dependent on the area and facility of researches. This phenomenon was also matched to several domestic researchs. In our survey, the genotype of type A rotavirus was mainly dependent on sampling area and the pattern of patients between sporadic cases and outbreak cases. Recently, the genotype of other virus also changed and sometimes induced outbreak by minor genotypes in Korea.

Genotype provide important information about evading strategy of enteric virus from host immune system and these also tactical information to diagnosis and prevent pathogens. Although we are not able to catch up the mutation rate of enteric virus, we must continuously follow up to decrease disease.
Transcriptional Profiling of Host Responses to *Mycobacterium avium* subsp. *paratuberculosis* Infection and Identifying Biomarker Candidates

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*Mycobacterium avium* subsp. *paratuberculosis* (Map) is the causative agent of bovine paratuberculosis, also known as Johne’s disease, which is considerable disease by economic losses in dairy industry and the possible relationship with Crohn’s disease in humans. Paratuberculosis has a prolonged subclinical stage that can continue to excrete the infective bacteria in feces without showing outward symptoms. Because these fecal shedders might act as sources of infection to other animals, the development of a diagnostic method that is useful in the early stage is very important to control the disease. Therefore, strategies for control of MAP have been focused on diagnosis and removal of the animals during early stage of the disease, thus leading to prevent new infection. However the control of the disease has been interfered with a lack of sensitive techniques for detection of asymptomatic paratuberculosis. Here, we aim to determine the most affected gene networks and pathways underlying the host immune response to Map infection in whole blood and provide potential transcriptional markers that distinguish according to the status of Map infection. Analysis of the transcriptome in response to MAP infection can provide valuable information for diagnosis and prognosis.

In the present study, we characterized the transcriptional profiles from whole-blood cells in cattle, which were identified and grouped according to the presence of Map-specific antibodies and shed Map bacteria. Three comparisons were done: 1) ELSIA negative and fecal-PCR positive; 2) ELSIA positive and fecal-PCR negative; 3) ELSIA positive and fecal-PCR positive, compared to uninfected control. The differentially expressed genes (DEG) (adjusted fold change ≥ 1.5 and \( p \leq 0.05 \)) were involved in the immune response and metabolic process function category, especially, showing significant inhibition of function related to “Free radical scavenging” and “Lipid metabolism” at the 1) case. In addition, the DEGs of 2) and 3) cases were involved in the same biological functions and canonical pathways (i.e. LXR/RXR activation and complement system), while IL-10 signaling was a unique feature at the 3) case.

This study demonstrates that the whole-blood transcriptional profiles of Map infection showed partitive functions in the immune response and metabolic process categories. The results showed down-regulation for production and metabolism of reactive oxygen species at the 1) case, activation of pathways related to the host-defense response against Map (LXR/RXR activation and complement system) at the 2) and 3) cases, and anti-inflammatory response (activation of IL-10 signaling pathway) at the 3) case. These data supported a balanced response that serves the immune-limiting mechanism while the host defense responses were progressing.
The Structural Basis of Lipopolysaccharide Recognition by the TLR4-MD-2 Complex

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The lipopolysaccharide (LPS) of Gram negative bacteria is a well-known inducer of the innate immune response. TLR4 and MD-2 form a heterodimer that recognizes a common “pattern” in structurally diverse LPS molecules. To understand the complex ligand specificity and receptor activation mechanism, we determined the crystal structure of the TLR4-MD-2-LPS complex. LPS binding induced the formation of an “m”-shaped receptor multimer composed of two copies of the TLR4-MD-2-LPS complex arranged in a symmetrical fashion. LPS interacts with a large hydrophobic pocket in MD-2 and directly bridges the two components of the multimer. Five of the six lipid chains of LPS are buried deep inside the pocket and the remaining chain is exposed to the surface of MD-2, forming a hydrophobic interaction with the conserved phenylalanines of TLR4. Comparison with the structures of antagonists bound to MD-2 indicates that two other lipid chains in LPS displace the phosphorylated glucosamine backbone towards the solvent area by ~5 angstrom. This structural shift allows phosphate groups of LPS to contribute to receptor multimerization by forming ionic interactions with a cluster of positively charged residues in TLR4 and MD-2. The TLR4-MD-2-LPS structure illustrates the remarkable versatility of the ligand recognition mechanisms employed by the TLR family, which is essential for defense against diverse microbial infection.
Symposium

S4 Fungal Diversity and Ecology

International Meeting of the Federation of Korean Microbiological Societies
**Microbial Diversity in Korean Traditional Fermenting Starter, **Nuruk, **Collected in 2013 and 2014**

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A total of sixty-six samples of Nuruk, a fermentation starter used to make the Korean traditional rice wine, Makgeolli, were collected from central and southern regions of Korea in 2013 and 2014. We classified two groups of the Nuruk samples, “commercial” and “home-made”, according to the manufacturing procedure and purpose of use. Commercial Nurusks were made in a controlled environment where the temperature and humidity are fixed and the final product is supplied to Makgeolli manufacturers. Home-made Nurusks were made under uncontrolled conditions in the naturally opened environment and were intended for use in the production of small amounts of home-brewed Makgeolli. We obtained more than five hundred isolates including filamentous fungi and yeasts from the Nuruk samples followed by identification of fungal species. Also we stored glycerol stocks of each single isolate at -70°C.

We identified the species of each isolate based on the sequences of ITS regions amplified with two different universal primer pairs. We also performed morphological characterization of the filamentous fungi and yeast species through observations under the microscope. We investigated the major fungal species of commercial and home-made Nurusks by counting the colony forming units (CFU) and analyzing the occurrence tendency of fungal species. While commercial Nurusks contained mostly high CFU of yeasts, home-made Nurusks showed relatively high occurrence of filamentous fungi. One of the representative Nuruk manufacturers used both domestic wheat bran and imported ones, mainly from US, as raw material. Depending on the source of ingredient, the fungal diversity was somewhat different. Another commercial Nuruk sample was collected twice, once in 2013 and again in 2014, and showed different diversity of fungal species in each year. Nurusks obtained from the southern regions of Korea and Jeju island showed high frequency of yeast such as _Saccharomyces fibuligera_ and _Pichia_ species as well as unique filamentous fungus, _Monascus_ species. _S. fibuligera_ was easily found in many Nuruk samples with high CFU. The major filamentous fungi were _Aspergillus_, _Lichtheimia_, _Mucor_ and _Penicillium_ species. In order to further our understanding of the isolates and their potential industrial applications, we assayed three enzymes, alpha amylase, glucoamylase and acid protease from 140 isolates out of about five hundred isolates and selected about 10 excellent strains with high enzyme activities. With these fungal isolates, we will perform omics analyses including genomics, transcriptomics, metabolic pathway analyses, and metabolomics followed by whole genome sequencing of unique isolates associated with the basic research of Nuruk and that also has applications in the Makgeolli making process.
Ultrastructure of the Epiphytic Sooty Mold *Capnodium* on Walnut Leaves

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Cellular aspects of sooty mold on walnut leaves were investigated by using light and electron microscopy. A black coating developed on the adaxial leaf surface of a walnut tree. No infestations were found on the abaxial leaf surface with peltate glandular trichomes. Light microscopy showed that fungal complexes from the leaf surface were composed of brown conidia and hyphae. Conidia, with longitudinal and transverse septa, were variable in length ranging from 10 to 30 μm, and commonly found in clusters, forming microsclerotia. Neither epidermal penetration nor hyphal entrance to host tissues was observed. Based on their morphological characteristics, the fungal complexes were assumed to be *Capnodium* species. An electron-dense melanized layer was present on the cell wall of multi-celled conidia. Concentric bodies in the fungal cytoplasm had an electron-translucent core surrounded by an electron-dense margin with a fibrillar sheath. Chloroplasts without starch granules in the palisade mesophyll cells of sooty leaves had electron-dense stromata and swollen plastoglobuli. These results suggest that the epiphytic growth of fungal complexes can be attributed to the melanized layer and concentric bodies against a water-deficient environment on the leaf surface. Ultrastructural characteristics of the sooty leaves indicate typical features of dark-adapted and non-photosynthetic shade leaves.
Cultural Characteristics of Ectomycorrhizal Mushrooms

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Ectomycorrhizal (ECM) mushrooms play a major role in plant growth promotion through symbiotic association with roots of forest trees. They also provide an economically important food resource to us and therefore they have been studied for their artificial cultivation for decades in Korea. We have secured bio-resources of ECM mushrooms from Korean forests and performed their physiological studies.

To investigate the cultural characteristics, the fungi were cultured under different conditions (medium, temperature, pH of the medium, inorganic nitrogen source). More than 90% of total 160 strains grew on three solid media (potato dextrose agar, PDA; sabouraud dextrose agar, SDA; modified Melin-Norkrans medium, MMN). The rate of mycelial growth on malt extract agar (MEA) was lower than those of three media (PDA, SDA, MMN). None of the Tricholomataceae strains grew on MEA. Many strains of ECM mushrooms were able to grow at the temperature range of 15~25°C on PDA, while they showed poor growth at 10°C or 30°C. In particular, the growth rates of both Gomphaceae and Tricholomataceae were significantly lower at 10°C than at 30°C. The optimal pH of many strains was pH 5.0 when they cultured in potato dextrose broth (PDB). Fifty-seven percent of tested strains grew well on medium containing ammonium source than nitrate source. Many strains of Tricholomataceae showed a notable growth on ammonium medium than nitrate medium. Twenty-three percent of strains preferred nitrate source than ammonium source for their mycelial growth. The production and activity of two enzymes (cellulase and laccase) by ECM fungi were also assayed on the enzyme screening media containing CMC or ABTS. Each strains exhibited different levels of enzymatic activities as well as enzyme production. The number of laccase-producing strains was less than that of cellulase-producing strains. We found that 77% of tested strains produced both cellulase and laccase, whereas 2% of strains did not produce any enzymes.

The morphological characteristics of mycelial colony were also examined on four different solid media. Yellow was a dominant color in mycelial colony and followed by white and brown on all culture media. ECM mushrooms formed mycelial colonies with a single or multiple colors within a culture medium depending on the strains and culture media. The most common shape of mycelial colony was a circular form on all media tested. Other families except for Amanitaceae formed an irregular colony on MMN than PDA. All strains of Tricholomataceae did not form a filamentous colony on all media. The pigmentation of culture media by mycelial colonies was observed in more than 50% of strains tested on both PDA and SDA. The degree of
pigmentation on PDA or SDA was higher than MMN and brown color was dominant than yellow color. The production of exudates from mycelial colony was higher on PDA than MMN. Brown exudates were mainly produced by many strains on PDA or SDA, whereas transparent exudates were mainly produced by strains on MMN. We observed the mycelial colonies with a single or multiple textures in just one culture plate. Wrinkled or uneven colony surfaces were remarkably observed in many strains on PDA or SDA, while an even colony surface was observed in many strains on MMN. Sixty percent of Tricholomaceae strains formed wrinkled surface on PDA. However, they did not form any wrinkle on MMN plate. Cottony texture was observed in mycelial colonies of many strains. Velvety texture was often observed in the mycelial colonies on SDA than PDA and accounted for 60% of Suillaceae strains on SDA.
Pathogenesis of Oak Wilt Disease Caused by *Raffaelea* Species

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Wilt disease in Oak trees occurs during summer season in Korea. Mass attack of trees by an ambrosia beetle (*Platypus koryoensis*) was the characteristic feature before appearance of the wilting symptoms. *Raffaelea* sp. caused the discoloration of xylem area called as wound heartwood. *Raffaelea* sp. was observed both on the body surfaces and inside the mycangia of the beetle *Platypus* sp. The scanning electron microscope (SEM) analysis showed that fungal spores were present within the wall of gallery and vessels that formed tyloses. The results revealed that the water movement in vessels was blocked as the fungus started to grow which caused the formation of tyloses thereby resulting wilt symptoms. We found that both female and male beetle *Platypus* sp. had fungi on their bodies and their large and small mycangia. This study confirmed that the fungus was transferred to oak trees by *Platypus* sp.
Symposium

S5 Infection, Inflammation, and Immunity
CCCTC-Binding Factor Controls the Homeostatic Maintenance and Migration of Langerhans Cells in the Skin

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Langerhans cells (LCs) are skin-resident dendritic cells (DCs) that orchestrate skin immunity. Regulation of LC homeostasis depends on several extra- and intra-cellular factors. CTCF is a highly-conserved DNA-binding protein that modulates cell differentiation and function through modifying chromatin architectures and subsequent gene expressions. A possible role of CTCF controlling LC homeostasis and function remains to be determined. Herein, by using conditional gene deletion mouse system, we show that CTCF is critically involved in homeostatic maintenance and migration of LCs in vivo. DC-specific CTCF deletion led to a reduced pool of systemic DCs, with LCs most severely affected. Decreases in epidermal LC number were specifically associated with self-turnover defects. Interestingly, CTCF-deficient LCs demonstrated impaired migration out of the epidermis. Whole-transcriptome analyses revealed that genes that promoted cell adhesion were highly expressed, but C-C chemokine receptor 7 was down-regulated in CTCF-depleted LCs. Hapten-induced CHS responses were more sustained in LC-specific CTCF-deficient mice while epicutaneous sensitization to protein antigen was attenuated, indicating that CTCF-dependent LC homeostasis is required for optimal immune function of LCs in a context-dependent manner. Our results show that CTCF positively regulates the homeostatic pool and the efficient emigration of LCs, which are required for modulating the functional immune network of the skin.
The HIF-1/Glial TIM-3 Axis Controls Inflammation-Associated Brain Damage Under Hypoxia

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Inflammation is closely related to the extent of damage following cerebral ischemia, and the targeting of this inflammation has emerged as a promising therapeutic strategy. Here, we present that hypoxia-induced glial T cell immunoglobulin and mucin domain protein (TIM)-3 can function as a modulator that links inflammation and subsequent brain damage after ischemia. We find that TIM-3 is highly expressed in hypoxic brain regions of a mouse cerebral hypoxia-ischemia (H/I) model. TIM-3 is distinctively up-regulated in activated microglia and astrocytes, brain resident immune cells, in a hypoxia-inducible factor (HIF)-1 dependent manner. Notably, blockade of TIM-3 markedly reduces infarct size, neuronal cell death, edema formation and neutrophil infiltration in H/I mice. Hypoxia-triggered neutrophil migration and infarction are also decreased in HIF-1\textalpha-deficient mice. Moreover, functional neurological deficits after H/I are significantly improved in both anti-TIM-3-treated mice and myeloid specific HIF-1\textalpha-deficient mice. Further understanding of these insights could serve as the basis for broadening the therapeutic scope against hypoxia-associated brain diseases.
New Insights into Antiviral Immunity by Repeated Low-Dose Viral Exposures

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Clearance of acute viral infection with systemic viremia can result in T cell-based immune protection upon reinfection. Hepatitis C virus (HCV)-specific T cells have also been detected in some high-risk individuals without any history of viral infection despite frequent exposures to trace amounts of virus; it has been suggested that these low-level viral exposures confer T cell-mediated immune protection to infection.

To confirm this hypothesis, we show in nonhuman primates that repeated exposure to human plasma with trace amounts of HCV induced HCV-specific T cells without systemic viremia, but did not protect upon subsequent HCV challenge. Rather, HCV-specific recall and de novo T cell responses as well as intrahepatic T cell recruitment and IFN-γ production were suppressed upon HCV challenge, concomitant to quantitative and qualitative changes in regulatory T (T_{reg}) cells. In vitro T_{reg} cell depletion restored HCV-specific T cell responses.

These findings might be relevant for vaccine research and for epidemiological studies because an increased T_{reg} cell number in frequently exposed individuals and in endemic areas might reduce the response to T cell-based vaccines. Therefore, strategies to reverse this exposure-induced immune suppression should be examined to aid the development of T cell-based vaccines.
Antagonism Among IL-12 Family Cytokines in Host Defense Against *Listeria monocytogenes* and Cancer

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Upon antigenic stimulation, naïve CD4+ T cells can be differentiated into Th1 and Th2 cells depending on the types of cytokines produced by antigen-presenting cells. In 2005, a subset of CD4+ T cells producing IL-17 (Th17) was identified as a new lineage of helper T cells distinct from Th1 and Th2 cells, and it is now well established that Th17 cells mediate a number of autoimmune diseases as well as host defense against extracellular pathogens by recruiting neutrophils to the site of inflammation and infection. Although the protective functions by Th17 cytokines against extracellular bacterial and fungal infection have been well documented, their importance against intracellular bacterial infection remains unclear. Here, we investigated the contribution of Th17 responses to host defense against intracellular bacteria *Listeria monocytogenes* and found that Th17 cell generation was suppressed in this model. Unexpectedly, mice lacking both p35 and EBI3 cleared *L. monocytogenes* as efficiently as wild-type mice, whereas p35-deficient mice failed to do so. Furthermore, both innate cells and pathogen-specific T cells from double-deficient mice produced significantly higher IL-17 and IL-22 compared to wild-type mice. The bacterial burden in the liver of double-deficient mice treated with anti-IL-17 was significantly increased compared to those receiving a control Ab. Transfer of Th17 cells specific for listeriolysin O as well as administration of IL-17 and IL-22 significantly suppressed bacterial growth in p35-deficient mice, indicating the critical contribution of Th17 responses to host defense against the intracellular pathogen in the absence of IL-12 and proper Th1 responses. To further investigate the role of IL-27EBI3 in tumor immunity, wild-type and EBI3-deficient mice were injected with B16 melanoma. The growth of the transplanted tumor cells was significantly delayed in the latter group. Notably, regulatory T cells in the tumor-bearing EBI3-deficient mice displayed different cell surface molecules which may explain the delayed tumor growth in the mice. Our findings unveil a novel immune evasion mechanism whereby the intracellular bacteria as well as cancerous cells exploit IL-27EBI3 to suppress Th17-mediated protective immunity.
Symposium

S6 Physiologies and Applications of Industrial Microorganisms

International Meeting of the Federation of Korean Microbiological Societies
S6-1

Engineering of *Corynebacterium glutamicum* for the Production of Value-added Biochemicals from Biomass

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*Corynebacterium glutamicum*, a non-pathogenic and Gram-positive bacterium, has been traditionally used for the industrial production of various amino acids, nucleotides and vitamins. In recent years, far beyond traditional amino acid production, there has been impressive progress in engineering *C. glutamicum* toward a broad product spectrum, including various chemicals (diamines, diols, polymers, etc.) and biofuels (ethanol, advanced alcohols, etc.). Due to numerous ideal intrinsic attributes and the recent development of metabolic and synthetic engineering, the potential of *C. glutamicum* as a microbial factory for the production of various value-added bio chemicals continues to grow. However, compared with *Escherichia coli*, the available genetic tools in *C. glutamicum* are quite limited and this limitation has been a notable obstacle for the synthetic engineering of *C. glutamicum*. In this talk, new genetic tools for gene expression in *C. glutamicum* will be presented. In addition, the engineering of *C. glutamicum* towards the production of value-added chemicals from biomass will be discussed.
Marker-free Genome Engineering in *Bacillus subtilis*

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Genome engineering without leaving foreign DNA behind requires an efficient counter-selectable marker system. Here, we developed a genome engineering method in *Bacillus subtilis* using a synthetic gene circuit as a counter-selectable marker system. The system contained two repressible promoters (*B. subtilis xylA* (P<sub>xyl</sub>) and *spac* (P<sub>spac</sub>)) and two repressor genes (*lacI* and *xylR*). P<sub>xyl</sub>-lacI was integrated into the *B. subtilis* genome with a target gene containing a desired mutation. The xylR and P<sub>spac</sub>-chloramphenicol resistant genes (*cat*) were located on a helper plasmid. In the presence of xylose, repression of XylR by xylose induced LacI expression, the LacIs repressed the P<sub>spac</sub> promoter and the cells become chloramphenicol sensitive. Thus, to survive in the presence of chloramphenicol, the cell must delete P<sub>xyl</sub>-lacI by recombination between the wild-type and mutated target genes. The recombination leads to mutation of the target gene. The remaining helper plasmid was removed easily under the chloramphenicol absent condition. In this study, we showed base insertion, deletion and point mutation of the *B. subtilis* genome without leaving any foreign DNA behind. Additionally, we successfully deleted a 2-kb gene (*amyE*) and a 38-kb operon (*ppsABCDE*). This method will be useful to construct designer *Bacillus* strains for various industrial applications.
Higher Fungi and Their Enzyme Systems in Bioremediation

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Higher fungi belonging to Basidiomycota and Ascomycota play key roles in the recycling of organic matter in nature. These fungi produce multiple oxidative enzymes in culture broth to attack ligninocellulosic substrates such as laccases, peroxidases, and cellulases. They also produce various cytochrome P450 monooxygenases in their cells in response to different substrates from more than 100 isogenic loci in their genomes. The power of fungal enzymes has been explored in the decomposition of recalcitrant aromatic compounds, including polycyclic aromatic hydrocarbons, dyes, and endocrine-disrupting compounds. Basidiomycetes laccase enzyme systems in \textit{Pycnoporus coccineus} and \textit{Pleurotus ostreatus} will be discussed in the removal of organic dyes and PAHs. Intracellular cytochrome P450 monooxygenases, particularly in Ascomycota, have shown to be responsible for the degradation of multiple substituted aromatic hydrocarbons.

In this study, we will compare the degradative pathways of substituted aromatic hydrocarbons in different fungal strains from \textit{Aspergillus}, \textit{Cordyceps}, and \textit{Trametes}. 
Yeast has long been used as a food microorganism for thousands of years. It has also become a commercial workhorse for the production of various recombinant pharmaceutical proteins and the production of engineered metabolites such as amorphadiene and vanillin. Recently, yeast has taken a special attention as a valuable host for renewable bioenergy and bio-based chemicals. For the production of such bulk chemicals cost-effectively, it is necessary to use sustainable, cheaper and commonly available carbon sources, especially lignocellulose. Unfortunately, yeast can utilize only certain mono- and di-sugars such as glucose, fructose, galactose, and sucrose. Furthermore, yeast robustness should be improved to be resistant from various stresses and a high concentration of product chemicals. To expand the narrow substrate spectrum of yeast, we have developed recombinant yeasts hyper-secreting enzymes for hydrolyzing various polymeric substrate using yeast TFP technology. Such engineered yeasts could improve the substrate spectrum, especially from lignocellulosic biomass. Identification and genetic application of a yeast transcriptional factor, UPC2 regulating ergosterol biosynthesis, greatly increased the acid-tolerance, resulting in a high level production of lactic acid at low pH. Demonstration of consolidated bioprocessing using recombinant yeasts developed in this study for the production of bioethanol, lactic acid and difructose anhydrides (DFA) from non-feedstock biomass will be presented.
Symposium

S8 Fungal Morphogenesis and Pathogenesis
A Rice Blast Fungus Alpha-N-Arabinofuranosidase B Elicits Host Defense in Rice

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Rice blast disease caused by *M. oryzae* is the most devastating fungal disease in rice. During the infection process, *M. oryzae* secretes a large number of glycosyl hydrolase (GH) proteins into the apoplast to digest host cell wall and assist fungal ingress into host tissues. In this study, we identified a novel *M. oryzae* arabinofuranosidase B (MoAbfB) which is secreted during fungal infection. Live-cell imaging exhibited that fluorescent labeled MoAbfB was highly accumulated in fungal invasive structures such as appressorium, tips of penetration peg, biotrophic interfacial complex (BIC), as well as invasive hyphal tip. Deletion of *MoAbfB* mutants extended biotrophic phase followed by enhanced disease severity, whereas, over-expression of *OsMoAbfB* mutant induced rapid defense responses and enhanced rice resistance to *M. oryzae* infection. Furthermore, exogenous treatment of MoAbfB protein showed inhibition of fungal infection via priming of defense gene expression. We later found that the extract of MoAbfB degraded rice cell wall fragments could also induce host defense activation, suggesting that not MoAbfB itself but oligosaccharides (OGs) derived from MoAbfB dissolved rice cell wall elicited rice innate immunity.
**Rpi-blb2 Gene-Mediated Late Blight Resistance in Plants**

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*Phytophthora infestans* is the causal agent of potato and tomato late blight, one of the most devastating plant diseases. *P. infestans* secretes effector proteins that are both modulators and targets of host plant immunity. Among these are the so-called RXLR effectors that function inside plant cells and are characterized by a conserved motif following the N-terminal signal peptide. In contrast, the effector activity is encoded by the C-terminal region that follows the RXLR domain. Recently, I performed *in planta* functional profiling of different RXLR effector alleles. These genes were amplified from a variety of *P. infestans* isolates and cloned into a Potato virus X (PVX) vector for transient *in planta* expression. I assayed for *R*-gene specific induction of hypersensitive cell death. The findings included the discovery of new effector with avirulence activity towards the *Solanum bulbocastanum* **Rpi-blb2** resistance gene.

The **Rpi-blb2** encodes a protein with a putative CC-NBS-LRR (a coiled-coil-nucleotide binding site and leucine-rich repeat) motif that confers *Phytophthora* late blight disease resistance. We examined the components required for **Rpi-blb2**-mediated resistance to *P. infestans* in *Nicotiana benthamiana*. Virus-induced gene silencing was used to repress candidate genes in *N. benthamiana* and to assay against *P. infestans* infections. NbSGT1 was required for disease resistance to *P. infestans* and hypersensitive responses (HRs) triggered by co-expression of **AVRblb2** and **Rpi-blb2** in *N. benthamiana*. **RAR1** and **HSP90** did not affect disease resistance or HRs in **Rpi-blb2**-transgenic plants. To elucidate the role of salicylic acid (SA) in **Rpi-blb2**-mediated resistance, we analyzed the response of **NahG**-transgenic plants following *P. infestans* infection. The increased susceptibility of **Rpi-blb2**-transgenic plants in the **NahG** background correlated with reduced SA and SA glucoside levels. Furthermore, **Rpi-blb2**-mediated HR cell death was associated with **H2O2**, but not SA, accumulation. SA affects basal defense and **Rpi-blb2**-mediated resistance against *P. infestans*. These findings provide evidence about the roles of SGT1 and SA signaling in **Rpi-blb2**-mediated resistance against *P. infestans*. 
Comparative Analysis of a Putative HLH Transcription Factor Responsible for Conidiation in *Aspergillus* Species

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Asexual reproduction or conidiation in aspergilli is a primary means to produce their progenies that is environmentally and genetically controlled tightly. Previously, intensive researches in the model fungus *Aspergillus nidulans* disclosed some genes playing important roles in asexual and sexual development. Among them, one gene encoding a putative helix-loop-helix (HLH) transcription factor, named *ndrA*, has been isolated and characterized as a downstream regulator of developmental master regulator NsdD. By using comparative genome search of *A. nidulans* NdrA protein, its orthologues have been identified in *A. fumigatus* and *A. flavus*, respectively (*AfudrnA* and *AfldrnA*). Deletion of the *ndrA* genes in both Aspergillus species made them unable to produce the conidia yet abundant production of sclerotia in *A. flavus*. Complementation of *ndrA* deletion strains by intact *ndrA* ORFs has restored the conidiation as in the control strains. In *A. fumigatus*, *ndrA* deletion also resulted in loss of conidiation phenotype. Northern analyses showed that the *ndrA* genes in both Aspergillus species are highly expressed at the early stage of the conidiation. Interestingly, the *ndrA* genes were found to be necessary for the proper expression of *brlA* genes. Antifungal sensitivity test revealed that the *ndrA* genes might be responsible for the sensitivity or resistance to some antifungal agents. However, *ndrA* deletion did not greatly influence the growth in both strains. And the *A. flavus* *ndrA* gene did not affect the aflatoxin production. Taken together, *ndrA* genes in *Aspergillus* species could be an important positive regulator of conidiation under the regulation of the *nsdD* gene yet upstream of the *brlA* gene.
Diversity of Fungi Isolated from Soil of Jeollabuk-do and Chungcheongbuk-do, Korea

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This study was conducted aiming with the assessment of fungal diversity in soil samples collected from different locations of Jeollabuk-do and Chungcheongbuk-do, Korea. Forty soil samples were collected in 2015 and fungi were isolated through serial dilution technique. Isolated fungi were purified and differentiated according to their morphological and microscopic characteristics. In total, 150 different representative isolates were recovered and the genomic DNA of each isolate was extracted by using QIAGEN® Plasmid Mini Kit (QIAGEN Sciences, USA) and the identification of fungi was carried out by sequence analysis of internal transcribed spacer (ITS) region of the 18S ribosomal DNA (18S rDNA). Recovered isolates belonged to 37 family, 67 genera and 108 species. Aspergillus spp., Penicillium spp., Trichoderma spp., Chaetomium spp. and Fusarium spp. were the most dominant taxa in this study. Out of total species, 20 species were identified as new records for Korea.
Symposium

S9 Antibiotic Resistome: from the Environment to the Clinic
Antibiotic resistome is referred to as a collection of all the antibiotic resistance genes and their precursors from pathogenic and non-pathogenic bacteria in the environment. Antibiotic resistance genes (ARGs) from pathogens comprise only a tiny fraction of the resistome of which a majority can be expanded in the natural environment. Recent metagenomic analysis of ancient DNA collected from Beringian permafrost demonstrated the presence of resistance genes for β-lactam, tetracycline and glycopeptide antibiotics, suggesting that the antibiotic resistome is ancient. In addition, there is no doubt that modern day selective pressure is shaping the current form of resistome as well as degree of mobility. Thus, there is growing concern by what routes and mechanisms the natural resistome is disseminated from the environment to the clinic. To address these questions, we explored the resistome in Han river from upper (pristine) to lower (estuarine) regions, which are considered to be affected by a gradient of anthropogenic activity of metropolitan Seoul. Both culture-dependent and independent methods were used. High-capacity quantitative PCR arrays detected 174 ARGs and mobile genetic elements among 5 of the Han river samples from upper to lower regions. The number of detected ARGs increased approximately 3-fold at the lower Han river (Seoul region). The abundance of total ARGs which was normalized by 16S rRNA gene copy number increased up to 29-fold at the lower Han river. Aminoglycoside and sulfonamide resistant genes increased 206- and 129-fold respectively. The potential for horizontal transfer of ARGs was evaluated by monitoring mobile genetic elements such as transposases and integrases. These also increased significantly up to 25-fold at downstream. Shotgun metagenomic analysis of DNA samples at the upper (Hwacheon) and lower (Seoul) regions also revealed that ARGs increased approximately 4.5-fold. 672 bacterial strains were isolated at these two regions by using the selective media supplemented with each of 11 different antibiotics and their 16S rRNA gene sequences were determined. Among these, 578 strains were tested for antibiotic susceptibility using disk diffusion assay. Except for aminoglycoside resistant bacteria, susceptibility of isolates significantly decreased at the lower Han river and these changes were antibiotic-specific. In the present study, phylogenetic, metagenomic, and quantitative PCR-based analysis of antibiotic resistome in Han river revealed that mobile genetic elements as well as ARGs might have been disseminated through the anthropogenic activity of metropolitan Seoul.
Prevalence of Antibiotic Resistant Bacteria in Stream

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According to the emergence of pathogens with antibiotic resistance, great concern has been raised regarding the prevalence of antibiotic resistant bacteria in natural environments as well. Microorganisms inhabiting natural environments are considered to be a reservoir of antibiotic resistant genes and share the resistant genes with clinical microbial pathogens. In this study, distribution of culturable antibiotic resistant bacteria (ARB) was investigated at 21 locations in Korea with ampicillin, lincomycin, tetracycline, kanamycin, erythromycin, cephalaxin, sulfamethoxazole, and ciprofloxin. Regional relationship with the prevalence of ARB was not observed. While higher populations of ARB were appeared against ampicillin, licomycin, kanamycin, and cephalaxin, relatively lower abundances of ARB were detected against tetracycline, erythromycin, sulfamethoxazole, and ciprofloxin. Ampicillin resistant bacteria were investigated for multidrug resistance. The 61.8% of them exhibited resistance on more than seven among tested fourteen antibiotics suggesting severe existence of multi-drug resistant bacteria in natural environments.

However, ARB against ampicillin, lincomycin, kanamycin, and cephalaxin were estimated abundantly more than ARB against other antibiotics used in this study. Ampicillin resistant bacteria were investigated for multidrug resistance.

As a result of classification, bacteria belonged to the following genus, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Kluyvera*, *Pantoea*, *Plesiomonas*, *Raoultella*, *Shigella* and a genus *Enterobacter* occupied 49% of identified isolates. In addition, 80% of them exhibited resistance on nine to eleven among tested fourteen antibiotics suggesting severe existence of multi-drug resistant bacteria in natural environments.

V. parahaemolyticus was detected from 47.7% of 966 samples (seawater 61.9%, seafoods 41.8%) analyzed using CHROMagarTM and TCBS agar plates as well as multiplex PCR. Among 13 antibiotics tested, resistance to vancomycin and ampicillin was observed in 97.3% and 87.3% of the isolates, respectively, and the ratios of them resistant to cephalothin (48.8%) and rifampin (46.1%) were also high. The isolates were most highly sensitive to chloramphenicol (91.7%) and trimethoprim-sulfamethoxazole (91.8%). The ratio of sensitivity for other antibiotics was also high in the descending order of gentamycin (82.3%), tobramycin (74.8%), nalidixic acid (71.6%), tetracyclin (69.4%), cefotaxime (63.0%). About 69% of the isolates showed multiple drug resistance toward 3 antibiotics including vancomycin and ampicillin. Two of them exhibited resistance for 11 antibiotics used in this study. Plasmid profile analysis of the isolates with antibiotic resistance revealed that 55.1% of them retained plasmids of 24 different types. However, no clear inter-relationship between the resistance and the plasmid profile has been observed.
**E. coli in the Yeongsan River Basin: Antibiotic Resistance and Virulence Factors**

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*Escherichia coli* is generally thought to be a commensal bacterium that inhabits the intestinal tract of warm-blooded animals, including humans. In addition, this bacterium often makes its way into soils and water resulting in fecal contamination of the environment. *E. coli* was adopted as one of the key indicator organisms to examine fecal contamination in freshwater environments. Several studies have suggested that there is a relationship between the genotypes of *E. coli* found among specific animal hosts and the geographical locations where they were isolated, and reported that surrounding environmental conditions may be one of the factors influencing the genotypic richness of *E. coli* in the extraintestinal environment.

Our findings from the studies of population genetics for *E. coli* strains in Yeongsan River indicate that the diversity of *E. coli* genotypes in the environment dynamically fluctuates due to the influence of various environmental factors, especially temperature. Therefore, in addition to issues of re-growth, persistence, secondary habitats, and the naturalization of *E. coli* in the environment, *E. coli* community dynamics must also be examined in order to fully understand that ecology of this bacterium and its use for monitoring fecal contamination in the environment. Furthermore, we also found that while *E. coli* strains could be clustered based on their genotypes and environment conditions, their phylogenetic groups did not change in relation to these same conditions. This result indicates that the distributional differences of phylogenetic groups among *E. coli* populations in different environments may be caused by different genomic adaptability and plasticity of *E. coli* strains belonging to each phylogenetic group.

Seasonal specificity of horizontal fluorophore-enhanced rep-PCR (HFERP) genotypes of 3,480 *E. coli* strains in the Yeongsan River basin has been examined to evaluate correlation between phylogenetic groups and HFERP genotypes of *E. coli* strains, and to figure out any clues of genomic plasticity of environmental *E. coli* isolates. Interestingly, genotypical separation between phylogenetic groups A and B1, and clustering of them were observed from multidimensional scaling (MDS) analysis based on their HFERP DNA fingerprints, suggesting presence of unique quality of genomic DNA structure of *E. coli* based on the different phylogenetic groups. Distribution patterns of *E. coli* phylogenetic groups depending on months and sampling locations revealed no regional but seasonal variations. Results of self-organized maps (SOMs) analysis also indicated that *E. coli* phylogenetic groups were seasonally affected by water temperature, for example high occurrences of phylogenetic
groups A and B1 in low and high temperature seasons, respectively. Occurrences of phylogenetic groups A and B1 were in inverse relation to each other. While phylogenetic groups A and B1 were majority (49.5% and 34.3%) among total E. coli strains, phylogenetic groups B2 and D were rarely observed (5.4% and 10.8%). The results of this study indicated that while E. coli strains could be genotypically clustered with change of the environment conditions, their phylogenetic groups would not be changed by the environmental conditions. The distributional differences of phylogenetic groups among E. coli populations in different environments may be caused by different genotypical adaptability and plasticity of E. coli strains belonging to each phylogenetic group.

Although Escherichia coli has been used as an indicator to examine fecal contamination of aquatic environment, it also has been reported to become naturalized to secondary habitats, including soil, water, and beach sand. A total of 2,880 E. coli isolates obtained from surface water and sediment samples from the Yeongsan River in 2013 were genotyped by using the horizontal fluorophore-enhanced rep-PCR (HFERP) DNA fingerprinting technique. Although different E. coli genotypic groups were observed between surface water and sediments in the dry season, they were mingled and undifferentiated from each other in the rainy season. This indicates that there are frequent sediment resuspension events in the river basin. Moreover, the genotypic composition of the E. coli population in the Yeongsan River basin changes over months and years, implying that genotypic structure of E. coli populations dynamically fluctuates in the river environment. Consequently, our data suggests that the use of E. coli libraries for fecal source tracking needs to be reassessed to account for the changing structure of riverine E. coli populations.

A total of 3564 E. coli isolates obtained from Yeongsan River basin of South Korea were investigated for their production of extended-spectrum β-lactamases (ESBLs) and potential pathogenicity in order to better understand the linkage between antibiotic production in the environment and their public health risks. About 2.5% of the strain (89 of 3564) were putative ESBL producers and antibiotic resistance analysis (ARA) and multiplex PCR was used to determine patterns of ESBL genes and the potential pathogenicity of diarrheagenic and extraintestinal pathogenic E. coli (ExPEC). Furthermore, both potential pathogenic ESBLs and non-ESBLs producers were examined for resistance to non-β-lactam antibiotics, and their genetic relatedness was determined by using the horizontal fluorophore-enhanced rep-PCR (HFERP) DNA fingerprinting technique. Interestingly, 60% (53 of 89) of the isolates producing ESBLs were determined to be potentially one or both of the two pathotypes, suggesting that trade-off between resistance and virulence of E. coli may not apply to this study. In addition, 67% (60 of 89) of the ESBL producers possessed more than one β-Lactamase gene, and most (59 of 63) had the CTX-M-14 enzyme, which is the most dominant ESBL and seems to be related with urban anthropogenic activities. About 68% (36 of 53) of the strains were resistant to more than 2 non-β-lactam antibiotics. Clustering of HFERP genotypes did not correlate with possession patterns of β-lactamase and virulence genes and phylogenetic groups of E. coli. Results from this study indicate that Yeongsan River basin has been contaminated with antibiotic resistant and potential pathogenic E. coli strains. This will likely impact
the dissemination of potential pathogenic *E. coli* producing ESBLs in the environment and suggests the need for further investigations of antibiotic resistant pathogens to prevent public health impacts in Yeongsan River basin.

While *E. coli* has been considered to be the best fecal indicator bacterium, its use for microbial source tracking technology is currently questioned since this bacterium have been reported to naturalize in environments and many current microbial source tracking (MST) methods rely on the use of genotypical/phenotypical library or specific molecular marker genes of fecal indicator bacteria to identify sources of fecal contamination, these methods often fail to determine all point and nonpoint contributors of fecal inputs into waterways. Thus, we developed a new library dependent MST method that uses pyrosequencing-derived shared operational taxonomy units (OTUs) to define sources of fecal contamination in waterways. A total 56,841 pyrosequencing reads of 16S rDNA obtained from the feces of humans and animals were evaluated and used to compare fecal microbial diversity in three freshwater samples obtained from the Yeongsan River basin in Jeonnam Province, South Korea. Analysis of OTUs shared between the fecal and environmental samples suggested that the potential sources of the fecal contamination at the sites were of human and swine origin. Our results indicated that analysis of shared OTUs derived from barcoded pyrosequencing reads provide the necessary resolution and discrimination to be useful as a next generation platform for microbial source tracking studies.
Current Status of Antimicrobial Resistance in Veterinary Medicine

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Antimicrobial resistance remains a major threat to public health world widely, and for the large part, the cause is attributed to overuse or misuse of antimicrobials in humans and animals. Selection of antimicrobial resistant zoonotic bacteria in animals can lead to transmission of these bacteria to humans by the consumption of contaminated animal products or by direct animal contact. Here, we summarize the current status on the antimicrobial usage and resistance trends, as well as zoonotic resistant bacteria in animals and animal products. In Korea, feed additive antimicrobials had been banned since 1995 and only the ionophores were allowed from July, 2011 because of the growing concern of the impact of antimicrobials used in animals on human health and food safety. We assessed the impacts of the phase out of feed additives on usage of antimicrobials and resistance in bacteria of animal origin. The overall consumption of antimicrobials in food producing animals and fisheries decreased especially antimicrobials that are used for feed additives namely, tetracycline, penicillin, and sulfonamides. While therapeutic use of some antimicrobials such as cephalosporins and phenicols have increased. The trends of antimicrobial resistance among indicator \textit{Escherichia coli} and \textit{Enterococcus} spp. isolated from healthy animals decreased with the decrease in consumption of these antimicrobials. Zoonotic resistant bacteria including extended-spectrum \(\beta\)-lactamases (ESBLs) producing \textit{Enterobacteriaceae} and methicillin resistant \textit{Staphylococcus aureus} (MRSA) have been isolated from various non-human sources such as pet, live pigs, carcasses, and bovine milk in Korea. Although a variety of CTX-M types was involved in the resistance against \textit{3}\textsuperscript{rd} generation cephalosporins, \textit{bla}\textsubscript{CTX-M-14} and \textit{bla}\textsubscript{CTX-M-15} were the most prevalent in non-human sources. Furthermore, identical PFGE patterns and conjugative IncFIIs and IncI1-I/\(\text{G}\) plasmids were detected in Non-Typhoidal \textit{Salmonella} (NTS) from both human and animal source. In Korea, two different lineages of MRSA were identified namely human associated type (ST5, ST59, and ST72) and livestock associated type (ST398, ST541, and ST692) in non-human sources. Until early 2000, human associated type was predominant, however, recently livestock associated type was prevalent in swine industry. Although the prevalence of MRSA in food animal products in Korea is still maintained at the low level compared to many other European countries, occurrence and increase in multiple resistant LA MRSA lineage and virulent HA MRSA lineage can be potential threat to public. In conclusion, the prevalence of zoonotic resistant bacteria in animals or food products is maintained still low in Korea, however, resistant genotypes similar to or identical with those of the human isolates were also found in non-human sources. Thus, to minimize antimicrobial resistant bacteria ensure that healthy animals enter the food chain, risk management interventions should be implemented.
International and Local Antimicrobial Resistant Clones in Korea

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Antimicrobial resistance is of great concern globally. Especially, high rates of antimicrobial resistance have been mainly due to dissemination of high-risk resistant clones. In this presentation, I introduce international or local antimicrobial resistant clones of several important bacterial pathogens (Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii) prevailing in Korea. Representatives of them are following; S. pneumoniae ST320 emerged after the introduction of polysaccharide conjugate vaccine (PCV), community-associated methicillin-resistant S. aureus (CA-MRSA) ST72, extended-spectrum β-lactamase (ESBL)-producing fluoroquinolone-resistant E. coli ST131, ESBL-producing K. pneumoniae ST11, carbapenem-resistant P. aeruginosa ST235, and CC92 clone of carbapenem-resistant A. baumannii. In addition to high resistance rates of them, they may show high virulence traits. Although some clones such as CA-MRSA ST72 is uniquely dominated in Korea, most of them belong to international clones. Thus, several factors including travel across country would contribute the globalization of resistant clones in Korea.
Symposium

S10 Host Responses against Infectious Agents
Drosophila Infection Models to Study Bacterial Pathogens

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Recent findings concerning the non-mammalian infection models using the fruit fly, Drosophila melanogaster, suggest that they are well suited to study the molecular mechanisms of host-pathogen interactions involving bacterial pathogens such as Pseudomonas aeruginosa. P. aeruginosa is an opportunistic human pathogen capable of both acute and chronic infections in humans, whose pathophysiology clearly differ involving a distinct set of virulence/persistence factors, especially in regards to the respiration mode (aerobic vs. anaerobic, respectively). Despite the physiological divergence between humans and insects, the modeling of both acute and chronic infections caused by P. aeruginosa is deemed realized using Drosophila, based on the two (i.e. systemic and local) infection models: the systemic infection primarily caused by injecting bacteria into the dorsal thorax of flies mimics the acute infection, whereas the local/epithelial infection instigated by feeding flies with bacteria into the gut mimics the chronic or persistent infection. Topics discussed will include the up-to-date information on Drosophila infection models and brief reviews on our previous studies on identification of novel virulence genes, verification of antibacterial efficacy of therapeutic phages in vivo and a high-throughput screen for selective antibacterials, based on these small-scale live animal infection models.
Role of Toll-Like Receptor 2 Ligands Existing in the Cell Wall of Staphylococcus Aureus in the Infection and Immunity

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Staphylococcus aureus is a representative Gram-positive bacterial pathogen that can cause various infectious diseases such as pneumonia, septic shock, and endocarditis. S. aureus gets a serious public concern due to the increasing emergence of various antibiotic resistant strains which are hardly curable. A new paradigm strategy for the control of S. aureus infection is to interfere with the interaction between S. aureus and the host. Thus, it is important to understand how S. aureus interacts with the host at molecular levels. Accumulating results suggest that Toll-like receptor 2 (TLR2) plays a crucial role in the staphylococcal infection and the host innate immune responses. However, it has been poorly understood which TLR2 ligands of S. aureus are involved in the infection and immunity. For the past decade, we have studied the representative cell-wall-associated TLR2 ligands of S. aureus, lipoteichoic acid (LTA) and lipoprotein, on their structure, function, and role in the infection and immunity by using highly-purified molecules together with S. aureus mutant strains deficient in the synthesis of LTA or lipoprotein. This lecture will describe the functional roles of LTA and lipoprotein on (i) how they interact with TLR2 for stimulation, (ii) how they regulate the activation and differentiation of phagocytic cells, and (iii) how they are differentially involved in the S. aureus infection in mouse infection models.
Toxoplasma Gondii GRA7-Induced TRAF6 Activation Contributes to Host Protective Immunity in Macrophages

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The intracellular parasite Toxoplasma gondii (T. gondii) has unique dense granule antigens (GRAs) that are crucial for host infection, although the role of GRAs in protection against T. gondii infection remains largely unknown. Emerging evidence suggests that the GRA7 of T. gondii is a promising serodiagnostic marker and an effective vaccine candidate against toxoplasmosis; however, little information is known about intracellular regulatory mechanisms involved in GRA7-induced host responses. Here we show that GRA7-induced MyD88 signaling through the activation of TRAF6 and production of reactive oxygen species (ROS) is required for the induction of NF-κB-mediated pro-inflammatory responses by macrophages. GRA7 stimulation resulted in the rapid activation of mitogen-activated protein kinases (MAPKs) and an early burst of ROS in macrophages in a MyD88-dependent manner. GRA7 induced a physical association between GRA7 and TRAF6 via MyD88. Remarkably, the C-terminal of GRA7 (GRA7-V) was sufficient for interaction and ubiquitination of RING domain of TRAF6, which is capable of inflammatory cytokine production. Interestingly, the generation of ROS and TRAF6 activation is mutually dependent on GRA7/MyD88-mediated signaling in macrophages. Furthermore, mice immunized with GRA7-V showed markedly increased Th1 immune responses and protective efficacy against T. gondii infection. Collectively, these results provide novel insight into the crucial role of GRA7-TRAF6 signaling in innate immune responses.
Innate Immune Response of Teleost to Pathogens: The Role of Toll-like Receptors and Antimicrobial Effectors

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Poly(I:C) is a structural analogue of double-stranded RNA that has been used widely in the study of immune responses associated with viral infection. Oligodeoxynucleotides (ODNs) containing dinucleotides with unmethylated cytosine-phosphate-guanine (CpG) motifs are a type of pathogen-associated molecular patterns (PAMPs) that are present commonly in the genomes of microbial pathogens. We investigated the signaling mechanisms of poly(I:C) and a CpG ODN (C7) in a model of Japanese flounder (\textit{Paralichthys olivaceus}) under pathogen infection. We found that (i) TLR3 as well as MDA5 knockdown reduced poly(I:C)-mediated immune response to significant extents, (ii) poly(I:C)-mediated antiviral activity was significantly decreased and increased when Myd88 was overexpressed and inhibited, respectively, in flounder, (iii) NF-\(\kappa\)B activity induced by poly(I:C) was upregulated in Myd88-overexpressing cells and unaffected in Myd88-inactivated cells; (iv) Myd88 overexpression inhibited and upregulated the expression of poly(I:C)-induced antiviral genes and inflammatory genes, respectively, (v) the CpG ODN C7 stimulated the proliferation/activation of flounder leukocytes, and C7-mediated immune response and antiviral activity required TLR9. In the study of antimicrobial molecules, we found that tongue sole (\textit{Cynoglossus semilaevis}) NK-lysin NKLP27 killed bacteria by destroying cell membrane integrity and inducing degradation of genomic DNA, and that NKLP27 possessed immunostimulatory property that promoted antibacterial and antiviral defense.
Symposium

S11 Pathogenesis of Bacterial Infection

International Meeting of the Federation of Korean Microbiological Societies
Unexpected Correlation Between Iron-Assimilating TonB Systems and Invasiveness in *Vibrio vulnificus* Pathogenesis

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TonB systems actively transport iron-bound substrates across the outer membrane of Gram-negative bacteria. *Vibrio vulnificus* CMCP6, a halophilic pathogen that causes fatal septicemia and necrotizing wound infections, possesses three active TonB systems. It is not known why *V. vulnificus* CMCP6 maintained three TonB systems throughout evolution. The TonB1 and TonB2 systems are relatively well characterized while the pathophysiological function of the TonB3 system is still elusive. A RT-PCR study showed that the *tonB1* and *tonB2* genes were preferentially induced *in vivo*, whereas the *tonB3* is persistently transcribed albeit at low expression levels under both *in vitro* and *in vivo* conditions. The present study was to elucidate the raison d’être of these three TonB systems. Differently from previous reports, we constructed in-frame single, double and triple deletion mutants of the entire structural genes in TonB loci, and the changes in various virulence-related phenotypes were evaluated to fulfill the Molecular Koch’s postulates. Surprisingly, only the *tonB123* mutant exhibited a significant delay in killing eukaryotic cells, which was complemented *in trans* with any TonB operons. Very interestingly, we discovered that the flagellum biogenesis was defective in the *tonB123* mutant. The loss of flagellation attributed to severe defects in motility and adhesion of the mutant. Because of difficulty to contact with host cells, the mutant manifested defective RtxA1 toxin production, which resulted in: impaired invasiveness, delayed cytotoxicity, and decreased lethality to mice. Taken together, a series of virulence defects all three TonB systems of *V. vulnificus* CMCP6 coordinately complement each other for iron assimilation and full virulence expression by ensuring flagellar biogenesis.
Macrophages are the primary target and an important reservoir of mycobacteria in the lungs, as indicated by the fact that virulent mycobacteria can survive and replicate within macrophages. Macrophage cell death is an important facet of host-mycobacteria interactions. Therefore, the properties of mycobacteria that are involved in modulating apoptosis have been extensively investigated. Most reports have indicated that the induction of apoptosis by Mycobacterium tuberculosis and M. avium is inversely proportional to bacterial virulence. However, apoptosis appears to act as a virulence mechanism for M. leprae and M. ulcerans. Therefore, induction of apoptosis can apparently serve different functions according to the mycobacterial strain and its infection course.

In general, apoptotic cell death is regarded an innate intracellular response to limit the multiplication of intracellular pathogens. Several apoptosis-inducing factors of M. tuberculosis have been identified: 19-kDa protein and lipoarabinomannan via TLR2, PE_PGRS33 with TNF-α inducing ability, 38-kDa protein with up-regulation of cell death receptor. In addition, we reported that M. tuberculosis HBHA may act as a strong pathogenic factor to cause apoptosis of professional phagocytes infected with M. tuberculosis. In fact, high intracellular burden of virulent M. tuberculosis induces apoptosis with rapid progression to necrosis as a mode of mycobacterial escape.

M. avium complex (MAC) and their sonic extracts induce a macrophage apoptosis. However, any components of MAC that are involved in inhibiting or triggering apoptosis are not identified. In recent, we found that that MAV205x protein induced significant apoptosis in macrophages through production of reactive oxygen species (ROS), loss of mitochondrial membrane potential (MMP), and the release of cytochrome c, and MAV205x was efficiently target to mitochondria of macrophages.

Mycobacterium abscessus (MAB) is a common respiratory pathogen. The rough (R) morphtype of MAB, lacking cell-surface glycopeptidolipids (GPLs), is associated with more severe and persistent infection than the smooth (S) type; however, the mechanisms underlying R-type virulence remain obscure. We found that the R type induced significantly more macrophage apoptosis than the S type, in which apoptosis is inhibited by the GPLs. The GPLs are targeted to mitochondria and interact with cyclophilin D, leading to preservation of the mitochondrial transmembrane potential and suppressed growth of R-type MAB in macrophages and mouse lung. Finally, GPLs enhanced the survival rate of mice infected with the R type. Our results provide insights into the cellular mechanism of R-type virulence and a potential strategy for the control of MAB infection through modulation of cell death.
PD-L1 Mediated Immune Modulation in Mesenchymal Stromal Cells

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The pathogenesis of inflammatory skin disease involves an interaction between immune cells and keratinocytes. Immune dysregulation results in the production of inflammatory cytokines and chemokines, which facilitate the development of the disease pathology. Psoriasis, for example, can be triggered by the dysregulation of T cell-mediated immune responses, and is characterized by hyperplasia of the epidermis, infiltration of leukocytes into both the dermis and epidermis, and the dilation and growth of blood vessels. Keratinocytes perform an important function in the regulation of inflammation, responding to environmental and pro-inflammatory stimuli such as the cytokines IL-17 and IL-22 released by Th17 cells. Mesenchymal stromal cells (MSCs) have known regulatory effects on immune and inflammatory responses. Bone marrow-derived MSCs (BM-MSCs) regulate the functions of immune cells such as T cells, dendritic cells (DCs), B cells, and natural killer cells. We previously isolated mesenchymal stromal cells from human tonsils (T-MSCs) and showed the immune modulatory effect on DCs, B cells, and T cells. In order to examine the interaction mechanism between Th17 and T-MSCs, we used imiquimod-induced skin inflammation in mice and evaluated the immunomodulatory effects of T-MSCs on Th17-mediated immune responses and characterized their mechanism of action. We found that T-MSCs induced Th17 suppression occurs via PD-L1 mediated immune responses. Therefore, PD-L1 might be used as functional marker of mesenchymal stromal cells for immune modulation.
A Single Microbiome Gene Determinant that Affects Host Susceptibility to Enteric Infection

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Indigenous microorganisms that reside in the mammalian intestine maintain a complex self-regulating community. These symbiotic microbes, collectively termed gut microbiota, exist as a highly adhesive biofilm-like community in the mucus layer lining the intestinal epithelium, where they act as a barrier against invading pathogens. The mechanisms by which these microbes interact with invading pathogens remain largely unknown, particularly at the species level. Here we identified a commensal species whose expansion predisposed the host to infection by *Vibrio cholerae*, a deadly human pathogen. An atypical *Escherichia coli* strain, which produces an extra catalase and thus exceptionally resistant to reactive oxygen species (ROS), proliferated substantially in antibiotic-treated adult mice, and increased their infection susceptibility. *V. cholerae* infection was more severe in neonatal mice transplanted with the atypical strain compared with those transplanted with a typical *E. coli* strain. Lower levels of intestinal ROS were detected in mice transplanted with the atypical *E. coli* cells, creating an environment for proliferation of ROS-sensitive *V. cholerae*. A mutant of the atypical *E. coli* strain defective in ROS degradation failed to facilitate *V. cholerae* infection when transplanted, suggesting that host infection susceptibility can be regulated by a single gene product of one particular commensal species. This is the first demonstration that a single microbiome gene determinant affects host susceptibility to enteric infection. Our results will stimulate further investigation to define microbiome gene functions to better understand the roles that commensal microbes play in the complicated ecosystem of the host intestine.
Symposium

S12 Industrial Application of Fungi
Identification and Functional Analysis of Mating Type Loci in the 
*Pleurotus eryngii*

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*Pleurotus eryngii* has recently become a major cultivated mushroom; it uses tetrapolar heterothallism as a part of its reproductive process. Sexual development progresses only when the A and B mating types are compatible. Such mating incompatibility occasionally limits the efficiency of breeding programs in which crossing within loci-shared strains or backcrossing strategies are employed. Therefore, understanding the mating system in edible mushroom fungi will help provide a short cut in the development of new strains. We isolated and identified pheromone and receptor genes in the B3 locus of *P. eryngii* and performed a functional analysis of the genes in the mating process by transformation. A genomic DNA library was constructed to map the entire mating-type locus. The B3 locus was found to contain four pheromone precursor genes and four receptor genes. Remarkably, receptor PESTE3.3.1 has just 34 amino acid residues in its C-terminal cytoplasmic region; therefore, it seems likely to be a receptor-like gene. Real-time quantitative RT-PCR (real-time qRT-PCR) revealed that most pheromone and receptor genes showed significantly higher expression in monokaryotic cells than dikaryotic cells. The pheromone genes PEphb3.1 and PEphb3.3 and the receptor gene PESTE3.3.1 were transformed into P5 (A3B4). The transformants were mated with a tester strain (A4B4), and the progeny showed clamp connections and a normal fruiting body, which indicates the proposed role of these genes in mating and fruiting processes. This result also confirms that PESTE3.3.1 is a receptor gene. In this study, we identified pheromone and receptor genes in the B3 locus of *P. eryngii* and found that some of those genes appear to play a role in the mating and fruiting processes. These results might help elucidate the mechanism of fruiting differentiation and improve breeding efficiency.
Study of Viral Effects of the Mycovirus (LeV) and Virus-Free Commercial Line in the Edible Mushroom *Lentinula Edodes*

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dsRNA was found in malformed cultures of *Lentinula edodes* strain FMRI0339, one of the three most popular sawdust cultivated commercial strains of shiitake, and was also found in healthy-looking fruiting bodies and actively growing mycelia. Cloning of the partial genome of the dsRNA revealed the presence of the RdRp sequence of a novel *L. edodes* mycovirus (LeV), and sequence comparison of the cloned amplicon showed an identical sequence to known RdRp genes of LeV found in strain HKA. The meiotic stability of dsRNA was examined by measuring the ratio of the presence of dsRNA among sexual monokaryotic progeny. More than 40% of the monokaryotic progeny still contained the dsRNA, indicating the persistence of dsRNA during sexual reproduction. Comparing the mycelia growth of monokaryotic progeny suggested that, although variations in the growth rate existed among progeny and virus infection was observed in highly actively growing progeny, there appeared to be a tendency toward a lower frequency of virus incidence in actively growing progeny.

This study attempted to cure the edible mushroom *L. edodes* strain FMRI0339 of the *L. edodes* mycovirus (LeV) in order to obtain an isogenic virus-free fungal strain as well as a virus-infected strain for comparison. Mycelial fragmentation, followed by being spread on a plate with serial dilutions resulted in a virus-free colony. Viral absence was confirmed with gel electrophoresis after dsRNA-specific virus purification, Northern blot analysis, and PCR using reverse transcriptase (RT-PCR). Once cured, all of fungal cultures remained virus-free over the next two years. Interestingly, the viral titer of LeV varied depending on the culture condition. The titer from the plate culture showed at least a 20-fold higher concentration than that grown in the liquid culture. However, the reduced virus titer in the liquid culture was recovered by transferring the mycelia to a plate containing the same medium. In addition, oxygen-depleted culture conditions resulted in a significant decrease of viral concentration, but not to the extent seen in the submerged liquid culture. Although no discernable phenotypic changes in colony morphology were observed, virus-cured strains showed significantly higher growth rates and mycelial mass than virus-infected strains. We were also explored effects of LeV on fruiting body formation and mushroom yield. The fruiting body formation yield of virus-free *L. edodes* was larger than...
virus-infected *L. edodes*. These results indicate that LeV infection has a deleterious effect on mycelial growth and fruiting body formation. In addition, we have been investigated host-parasite interaction between *L. edodes* and its mycovirus interaction to study viral mechanism by establishment of proteomics.
Recreation of Korean Traditional Nuruk and the Analysis of Metabolomic Characteristics

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Korean traditional Nuruk has been developed with various materials and shapes according to geographical environments and climates of their origins. Nuruk is also known as kokju in Korea, reflecting the understanding that microorganisms such as wild fungi, yeasts, and lactobacillus bacteria are naturally inoculated and reproduced. The objective of this study is to identify the characteristics of traditional Nuruk through recreating traditional production methods detailed in ancient Korean documents. In the present study, a total of 58 different kinds of Korean traditional Nuruk were prepared, including 46 kinds of recreated products. Each Nuruk sample was evaluated for its enzymatic activities, including glucoamylase, protease, and glucanase. Their suitability for alcoholic beverage production were compared to each other. To isolate valuable microorganisms from Nuruk samples, alcoholic beverages produced using each sample were subjected to sensory evaluation to determine their taste. In addition, metabolite changes in traditional alcoholic beverages fermented with different kinds of Nuruk were analyzed through mass-based metabolomics approach. This study presents, for the first time, the traditional production methods written in ancient Korean documents using workable production methods supported by modern technologies. In addition, this study analyzed the characteristics of reproduced Nuruk. It could be utilized as a basis for studying traditional Korean traditional alcoholic beverages and their valuable microorganisms.
Usability of DNA Sequence Data: from Taxonomy over Barcoding to Field Detection. A Case Study of Oomycete Pathogens

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Oomycetes belong to the kingdom Straminipila, a remarkably diverse group which includes brown algae and planktonic diatoms, although they have previously been classified under the kingdom Fungi. These organisms have evolved both saprophytic and pathogenic lifestyles, and more than 60% of the known species are pathogens on plants, the majority of which are classified into the order Peronosporales (includes downy mildews, Phytophthora, and Pythium). Recent phylogenetic investigations based on DNA sequences have revealed that the diversity of oomycetes has been largely underestimated. Although morphology is the most valuable criterion for their identification and diversity, morphological species identification is time-consuming and in some groups very difficult, especially for non-taxonomists.

DNA barcoding is a fast and reliable tool for identification of species, enabling us to unravel the diversity and distribution of oomycetes. Accurate species determination of plant pathogens is a prerequisite for their control and quarantine, and further for assessing their potential threat to crops. The mitochondrial cox2 gene has been widely used for identification, taxonomy and phylogeny of various oomycete groups. However, recently the cox1 gene was proposed as a DNA barcode marker instead, together with ITS rDNA. To determine which out of cox1 or cox2 is best suited as universal oomycete barcode, we compared these two genes in terms of (1) PCR efficiency for 31 representative genera, as well as for historic herbarium specimens, and (2) in terms of sequence polymorphism, intra- and interspecific divergence. The primer sets for cox2 successfully amplified all oomycete genera tested, while cox1 failed to amplify three genera. In addition, cox2 exhibited higher PCR efficiency for historic herbarium specimens, providing easier access to barcoding type material. In addition, cox2 yielded higher species identification success, with higher interspecific and lower intraspecific divergences than cox1. Therefore, cox2 is suggested as a partner DNA barcode along with ITS rDNA instead of cox1.

Including the two barcoding markers, ITS rDNA and cox2 mtDNA, the multi-locus phylogenetic analyses were performed to resolve two complex clades, Bremia lactucae (lettuce downy mildew) and Peronospora effusa (spinach downy mildew) at the species level and to infer evolutionary relationships within them. The approaches discriminated all currently accepted species and revealed several previously unrecognized lineages, which are
specific to a host genus or species. The sequence polymorphisms were useful to develop a real-time quantitative PCR (qPCR) assay for detection of airborne inoculum of *B. lactucae* and *P. effusa*. Specificity tests revealed that the qPCR assay is specific for detection of each species. This assay is sensitive, enabling detection of very low levels of inoculum that may be present in the field. Early detection of the pathogen, coupled with knowledge of other factors that favor downy mildew outbreaks, may enable disease forecasting for judicious timing of fungicide applications.
Symposium

S13 Current Topics in Pathogens
Enterohemorrhagic *Escherichia coli* Shiga Toxins as Multi-functional Proteins are not just Cytotoxins

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Shiga toxins (Stxs) produced by Shiga toxin-producing bacteria *Shigella dysenteriae* serotype 1 and select serotypes of *Escherichia coli* are primary virulence factors in the pathogenesis of hemorrhagic colitis progressing to potentially fatal systemic complications such as the hemolytic uremic syndrome and central nervous system abnormalities. Current therapeutic options to treat patients infected with toxin-producing bacteria are limited. The structure of Stxs, toxin-receptor binding, intracellular transport, and mode of action of the toxins have been well defined. However, in the last decade, numerous studies have demonstrated that in addition to being potent protein synthesis inhibitors, Stxs are also multifunctional proteins capable of activating multiple cell stress signaling pathways which may result in apoptosis, autophagy, or activation of the innate immune response. Here we briefly present a current understanding of Stx-activated signaling pathways in host cells.
Induction of Airway Mucus Hypersecretion and Glycosylation by *Pseudomonas aeruginosa* Virulence

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Cystic fibrosis (CF), non-CF bronchiectasis and chronic obstructive pulmonary disease (COPD) are debilitating airway diseases characterized by dilated bronchi with chronic inflammation, infection and mucus hypersecretion. They affect millions of individuals with health and social costs up to billions of dollars. *Pseudomonas aeruginosa* is a major pathogen in patients with CF and advanced COPD. Antibiotic-resistant *P. aeruginosa* is widespread, necessitating a non-traditional approach to limit the exacerbation of resistance. Repeated cycles of *P. aeruginosa* infection exacerbate mucus hypersecretion and glycosylation, and airway obstruction. In turn, the thick glycosylated mucus provides a niche for *P. aeruginosa* to thrive. In this talk, I summarized our recent findings on the roles of *P. aeruginosa* virulence factors, especially the redox-active pyocyanin, in the induction of mucin hypersecretion and glycosylation. Pyocyanin causes oxidative stress, and can be recovered in 100 μM concentrations in the sputa of bronchiectasis patients chronically infected with *P. aeruginosa*. However, the importance of pyocyanin within bronchiectatic airways colonized by *P. aeruginosa* remains poorly studied. Recently, we have shown that pyocyanin is important for chronic *P. aeruginosa* lung infection in mice, and that chronic instillation of pyocyanin induces goblet cell hyperplasia and metaplasia, pulmonary fibrosis, emphysema, and influx of immune cells in mouse airways. Many of these pathological features are strikingly similar to the mouse airways devoid of functional FOXA2, a transcriptional repressor of GCHM and mucus biosynthesis. We present evidence that pyocyanin causes GCHM and mucus hypersecretion in bronchiectatic airways chronically infected by *P. aeruginosa* by inactivating FOXA2 through the upregulation of two inhibitory signaling pathways STAT6 and EGFR, both of which repress the expression of FOXA2. Furthermore, pyocyanin-generated ROS and RNS caused nitrosylation, acetylation, ubiquitination and degradation of FOXA2. Modified FOXA2 had reduced ability to bind the promoter of the MUC5B gene. The antioxidant glutathione alleviated the modification of FOXA2 by pyocyanin, and inhibited the overexpression of MUC5AC and MUC5B mucins. Finally, among the virulence factors of *P. aeruginosa* known to induce GCHM and mucus hypersecretion, pyocyanin is most potent in inducing mucin sialylation in both mouse airways and in primary and immortalized CF and non-CF human airway epithelial cells. Pyocyanin increased the expression of C2/4GnT and ST3Gal-IV, two of the glycosyltransferases responsible for the stepwise biosynthesis of sialyl-Lewisx, through a TNF-α-mediated phosphoinositol-specific phospholipase C dependent pathway. Furthermore, *P. aeruginosa* bound more efficiently...
to airway epithelial cells pre-exposed to pyocyanin through a flagellar cap-dependent manner. Importantly, antibodies against sialyl-Lewisx and anti-TNF-α attenuated *P. aeruginosa* binding. Collectively, our results indicate that *P. aeruginosa* secretes pyocyanin to induce a favorable environment for chronic colonization of CF lungs by increasing the mucus hypersecretion and glycosylation.
Exportation of the *Pseudomonas* Quinolone Signal (PQS) Synthase PqsH: a New Puzzle Piece Supporting That Extracellular 4-Hydroxy-2-Heptylquinoline (HHQ) is a Sentinel Signal Precursor

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*Pseudomonas aeruginosa* is an opportunistic bacterial pathogen responsible diseases in immuno-compromized individuals. Its infectious potential relies on virulence factors that can be synthesized simultaneously by all bacterial cells within a given population. This coordination phenomenon, known as quorum sensing (QS), depends on transcriptional regulators LasR, RhlR, MvfR (PqsR) and their cognate autoinducers (3-oxo-C₁₀-homoserine lactone [HSL], C₄-HSL and 3,4-dihydroxy-2-heptylquinoline [*Pseudomonas* Quinolone Signal; PQS], respectively). Autoinducers are secreted in the environment and when threshold concentrations are reached, regulator/autoinducer complexes bind to QS-dependent gene promoters to regulate gene expression. Interestingly, PQS is first synthesized as a precursor. Indeed, 4-hydroxy-2-heptylquinoline (HHQ) is produced by the MvfR-dependent enzymes PqsABCD and then, converted into PQS by the LasR-dependent enzyme PqsH. Furthermore, *mvfR* transcription itself relies on LasR. Likewise, PqsH as well as HHQ are synthesized earlier than PQS accumulates and are both believed to accumulate into the cytoplasm simultaneously. In contrast to acyl-homoserine lactones, which can be secreted by efflux pumps, we have shown that HHQ can be pumped out only in constitutive MexEF-OprN efflux *nfxC* mutants. Together, these observations prompt us to look for the sorting mechanisms for HHQ (endogenous versus exogenous), considering that PqsH has rapidly access to plenty amount of HHQ whereas no PQS synthesis occurs. We show that PqsH is actually translocated into the periplasm, owing to a C-terminal alpha-helix. We present an integrative model showing the central position of PqsH in *P. aeruginosa*’s QS circuitry, revealing its central role in MvfR QS regulation. This research redefines hierarchical positions of *P. aeruginosa* QS players and relates this new model to pathogenesis.
Functional, Biochemical, and Proteomic Analyses of a Prokaryotic Tyrosine Sulfotranferase

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Tyrosine sulfation, a well-characterized post-translation modification in eukaryotes, has not previously been reported in prokaryotes. Xanthomonas oryzae pv. oryzae (Xoo), a causal agent of rice blight disease, is a Gram-negative bacterium and possesses a homolog of tyrosine sulfotransferase, raxST. To test RaxST have tyrosine sulfotransferase activity, a newly developed sulfotransferase assay and a novel UV photodissociation LC-MS/MS method were employed and demonstrated that RaxST catalyzed sulfation of tyrosine 22 of the Xoo Omp1X (outer membrane proteins 1 in Xoo). Structural prediction performed on the protein sequence of Omp1X reveals that it encodes a putative porin-like protein, possessing a β-barrel domain with 10 β-strands and a signal peptide at the N-terminus. The protein expression level in a knock out mutant of Omp1X (XooΔomp1X) was compared in the wild type using a label-free shotgun proteomic approach. Clusters of Orthologous Groups analysis revealed that differentially expressed proteins (1.5 fold) were most likely involved in cell motility. Phenotypic analysis also demonstrated that motility and biofilm formation in XooΔomp1X are lower than the wild type, indicating the Omp1X is involved in bacterial movement. Furthermore, a comparative proteomic analysis with a RaxST knock out mutant (XooΔraxST) also showed that proteins related in cell motility were mostly abundant in differentially expressed proteins (1.5 fold). The observed motility of XooΔraxST was similar with that of XooΔomp1X but not the wild type, pointing out sulfation by RaxST is also required for bacterial movement. These results provide new insights into the functions of prokaryotic sulfation in Gram-negative bacteria.
Investigation of the Role of *Mycobacterium tuberculosis* Rv2019-Rv2018 Toxin-antitoxin System in *E. coli*

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Toxin-antitoxin (TA) systems composed of an intracellular toxin and its antidote (antitoxin) are ubiquitous genetic modules in prokaryotes. Commonly, activity of toxin is inhibited by antitoxin under normal growth condition. However, antitoxins are degraded in response to environmental stress conditions and toxins liberated from antitoxin consequently induce cell death or growth inhibition. In free-living prokaryotes, TA systems often present in large numbers are considered to be associated with adaptation of pathogenic bacteria or extremophiles to various unfavorable environments by shifting into slowly growing state. By performing BLAST searches of the Mycobacteria genome, we found a large number of TA systems in human pathogen *Mycobacterium tuberculosis* H37Rv (*Mtb*). Accordingly, we investigated uncharacterized five TA systems (Rv2019-Rv2018, Rv3697c-Rv3697A, Rv3180c-Rv3181c, Rv0299-Rv0298, and Rv3749c-Rv3750c) in *Mtb*. Among them, expression of Rv2019 toxin caused growth inhibition of *Escherichia coli* and this growth defect induced by Rv2019 toxin was recovered by expression of Rv2018 antitoxin in *E. coli*. *In vivo* and *in vitro* studies showed ribosomal RNA (rRNA) cleavage activity of Rv2019 toxin via a divalent metal ion-independent manner *in vitro*. Consequently, we concluded that ribonuclease activity of Rv2019 toxin triggers growth defect in *E. coli* and Rv2018 antitoxin inhibits ribonuclease activity of Rv2019 toxin.
Symposium

S14 Norovirus Detection and Field Applications

International Meeting of the Federation of Korean Microbiological Societies
Application of Macrofoam Swab Based Sampling Method for the Investigation and Control of Human Norovirus Infections

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Human noroviruses (HuNoV) are the leading cause of epidemic and sporadic acute gastroenteritis worldwide. Most outbreaks are reported in semi-closed environments such as long-term care facilities, hospitals and schools. Because the majority of infections are either spread directly via person-to-person route or indirectly through environmental surfaces or food, the role of contaminated fomites and inanimate surfaces are regarded as important vehicles for the spread of these viruses during outbreaks. Since there are no recommended vaccines for HuNoV, personal and environmental hygiene are suggested as key measures to interrupt the cycle of HuNoV infections.

Unfortunately, little is known about the correlation between the level of surface contamination and increased risk of norovirus infection. This lack of understanding may affect the implementation of adequate hygiene practices. Recently, we have establish a macrofoam swab based surface sampling protocol to detect norovirus contamination on the environmental surfaces. Field application of macrofoam swabs at long term care facilities and cruise ships allowed us to investigate norovirus outbreaks for determination of bioburdens and distributions of HuNoV. Also, we were able to identify circulating strains even when there were no clinical specimen available. Overall, our macrofoam swab based surface sampling could serve as an effective norovirus tracking tool. Furthermore, surface sampling results would be useful in the understanding of transmission dynamic of HuNoV and would help in the improvement of current HuNoV control strategy.
Super Resolution Microscopy for Studying Virus Infection

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Molecular Imaging is a new discipline that unites molecular & cellular biology and \textit{in vivo} imaging. It enables the visualization of the cellular function and the follow-up of the molecular process in living organisms without perturbing them.

Super-resolution optical microscopy allows the capture of images with a higher resolution than the diffraction limit of light microscope, allowing visualization of bridging the gap between conventional fluorescence microscopy and electron microscopy. We summarize here findings on norovirus using super resolution microscopic technique.
Norovirus Detection and Field Applications

Development of Foodborne Virus Concentration and Detection Methods

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Norovirus (NoV), a non-enveloped single-stranded RNA virus, causes acute gastroenteritis in humans. Considerable attention has been given to develop a rapid and sensitive method for the detection of the virus. Immunomagnetic separation (IMS) removes PCR inhibitors and reduces time-consuming concentration steps for the detection of virus in food sample, however, this method requires expensive antibodies for the binding of virus. To identify an alternative for NoV-specific antibody dependent IMS, the relative binding affinities of NoV genotype GII.4 to eight lectins, Con A, DBA, HPA, PNA, PTA, UEA, and WGA were evaluated using an ELISA, and confirmed the affinities of different subtype of NoV using surface plasmon resonance. Con A from jack-bean showed significantly higher (relative binding: >2-fold of control) relative binding affinity to NoV than other lectins tested. The SPR analysis showed that the equilibrium dissociation constants of Con A to NoV genotypes GII.4, GI.4, and GII.3 were 2.19, 4.47, 238 nM, respectively, indicating that NoV genotype, GII.4 bound Con A with higher affinity than other genotypes and con A was capable to substitute the antibodies required for NoV attachment. A new Con A-linked magnetic bead combined with quantitative reverse transcription (RT)-PCR assay detected NoV RNA level of $10^1$ to $10^6$ copies/ml. This method is a rapid, sensitive and cost-effective to detect NoV from artificially and naturally contaminated food.
Speeding up Norovirus Diagnostics, SolGent Norovirus Detection System

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Noroviruses are estimated to be responsible for 60 to 80% of all human gastroenteritis outbreaks worldwide. Although the main mode of transmission in health institutions is person to person, norovirus are also efficiently transmitted via food, water, and contaminated surfaces. Members of the Caliciviridae family, they are subdivided into five genogroups (genogroup I [GI], GII, GIII, GIV, and GV), and GI, GII, and GIV have been detected in humans. GII has been shown to account for the majority (up to 92%) of reported norovirus gastroenteritis cases, and GI accounts for the large majority of the remaining cases.

Let us introduce a novel “NoroGlue protein”. It is able to concentrate norovirus rapidly and replace expensive anti-norovirus antibody and other methods. The recent advances proved that Concanavalin A (Con A) called as NoroGlue protein has a specific strong interaction with human norovirus and can be applied to concentration and detection. The binding affinity of NoroGlue protein with human norovirus is demonstrated by isothermal titration calorimetry for protein-protein binding strength measurement. Specific interaction and strong binding are the key aspects for Norovirus diagnosis. Our enrichment system is designed for the disposable solid phase extraction type syringe. NoroGlue protein immobilized resin can do concentrate norovirus rapidly from multiple liquid samples. Our system only takes 15 min to concentrate norovirus from 200 ml liquid samples.

SolGent is a molecular diagnostics company based on research reagent and genome analysis service. Adhering to global standards (ISO9001:2008, ISO13485:2003) we produce and maintain directly the most important element in the reagent, high-quality enzyme for enhancing the precision and price competitiveness. SolGent provides Molecular Diagnostics Kits based on Multiplex PCR. They can detect multiple specific target genes in a single PCR. SolGent Molecular Diagnostics Kits has ultra-high specificity & sensitivity using o“HotStart DNA polymerase” and specialized novel buffer system.
Dengue virus (DENV) is the leading cause of vector-borne viral disease globally with an estimated 390 million infections per year of which approximately 96 million lead to symptomatic disease. The burden of dengue has been increasing over the past few decades presumed to be due to increased urbanization, ease of global travel, ecological changes, and perhaps climate effects. Severe forms of dengue which were rare outside of Southeast Asia in the 1970’s are now more commonly seen in dengue endemic countries as these areas become hyperendemic with more than one DENV serotype. Vector control measures have been implemented in some form in most affected countries, but the impact of such efforts have been limited. The general consensus is that an integrated approach involving both vector control and vaccine introduction will be needed to reverse the dengue pandemic. Currently, six different dengue vaccine candidates have been tested in various phases of human clinical trials. The most advanced candidate, CYD-TDV, sponsored by Sanofi Pasteur, is a live attenuated tetravalent chimeric vaccine consisting of a 17D yellow fever backbone with DENV pre-membrane and envelope proteins from the four different DENV serotypes. CYD-TDV has undergone phase III clinical trials in Asia and Latin America with mixed results. In particular, the vaccine had poor efficacy against serotype-2 and marginal efficacy against serotype-1 despite having good immunogenicity to all four serotypes based on neutralization antibody assays. In addition, an increased risk of dengue hospitalization was noted in very young vaccinated children during the third year of the Asian phase III trial. Nevertheless, the vaccine appeared to have greater overall efficacy against more severe disease with no apparent safety signal among older vaccinated children. This has led to the possibility of limited vaccine introduction in older children and adults in some dengue endemic countries. Questions linger, however, about the reasons for the suboptimal efficacy and safety signal in very young children which may have implications not only for CYD-TDV but also for other vaccine candidates. Two other dengue vaccine candidates in addition to CYD-TDV are close to entering phase III clinical trials. DENVax, sponsored by Takeda, is a live attenuated chimeric vaccine that uses a DENV-2 backbone with pre-membrane and envelope proteins from the four serotypes. TV005, developed by U.S. NIH, is a live attenuated tetravalent vaccine which has undergone direct mutagenesis for three serotypes while the fourth serotype consists of a DENV-DENV chimera. Whether these and other vaccine candidates in development will have different results from CYD-TDV is unclear. However, the next few years are sure to bring new developments that will impact the global effort to control dengue.
Vaccination is a major intervention method used to mitigate the disease burden of influenza in humans. However, the effective spectrum of influenza vaccine is too narrow to offer protection against antigenically drifted strains. This incurs a limited vaccine effectiveness, as was observed against influenza H3N2 viruses in Korea in the 2011-2012 season. To investigate the molecular evidence of reduced vaccine effectiveness in recent years, here we show the genetic mutations and evolutionary dynamics of H3N2 viruses circulating between 2008 and 2015. The results of hemagglutination inhibition assay and genealogical relationship suggested a substantial distance between vaccine and circulating H3N2 strains. The distance was then quantified by a large number of nonsynonymous mutations within the antigenic sites (A, B, C, D, and E) of the hemagglutinin (HA) protein. With seasonal variations of its association, the epitope distance detected in the antigenic sites B and D exhibited the sustained levels of nonsynonymous mutations. Of the mutations that appeared positively selected, the genetic signature in relation to anchoring N-linked sugar side chains showed an increasing evolutionary competence with changing vaccine formulations. While having been introduced or lost, the overall glycosylation status also appeared to co-evolve with the selected sites in the epitope regions. By providing specific measurements of epitope distances involving changing glycosylation patterns and co-evolving genetic alterations, our work contributes substantially to our understanding of vaccine effectiveness and the genetic network of epitope dynamics engraved in the HA of influenza viruses.
World-wide Threat of Coronaviruses (PEDV and MERS-CoV)

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Coronaviruses may infect many different animals and cause them to have respiratory, gastrointestinal, liver, and neurologic diseases. Most of these coronaviruses usually infect only one animal species or, at most, a small number of closely related species. However, some coronaviruses, like the one that caused SARS and MERS can infect people and animals. In field of veterinary virology, coronaviruses have caused damage to livestock industry continuously. Porcine epidemic diarrhea virus (PEDV), an Alphacoronavirus in the family Coronaviridae, causes acute diarrhea, vomiting, dehydration, and high mortality rates in neonatal piglets. PEDV can also cause diarrhea, agalactia, and abnormal reproductive cycles in pregnant sows. Although PEDV was first identified in Europe, it has resulted in significant economic losses in many Asian swine-raising countries, including Korea, China, Japan, Vietnam, and the Philippines. However, from April 2013 to the present, major outbreaks of PEDV have been reported in the USA, Canada, and Mexico. Moreover, intercontinental transmission of PEDV has increased mortality rates in seronegative neonatal piglets, resulting in 10% loss of the US pig population. The emergence and re-emergence of PEDV indicates that the virus is able to evade current vaccine strategies. Continuous emergence of multiple mutant strains from several regions has aggravated PED endemic conditions and highlighted the need for new vaccines based on the current circulating PEDV.

Coronavirus of Middle East was also identified in animals, mainly in camels, and it infected people causing a respiratory syndrome, MERS (Middle East Respiratory Syndrome), in several countries. The MERS-CoV spread to other countries and caused grave damage to South Korea recently. To prevent new emerging zoonosis in advance, the development of diagnosis is strongly emphasized. We present here a rapid immunochromatographic assay for the detection of Middle East respiratory syndrome coronavirus (MERS-CoV) antigen in the nasal swabs of dromedary camels. The assay is based on the detection of MERS-CoV nucleocapsid protein in a short time frame using highly selective monoclonal antibodies at room temperature. The relative sensitivity and specificity of the assay were found to be 93.90% and 100%, respectively, compared to that of the UpE and open reading frame 1A (Orf1A) real-time reverse transcriptase PCR (RT-PCR). The results suggest that the assay developed here is a useful tool for the rapid diagnosis and epidemiological surveillance of MERS-CoV infection in dromedary camels.
Therapeutic Effects of Plasmapheresis on Severe Fever with Thrombocytopenia Syndrome

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Background: Severe fever with thrombocytopenia syndrome (SFTS) has been an emerging hemorrhagic fever disease in Korea since 2013. The mortality rate in South Korea was about 47.2% in 2013. The number of infections and resulting deaths has been growing, but no effective treatment has been determined yet.

Objectives: We introduced this study to investigate the efficacy of therapeutic plasmapheresis in confirmed SFTS patients in a tertiary hospital between May 2013 and July 2015.

Study Design: We were conducted an observational study from 2013 to 2015 on Jeju Island in South Korea. A confirmed SFTS patients underwent plasmapheresis among severe cases.

Results: A total of 21 confirmed SFTS patients, who ranged in age from 45 to 82, had mostly been performing outdoor activities before admission. Laboratory findings showed decreased white blood cell counts, neutrophils and platelets and elevated activated partial thromboplastin time, aspartate aminotransferase, lactate dehydrogenase, and creatine kinase. Thirteen patients who underwent plasmapheresis treatment showed improved a clinical course and laboratory results including viral loads decrease.

Conclusions: Our data suggest that there might be a role of using plasmapheresis in patients who severe SFTS to improve their clinical course and survival.
Symposium

S16 Microbiome and Diseases

International Meeting of the Federation of Korean Microbiological Societies
Regulation of Insulin Sensitivity by Gut Microbiota Alteration via GLP-1

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Firmicutes and Bacteroidetes, 2 major phyla of gut microbiota, are involved in lipid and bile acid metabolism to maintain systemic energy homeostasis in host. Recently, accumulating evidence has suggested that dietary changes promptly induce the alteration of abundance of both Firmicutes and Bacteroidetes in obesity and its related metabolic diseases. Nevertheless, the metabolic roles of Firmicutes and Bacteroidetes on such disease states remain unclear. In this study, we would like to determine the effects of antibiotic-induced depletion of Firmicutes and Bacteroidetes on dysregulation of energy homeostasis in obesity. Treatment of C57BL/6J mice with the antibiotics (vancomycin [V] and bacitracin [B]), in the drinking water, before diet-induced obesity (DIO) greatly decreased both Firmicutes and Bacteroidetes in the gut as revealed by pyrosequencing of the microbial 16S rRNA gene. Concomitantly, systemic glucose intolerance, hyperinsulinemia, and insulin resistance in DIO were ameliorated via augmentation of GLP-1 secretion independently of obesity as compared with untreated DIO controls. Furthermore, there were increases in metabolically beneficial metabolites derived from the gut. Together, our data suggest that Firmicutes and Bacteroidetes potentially mediate insulin resistance through modulation of GLP-1 secretion in obesity.
Recent molecular studies into the microbial diversity of the human intestine revealed a much higher diversity than previously recognized. One of the most dominant intestinal microbes, bifidobacteria, has been suggested to be associated with good intestinal health given their most dominance in the feces of breast-fed infants. While many dairy products containing bifidobacteria have been consumed world-wide for health promotion purpose, recent clinical feeding studies suggested that they cannot remain in the human gut. To answer the question, complete genome sequencing and comparative genome analysis of intestinal and commercial bifidobacteria were conducted, revealing that commercial bifidobacteria may lose competitive fitness when grown outside the human gut. Subsequent genome analysis and additional experiments showed that commercial bifidobacteria have lost important functional genes for intestinal survival, such as multiple oligosaccharide utilization gene clusters, arsenic resistance operon, and lantibiotic operon, probably due to their rapid genome adaptation capabilities. This rapid genome adaptation of bifidobacteria may be derived from hyperactivity of IS30 in their genomes. Therefore, to preserve those important functional genes in bifidobacterial genomes, development of new concepts for incubation culture and storage methods of bifidobacteria is required, probably mimicking human intestinal environments.

To elucidate the roles of bifidobacteria and initial intestinal microbiota in new-born infants, composition and development of initial infantile intestinal microbiota should be understood. Therefore, three kinds of meconium samples from breast-fed and bottle-fed infants were collected and composition of their intestinal microbiota were analyzed using random cloning/sequencing and subsequent metagenomic analysis of 16S rRNA PCR products using newly developed 16S universal PCR primers. Interestingly, more than 70% of infantile intestinal microbiota in 1-week-old infantile fecal samples is *B. longum* and *Streptococcus salivarius*, unlike previous reports reporting that more than 90% is *B. infantis*. Probably, these two major bacteria detected in initial intestinal fecal samples may be derived from mothers’ vagina during delivery.
Post-fermented Green Tea Extract Reduces Body Weight, Alleviates Glucose Intolerance and Fatty Liver, and Alters Gut Microbiota Composition in Diet-induced Obese Mice

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Obesity, a medical condition of excess fat mass due to increase of calorie intake or decrease of energy expenditure, is a risk factor of numerous metabolic disorders including hyperglycemia, hyperlipidemia, hypercholesterolemia, type 2 diabetes, and cardiovascular diseases. Among the causal factors of obesity, compositional change of gut microbiota is reported to be closely associated with the development of obesity and obesity-related metabolic disorders. Here we propose that post-fermented green tea extract (FGT) exhibits anti-obesity effect and changes gut microbiota composition in obese mice. FGT reduced body weight gain and decreased fat mass without food intake change in high fat-fed obese mice. mRNA expression of lipogenic genes and inflammatory genes was also down-regulated in white adipose tissue of FGT-administered high fat-fed mice. Additionally, FGT treatment alleviated glucose intolerance and fatty liver, common complications of obesity. FGT also restored compositional change of gut microbiota (e.g. Firmicutes/Bacteroidetes ratio and Bacteroides/Prevotella ratio) induced by high-fat diet. Collectively, FGT would be a useful agent to treat obesity and related complications by modulating gut microbiota and lipid metabolism.
A significant advancement in life sciences in recent years is the discovery of humans being symbionts with microbes. These microbes play a pivotal role in human physiology and their malfunction is linked to modern diseases, including allergic diseases, inflammatory bowel diseases, metabolic syndrome, obesity, and many more. This finding triggered a paradigm shift in human biology and medicine. In my laboratory, we are dissecting the interrelationship between aberrant functions in microbiome (microbial community and their collective genomes) and human diseases. We recently discovered that strain-level dysbiosis in microbiome underlies the onset and/or progression of atopic dermatitis. Such understanding of microbiome will be further developed and eventually translated into diagnostic means and therapy for the modern diseases that are currently difficult to cure.
Symposium

S17 Cell Signaling in Host-Microbe Interactions
Signal-specific Temporal Response by the *Salmonella* PhoP/PhoQ System: Regulatory Mechanism and a Distinct Type of Behavior

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The two-component system PhoP/PhoQ controls a large number of genes responsible for a variety of physiological and virulence functions in *Salmonella enterica* serovar Typhimurium. The sensor PhoQ, responding to extracytoplasmic low Mg\(^{2+}\), mildly acidic pH, and antimicrobial peptides, promotes the active form (i.e. PhoP-P) of the regulator PhoP. PhoP-P binds to its target promoters, recruiting RNA polymerase, thereby advancing transcription of the PhoP-activated genes. We show that full transcription of a subset of PhoP-activated genes requires the Mg\(^{2+}\) transporter MgtA when the PhoQ inducing signal is low Mg\(^{2+}\), but not mildly acidic pH or the antimicrobial peptide C18G. MgtA enhances PhoP-P levels by transporting Mg\(^{2+}\) away the periplasm, where it functions as a repressing signal for PhoQ. Because transcription elongation into the *mgtA* coding region is controlled by the Mg\(^{2+}\)-responding *mgtA* leader, MgtA protein and the resulting higher levels of PhoP-P are produced only when cytoplasmic Mg\(^{2+}\) levels drop to below a certain threshold. This regulatory architecture creates a two-tiered temporal response among PhoP-activated genes based on their requirement of MgtA for maximal expression. The PhoP-activated genes most dependent on the MgtA protein specify proteins of unknown function, and are not required for virulence, suggesting that the investigation of MgtA-dependent phenotype might reveal a novel aspect(s) of *Salmonella*’s lifestyle outside animal. We show that MgtA-dependent pathway governs a form of surface migration that does not appear to involve flagella or fimbriae. We establish that this motility requires the PhoP- and MgtA-activated *pagM* gene specifying a small protein of unknown function. The *pagM* gene is rarely found outside subspecies I of *S. enterica* and often present in nonfunctional allelic forms in organisms lacking the identified motility. Deletion of the *pagM* gene reduced bacterial replication on 0.3% agarose low Mg\(^{2+}\) media but not in low Mg\(^{2+}\) liquid media. Our findings define a form of motility that allows *Salmonella* to scavenge nutrients and to escape toxic compounds under conditions unfavorable to flagella-mediated motility.
Characterization of Prohibitin 1 as a Host Partner of Vibrio Vulnificus RtxA1 Toxin

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Vibrio vulnificus, an opportunistic human pathogen, causes fatal septicemia and necrotic wound infections that result in death within a few days. V. vulnificus is highly cytotoxic to eukaryotic cells, and this cytotoxicity is regarded as a hallmark of the pathogenicity. V. vulnificus produces 3 major cytotoxins, namely, elastolytic protease, cytolitic hemolysin, and RTX (RtxA1) toxin. RtxA1 is a multifunctional cytotoxin that plays an essential role in V. vulnificus infection. V. vulnificus RtxA1 is a composite toxin comprised of repeat-containing regions and five centrally located putative effector domains and their distinct roles have been demonstrated. Although V. vulnificus RtxA1 is highly homologous to V. cholerae MARTX toxin, a domain (named as RtxA1-D2), which corresponds to amino acids 1950-2574 of V. vulnificus 29307 RtxA1 containing domain 1 of unknown function (DUF1), did not show similarity to known proteins by BLAST search. Therefore, we hypothesized that RtxA1-D2 may confer different biological functions to V. vulnificus RtxA1 compared with those of V. cholerae MARTX. HeLa cells expressing green fluorescent protein-RtxA1-D2 became round and lost their viability. A yeast 2-hybrid system identified prohibitin (PHB) 1 as the host partner of RtxA1-D2. The specific interaction of RtxA1-D2 with PHB1 was confirmed by performing immunoprecipitation. Interestingly, V. vulnificus RtxA1 up-regulated PHB1 expression on the cytoplasmic membrane of host cells. Extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways were confirmed as being important in the up-regulation of PHB1 by using inhibitors. Down-regulation of PHB1 by small interfering RNAs decreased the cytotoxicity of RtxA1-D2 against HeLa cells. The pretreatment of an anti-PHB1 antibody impaired the cytotoxicity of V. vulnificus RtxA1. These results suggest that the involvement PHB1 in the RtxA1 cytotoxicity has significant implications for the pathogenesis of V. vulnificus infections.
Roles of Innate Immune Regulators against Viral Infection

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RIG-I is a key cytosolic sensor that detects RNA viruses through its C-terminal region and activates the production of antiviral interferons (IFNs) and proinflammatory cytokines. While post-translational modification has been demonstrated to regulate RIG-I signaling activity, its significance for the sensing of viral RNAs remains unclear. Here, we first show that the RIG-I C-terminal region undergoes deacetylation to regulate its viral RNA sensing activity and that the HDAC6-mediated deacetylation of RIG-I is critical for viral RNA detection. HDAC6 transiently bound to RIG-I and removed the lysine 909 acetylation in the presence of viral RNAs, promoting RIG-I sensing of viral RNAs. Depletion of HDAC6 expression led to impaired antiviral responses against RNA viruses, but not against DNA viruses. Consequently, HDAC6 knockout mice were highly susceptible to RNA virus infections compared to wild-type mice. These findings underscore the critical role of HDAC6 in the modulation of the RIG-I-mediated antiviral sensing pathway.
Defensive Function of Aminoacyl-tRNA Synthetases Against Viral Infection

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Aminoacyl-tRNA synthetases (aaRSs) are ubiquitous housekeeping enzymes responsible for charging amino acids to their cognate tRNAs and providing the substrates for global protein synthesis. During the evolution of higher eukaryotes, cytoplasmic aaRSs have undergone significant changes to add new domains which are associated with a broad range of biological functions beyond protein synthesis. Among these aaRSs, nine aaRSs in association with three auxiliary proteins are organized in a multiprotein complex called multi-tRNA synthetase complex (MSC). The MSC assembly is in most cases mediated by the evolutionarily obtained new domains. Under stress conditions, some MSC components, such as GluProRS, LysRS, AIMP2 and AIMP3, are released from the complex through their posttranslational modifications to provide nontranslational functions including inflammation, angiogenesis and tumorigenesis. Recently, we performed a global transcriptome analysis to assess the MSC gene expression profile in the influenza virus-infected mucosal cells. Heterogeneous expression and temporal fluctuation of the MSC genes were observed in the infected cells, suggesting their defensive roles against viral infection. Among the MSC members tested, several proteins showed potential functions to induce type I interferons, leading to antiviral immune responses. This presentation will focus mainly on the function of GluProRS to protect cells against viral infection.
Symposium

S18 Host Defense against Viral Infection

International Meeting of the Federation of Korean Microbiological Societies
IL-4-Induced Innate CD8+ T Cells Control Persistent Viral Infection

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Memory-like CD8+ T cells expressing eomesodermin are a subset of innate T cells initially identified in a number of genetically modified mice, and also exist in wild mice and human. The acquisition of memory phenotype and function by these T cells is dependent on IL-4 produced by PLZF+ innate T cells; however, their physiologic function is still not known. Here we found that these IL-4-induced innate CD8+ T cells are critical for accelerating the control of chronic virus infection. In CIITA-transgenic mice, which have a substantial population of IL-4-induced innate CD8+ T cells, this population facilitated rapid control of viremia and induction of functional anti-viral T-cell responses during infection with chronic form of lymphocytic choriomeningitis virus. Characteristically, anti-viral innate CD8+ T cells accumulated sufficiently during early phase of infection. They produced a robust amount of IFN-γ and TNF-α with enhanced expression of a degranulation marker. Furthermore, this finding was confirmed in wild-type mice. Taken together, the results from our study show that innate CD8+ T cells works as an early defense mechanism against chronic viral infection.
ZBTB32 and Blimp-1 Cooperate in the Epigenetic Programming of Both CD8+ Effector and Memory T Cells During LCMV Infection.

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Virus infections induce CD8+ T cell responses comprised of a large population of terminal effector cells and a smaller subset of long-lived memory cells. We have previously shown that a transcription factor Blimp-1 (encoded by Prdm1) acts as an epigenetic regulator and enhances the numbers of short-lived effector cells, while suppressing the development of memory-precursor CD8+ T cells. Recently, we found a second transcription factor as one of IL-2-induced factors in CD8+ T cells, which was the transcription factor ZBTB32/ROG. Several members of the POK family have been implicated in T and B cell differentiation. Here we show that one of the members, ZBTB32 plays a unique non-redundant role in negatively regulating T cell response and memory generation during acute LCMV infection. The transcription factor ZBTB32 was transiently expressed following CD8+ T cell activation. In the absence of ZBTB32, virus-specific CD8+ T cell effector responses were increased and memory generation was enhanced, whereas persistent expression of ZBTB32 suppressed memory cell formation. ZBTB32 recruited histone modifying enzymes and bound cooperatively with Blimp-1 to repress target genes. Genome-wide analysis indicated that ~60% of genes differentially expressed in responding virus-specific CD8+ T cells shared binding sites for Blimp-1 and ZBTB32. The disregulation of CD8+ T cell responses in the absence of ZBTB32 was catastrophic, as Zbtb32−/− mice succumbed to a systemic viral infection and showed evidence of severe lung pathology.
Microneedle Patches for Improved Influenza Vaccination

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Influenza is a vaccine-preventable disease, but remains a major health problem world-wide. Morbidity and mortality due to influenza could be reduced by development of simple and effective vaccination methods. Immunization via the skin is attractive, because, in large part, the skin is replete with antigen-presenting cells such as Langerhans and dermal dendritic cells. Arrays of metal micron-scale needles were coated with influenza inactivated virus vaccines suitable for simple, manual application. A single dose of influenza vaccine from microneedles (MNs) generated strong antibody and cellular immune responses in mice and provided superior protection against lethal viral challenge at the main site of viral replication in the lung, as evidenced by virus clearance below the detection limit. Additionally, microneedle vaccination resulted in enhanced cellular recall responses after challenge. In contrast to conventional egg-based vaccine production, cell-based vaccines are being developed to expedite vaccine manufacturing and thereby reduce the threat of insufficient supply. Virus like particles (VLPs) and DNA vaccines are attractive cell-based vaccines and the vaccinations using MN patch coated with VLP or DNA demonstrated dose-sparing effects of influenza vaccine. Apart from immunologic advantages, microneedles also offer potential logistic opportunities. The small size of microneedles should facilitate storage, stockpiling and transportation of influenza vaccines. Vaccination should be faster and simpler because microneedles are painless and suitable for self administration. Mass-produced microneedles would be cost-competitive with hypodermic needle and syringe. In summary, microneedle vaccination may provide a new modality to increase patient coverage and improve immunogenicity of influenza and other vaccines.
Baculovirus-based VLP Forming DNA Vaccine for Influenza pdmH1N1

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The first identification of swine-originated influenza A/CA/04/2009 (pH1N1) as the cause of an outbreak of human influenza accelerated efforts to develop vaccines to prevent and control influenza viruses. Here, we constructed a human endogenous retrovirus (HERV) envelope-coated, non-replicable, baculovirus-based virus like particle (VLP) forming DNA vaccine against pandemic influenza A/California/04/2009 (pH1N1). Previous we reported the efficacy of influenza HA DNA vaccine using a baculoviral delivering system (AcHERV-HA). However, AcHERV-HA vaccine only elicits immune response against HA antigen, and showed a weak immunogenicity compare to that of the whole killed vaccine. For that reason, we constructed a recombinant baculovirus carrying HA, NA and M genes for forming VLP in host cell. AcHERV HA-NA-M showed VLP formation in mammalian cell and induced high level of humoral and cellular immune response in mice. In challenge test, mice immunized with the AcHERV HA-NA-M were protected from lethal dose of challenge with pH1N1 and showed better clinical signs comparable to that of killed virus vaccines. These results suggested that VLP forming DNA vaccine (AcHERV HA-NA-M) could be a potential prophylactic vaccine candidate against influenza H1N1.

Keywords: Influenza, Recombinant baculovirus, Virus-like particle, Immune response, DNA Vaccine
Symposium

S19 Yeast System for Comparative Functional Genomics
Structural Mechanism of Ergosterol Regulation and Antifungal Resistance by Fungal Sterol Transcription Factor Upc2

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Azole drugs widely used for the treatment of fungal infections inhibit the biosynthesis of ergosterol, an essential component of fungal plasma membrane. Transcriptional regulation of ergosterol biosynthesis in fungi is crucial for sterol homeostasis and for resistance to azole drugs. In *Candida* species and *Saccharomyces cerevisiae*, the Upc2 transcription factor activates the expression of related genes in response to sterol depletion by poorly understood mechanisms.

In this study, in order to obtain a structural insight into the regulatory mechanism of Upc2, we have determined the structure of the C-terminal domain (CTD) of Upc2, which displays a novel α-helical fold with a deep hydrophobic pocket. We discovered that the conserved CTD is a ligand-binding domain and senses the ergosterol level in the cell. Ergosterol binding represses its transcription activity while dissociation of ligand leads to relocalization of Upc2 from cytosol to nucleus for transcriptional activation.

Our findings highlight Upc2 represents a novel class of fungal zinc cluster transcription factors which can serve as a target for the developments of anti-fungal therapeutics. Upc2 LBD displays a novel fold of ligand binding domain and a deep hydrophobic pocket which could serve as an excellent pharmacopore for the design of small molecule inhibitors. Inhibition of Upc2, which subsequently suppresses the adaptive responses of fungal cells to azoles, could be a novel strategy to improve the combined therapy with antifungal agents.
**S19-2**

**Direct Interaction of Ste11 and Mkk1/2 through Nst1 Integrates High Osmolarity Glycerol to the Cell Wall Integrity MAPK Pathway in Budding Yeast *S. cerevisiae***

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Environment stresses cause cellular responses through activating mitogen-activated protein kinase (MAPK) pathways in *Saccharomyces cerevisiae*. The coordination and cross-talks of MAPK pathways are critical for signaling efficiency, but their mechanisms are not well understood. The cell wall integrity MAPK pathway (CWI pathway) is comprised of Pkc1, Bck1, Mkk1/Mkk2 and Slt2, and activated by heat stress. However, Slt2 is also known to be activated in the absence of upstream Pkc1 or Bck1, suggesting the presence of another mechanism to activate Slt2. Nst1 is a novel protein in *S. cerevisiae*. In this study, we showed that Δnst1 is hypersensitive to heat stress. The activation of Slt2 is delayed in Δnst1 in response to heat stress. We also revealed that the upstream components of HOG pathway, Sho1, Ste20, Ste50 and Ste11, are involved in heat stress response. Nst1 physically and physiologically connects Ste11 to Mkk1. These results suggest that Sho1-Ste20-Ste50-Ste11 branch of the HOG pathway is connected to Mkk1/Mkk2-Slt2 of the CWI pathway with the help of Nst1. Notably, we also found two new phosphorylation residues Ser407 and Thr411 on Mkk1 that are likely phosphorylated by Ste11. Taken together, Nst1, as a novel adaptor protein, might assemble a novel MAPK module which connects HOG and CWI MAPK pathway.
Lifespan Regulation by Actin Dynamics Regulation and Amino Acids Metabolism

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Main research interests of our lab are studies on basic mechanisms of aging. One of them is to study the cause of premature aging such as Cockayne syndrome (CS). RAD2, a yeast homolog of human XPG gene, is a nucleotide excision repair gene. Mutations in XPG gene cause xeroderma pigmentosum (XP) or CS depends on the mutations. XP is a human hereditary genetic disorder, that is characterized by photosensitivity and increased skin cancer incidence in sun exposed areas. On the other hand, CS is characterized by retarded growth, impaired neurological development, mental retardation, and premature aging. We have found the role of Rad2p-PCNA interaction in UV-induced mutagenesis. Mutagenesis regulation by Rad2p-PCNA interaction implicates that XPG-PCNA interaction might be the cause of high cancer incidence in XPG patients. We also reported that the Rad2p C-terminal region is pivotal for post-UV actin dynamics regulation. These results provide insights into the role of RAD2 in post-UV irradiation cell cycle regulation and actin assembly, which may be an underlying cause of XPG/CS.

Another main research interest of my lab is to study the role of amino acids metabolism on the regulation of yeast lifespan. Using metabolomics and genetic approaches, we identified metabolites affecting calorie restriction (CR)-induced chronological life span in yeast, Saccharomyces cerevisiae. Among 23 metabolites with altered profile by CR, we found that alanine level is inversely correlated with yeast chronological life span. Later analysis using deletion mutant of ALT1, the gene encoding the major alanine transaminase, revealed that lifespan modulation by ALT1 is TORI-independent. Further studies showed that alt1Δ completely suppresses cytochrome C oxidase subunit 2 expression. This study shows the importance of small molecule metabolism and relevance of metabolomics combined with genetic approach in the mechanistic studies in life span.
The Regulation for Histone Modifications During Transcription Elongation

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Chromatin structure is very important for the regulation of DNA-templated gene expression and can be regulated by several histone modifications, including phosphorylation, acetylation, ubiquitination, sumoylation and methylation. Many of these modifications can influence other modifications in a process called histone crosstalk. The best characterized histone crosstalk is the requirement of histone H2B monoubiquitination for H3K4 trimethylation by COMPASS, which is only H3K4 methyltransferase in *Saccharomyces cerevisiae*. We previously showed that one of COMPASS component, Cps35/Swd2, is a key regulator for translating signal of H2B monoubiquitination to H3K4 trimethylation in *S. cerevisiae*. However, it is poorly understood the mechanism of how Cps35/Swd2 regulate Set1’s H3K4 methylating activity depending on H2B ubiquitination. Recently it is reported that truncated Set1, which doesn’t bind Cps35/Swd2, showed H3K4 trimethylating activity. However, we showed that the localization of this H3K4 trimethylation containing truncated Set1 is different from normal localization. We suggested that histone H2B monoubiquitination and Cps35/Swd2 function to focus COPMASS’s H3K4me3 activity at promoter-proximal regions in a context-dependent manner.
Symposium

S21 Viral Pathogenesis
New Insights into the Biology of Hepatitis B Virus: Viral Entry and Viral Oncogenesis

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Chronic hepatitis B virus (HBV) infection leads to severe liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). However, the mechanism underlying the HBV-associated HCC remained elusive. HBx, a viral regulatory protein, has been a focus, because HBx induces multiple cancer-related cytoplasmic signaling such as NF-kB, Ras-Raf-MAPK, and Wnt signaling. In addition, HBx-induced Myc stabilization has been reported; however, the mechanism by which HBx induces Myc stabilization has been unclear. We found that HBx induces Myc stabilization via directing binding on Myc oncoprotein. Mechanistically, the HBx-induced Myc stabilization is achieved by inhibiting the SCF^{Skp2} ubiquitin E3 ligase-mediated Myc ubiquitination. Moreover, we found the HBV-induced Myc elevation in HCC tissues, validating that HBx-induced Myc stabilization is attributable for the HBV-induced HCC. Importantly, we defined the Myc binding site of HBx polypeptide into four hydrophobic residues near the carboxyl-terminus (i.e., VFVL). Work is in progress to exploit this peptide segment for the treatment of the HCC.

On the other hand, little was known about early steps of HBV life cycle due to the lack of susceptible cells permissive for viral replication. Recent discovery of NTCP (sodium taurocholate co-transporting polypeptide) or the cellular receptor for HBV entry opened avenues of investigation. We established HepG2-NTCP cell line that supports robust HBV infection. By using HBV infection system, we demonstrated that pharmacological inhibitors of the NTCP inhibit the HBV entry, implicated that NTCP represents an attractive molecular target for therapeutic intervention of chronic HBV infection. Work in progress is to define the biochemical steps involved in the covalently closed circular DNA (cccDNA), which is a hallmark of chronic HBV infection.
MiR-BART 20-5p Targets Both Viral and Cellular Genes in Epstein-Barr Virus-associated Tumors

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Although Epstein-Barr virus (EBV) BamHI A rightward transcript (BART) microRNAs (miRNAs) are ubiquitously expressed in EBV-associated tumors, the role of most BART miRNAs is unclear. In this study, we tried to identify cellular and viral target genes of BART miRNAs. Bioinformatic analysis predicted that 12 different BART miRNAs would target an EBV immediate early gene, BRLF1. Of these, the results of a luciferase reporter assay indicated that only one interacted with the 3’ untranslated region (3’UTR) of BRLF1: miR-BART20-5p. miR-BART20-5p’s effect on gene expression involved two putative seed match sites in the BRLF1 3’UTR, but a mutant version of the miRNA, miR-BART20-5p(m), had no effect on expression. As expected from the fact that BZLF1 and BRLF1 are part of a bicistronic transcript and both genes in the transcripts share a 3’UTR, miR-BART20-5p interacted with the 3’UTR of BZLF1 as well. BZLF1 and BRLF1 mRNA and protein expression was suppressed in AGS-EBV cells transfected with a miR-BART20-5p mimic. The expression of various EBV early proteins was also suppressed by the miR-BART20-5p mimic. By contrast, BZLF1 and BRLF1 expression in AGS-EBV cells transfected with a miR-BART20-5p inhibitor was enhanced. We also found that miR-BART20-5p directly targets Bcl-2-associated death promoter (BAD), causing increased cell proliferation and reduced apoptosis. miR-BART20-5p increased chemoresistance to 5-fluorouracil and docetaxel as well. Progeny virus production was suppressed by the BART20-5p mimic, while enhanced by the miR-BART20-5p inhibitor in AGS-EBV cells in which the lytic cycle had been induced. Our data suggest that miR-BART20-5p plays a key role in latency maintenance and tumor cell growth in EBV-associated tumors by directly targeting immediate early genes and BAD.
Hepatitis B Virus, DNA Methylation, and Hepatocellular Carcinoma


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DNA methylation of tumor suppressor genes is frequently detected in hepatocellular carcinoma; however, the mechanism and its biological significance were unclear until we found that HBx, the major oncogenic protein of hepatitis B virus (HBV), upregulates levels of DNA methyltransferases 1, 3a, and 3b. HBx induces DNA methylation of several tumor suppressor genes, including E-cadherin, p16, p21, and RAR-beta2, etc. and represses their gene expression, resulting in modulation of cell cycle progression, apoptosis, cellular senescence, and epithelial-mesenchymal transition, etc. in human hepatocytes. Recently, we also found that HBx stimulates HBV replication by interfering the action of a negative regulatory element binding protein on the HBV core promoter via DNA methylation.
Viral Modulation of Poly (ADP-ribose) Polymerase 1 to Facilitate Lytic Replication of Gammaherpesviruses

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Poly (ADP-ribose) polymerase-1 (PARP-1) is an abundant nuclear protein that catalyzes the serial transfer of the ADP-ribose moiety of NAD+ to various target proteins. PARP-1 participates in regulation of diverse cellular activities including DNA repair and transcription. In gammaherpesviruses such as Kaposi’s sarcoma-associated herpesvirus (KSHV) and murine gammaherpesvirus 68 (MHV-68), PARP-1 acts as a negative regulator of lytic replication via poly ADP-ribosylating RTA, leading to the repression of the RTA activity. We have identified viral strategies to modulate PARP-1, facilitating virus lytic replication. Our genome-wide screening of MHV-68 mutants revealed that ORF49, a novel virion protein, promoted MHV-68 lytic replication by enhancing RTA-mediated transactivation. ORF49 protein was able to disrupt the interaction between RTA and PARP-1, thereby acting as a derepressor of RTA. Furthermore, gammaherpesvirus lytic replication down-regulated the PARP-1 protein upon reactivation as well as de novo infection. Among lytic genes, KSHV ORF59 encoding viral processivity factor (PF-8) interacted with PARP-1 and promoted PARP-1 degradation in a proteasome-dependent manner. Moreover, KSHV PF-8, a downstream gene of RTA, enhanced RTA-mediated transactivation of the lytic viral promoters, suggesting an intricate feed-forward loop of lytic replication. Taken together, our results provide an insight of how gammaherpesviruses overcome the inhibitory effect of PARP-1 during different stages of virus replication.
Korea Mushroom Resource Bank for Wider Application

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The Korea Mushroom Resource Bank (KMRB) was launched as a national research resource bank in 2015 by the Ministry of Science, ICT and Future Planning. The main goal of the KMRB is to secure important biological resources, mushroom-forming basidiomycota, significant sources of fundamental and novel substances and materials, as dried specimen, cultures, and genomic DNA. For wider application of fungal resources in education, medicinal and industrial uses, the KMRB will undertake following tasks: 1) Survey natural environments across Korea to catalogue mushroom diversity, 2) Establish resource management system based on accurate identification of mushroom, 3) Evaluate the usefulness of the discovered mushroom, 4) Create a secure preservation and loan system. With a global focus on utilizing natural resources, mushroom resources provide excellent opportunities for academic research, and discovering novel substances for use as medicine and energy.
Microbial Carbohydrate-directed Molecular Complexation for Biotechnological Applications

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Microbial carbohydrates are one of the general constituents in microorganisms. They act as both structural and functional components as forms of oligosaccharides or polysaccharides. Those microbial carbohydrates have been classified into several families based on the structural features of their backbones or configurations and they can be sometimes modified by a variety of non-sugar substituents. Molecular complexation by those microbial carbohydrates with special cyclic or linear configurations could induce the change of physico-chemical properties of target guest chemicals such as poorly soluble drugs, chiral enantiomers or environmental toxic pollutants. Various potential biotechnological applications related with chiral separations, solubility enhancement, bioavailability enhancement and drug delivery system will be suggested by the microbial carbohydrate-directed molecular complexation.
Antimicrobial Mechanisms of Oleanolic Acid and Ursolic Acid against *Streptococcus mutans* UA159

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Oleanolic acid (OA, 3β-3-hydroxyolean-12-en-28-oic acid) and ursolic acid (UA, (3β)-hydroxy-urs-12-en-28-oic acid), and betulinic acid (BA, 3β-3-hydroxy-lup-20(29)-en-28-oic acid) are derivatives of triterpenoid saponins. In previous study, we reported that OA and UA have strong antimicrobial activity against *Streptococcus mutans* and *Streptococcus sobrinus* but BA had no antimicrobial activity against *S. mutans* and *S. sobrinus*. However, the antimicrobial mechanisms of OA and UA are unknown. The aim of this study was to investigate the antimicrobial mechanism of OA and UA against *S. mutans UA19* using microarray analysis method. Overnight cultures (1 ml) of *S. mutans* UA 159 were transferred to 9 ml BHI broth and grown at 37°C to the mid-log phase (OD$_{600}$ = 0.35). OA, UA, or BA solutions were added to each tube to the final concentration of 64 μg/ml. The bacterial culture solutions were incubated in a 37°C incubator for 90 min, harvested by centrifugation at 10,000 × g for 10 min at 4°C, and then extracted total RNA from the bacterial pellets. The oligonucleotide microarray was designed using the server-based eArray platform from Agilent Technologies. Agilent eArray system (http://earray.chem.agilent.com/earray/) was used to design oligonucleotide probes (45-mers, three probes for each gene) based on 1,928 (out of 1,963) genes for *Streptococcus mutans* UA159. Total RNA (30 µg) was converted to cyanine 3-labeled cDNA by post-synthesis labeling method. Labeled cDNA was hybridized with Agilent oligonucleotide microarray (*Streptococcus mutans* UA159, GE 4x44K). The hybridization images were analyzed by Agilent DNA microarray Scanner and the data quantification was performed using Agilent Feature Extraction software. All data normalization and selection of fold-changed genes were performed using GeneSpring GX 7.3. The data showed that stress-related molecular chaperone genes, *groES*, *groEL*, *dnaJ*, *dnaK*, and *grpE*, were highly expressed in both OA- and UA-treated groups compared to control group, whereas these genes were down-regulated in BA-treated one. The *liaS* and *ciaH*, which are the major sensor kinases in the stress tolerance response of *S. mutans* UA159, were also over-expressed in just both OA- and UA-treated groups. Of the energy metabolic pathways, ATP and fatty acid syntheses-related genes were also highly down-regulated in both OA- and UA-treated groups, whereas the pyruvate fermentation-related genes of pyruvate dehydrogenase (PDH) complex and pyruvate formate lyase (PFL) pathway were significantly over-expressed in UA-treated group compared to the other groups. The acetyl-CoA molecules, produced by the PDH complex and PFL
pathway, could be used in the fermentation and in the citrate synthesis by citrate synthase with oxaloacetate substrate. In pyruvate heterofermentation, ATPs are generated by the acetate kinase and these ATPs might be used in the pumping out the intracellular protons. The citrates are converted to isocitric and α-ketoglutarates by aconitase and isocitrate dehydrogenase, respectively. NADHs are produced by isocitrate dehydrogenase and they could be reused in the pyruvate fermentation as proton donors because the transcriptional level of lactate dehydrogenase was not changed in all groups. In addition, the genes related to the peptidoglycan biosynthesis were significantly down-regulated in the OA- and UA-treated groups compared to BA-treated one. These results indicate the OA and UA, but not BA, induced acid stress in *S. mutans* UA159. The *S. mutans* UA159 over-expressed the acid tolerance-related genes to overcome the intracellular acidosis. However, the ATPs were not enough to completely pumping out the intracellular protons because the F-type ATPase genes were significantly down-regulated in OA- and UA-treated groups. These results suggest that the main antimicrobial mechanisms of OA and UA against *S. mutans* UA159 are the inhibition of the ATP, fatty acid, and peptidoglycan syntheses. However, this study was performed using only one strain, investigating change of gene expression in a transcriptional level, and was not evaluating the intracellular pH changes in all groups. To completely understand the antimicrobial mechanism of OA and UA, proteomic studies and phenotype characterizing studies should be need in the future.
Anti-Desertification Using Artificial Induction of Biological Soil Crust

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This study was conducted to rapidly stabilize the initial stage of artificial cyanobacterial soil crust using combined treatments of cyanobacteria with soil fixing chemicals. PVA (polyvinyl alcohol) and TKS chemicals were examined under laboratory conditions. TKS7 showed noticeable aggregate stability among the examined chemicals. Combined application of cyanobacteria with different concentrations of TKS7 (CT1-CT5) also showed high aggregate stability with MWD (mean weight diameter) values of 0.58-0.69 mm relative to single application of TKS7 (0.18-0.40 mm). For the field trial, SAP (superabsorbent polymer) was also applied as a water-holding material and nutrient supplement. Application of cyanobacteria with SAP and TKS7 (CST) remarkably improved soil stability and biological activity in the field at 4 months after induction. These results suggest that combined application of cyanobacteria with soil fixing agent rapidly induced biological soil crust (BSC) formation in the field within a few months, and the soil properties and biological activities of the induced BSC reached 55-88% of that of natural cyanobacterial crust aged approximately 20 years. The novel method presented herein makes it feasible to artificially induce BSC formation in a very short term with improved stabilization of cyanobacterial soil crust properties during the initial stages of BSC formation.
Impacts of Climate Change on Microbial Pathogenic Characteristics and Behavior: Inevitable Paradigm Shift

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Climate change has brought various types of natural disasters around the globe in an unprecedented pace. The scope of its impact is so deeply woven in our daily lives that almost all aspects of our lives and our environment including ecosystems are now affected. With carbon dioxide level reaching over 400 ppm, maintaining the environmental conditions in sustainable ways and their influences on all living organisms and humans has become a great challenge. Consequently, many scientific and engineering concepts for global scale of adaptation to major anticipated climate-change-induced changes have been tested and even hastily implemented with various outcomes. The direct threat to human survival from sea level rising, drought, wild fires, environmental pollutants, and disruption of food and water supply coming from climate change are all real. Many ecosystems around the world have been disrupted to the level of beyond repair. Understanding the adaptation processes of existing pathogens in our environment and their influences on human health and illness, crop production, and animal welfare is a timely relevant task to all of us. Several major research tasks globally shared in scientific and engineering communities in climate change perspective include: 1) Changes in virulence properties among plant pathogens, 2) microbe adaptation in vectors in zoonotic illness, 3) Adaptation of normal flora to virulent forms of incoming pathogens with modified virulence properties, 4) Microbial impact on animal mass kill or extinction, 5) microbial adaptation process in the presence of multiple insults including engineered nanomaterial and other existing conditions, and 6) the role of microbes in climate change in the coming years. For each category, a representative case study will be presented with historical background information and how each case could be relevant to both scientists and engineer working in the fields of exposure and risk assessments and remediation in different regions of the world.
Microbiological Assessment of Indoor Air Quality

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There are numerous microorganisms in the air we breathe, including viruses, bacteria, and fungi. Both the metabolically active and inactive airborne microbes have clinically important effects on human health. The microbial content of the bioaerosol is a great interest, because metabolically active human pathogens cause infectious diseases. The amount of microbial load in the bioaerosol is also a main concern, because inactive fungal components can cause allergic diseases like as asthma and rhinitis. The traditional culture-based evaluation methods have applied in various environmental air samples and have enabled direct observation of airborne bacteria and fungi. However, culture-based method had limitation in estimating the true diversity of microorganisms. Thus, to figure out the microbial content of the air, we applied the culture-independent metagenomics technique to the assessment of indoor air quality. The profile of microorganisms present in the air of built environment including daycare center, elementary schools, and hospitals were analyzed using pyrosequencing of 16S rRNA and ITS gene sequences. The year round fluctuation of microbial load in the air was also evaluated indirectly throughout the traditional culture-dependent methods. The determined microbial communities contained a wide variety of taxa previously not identified in built environment. The dominant species were different from those found in previous studies based on culture methods. The bacterial community in the indoor air appeared to contain diverse bacteria associated with both humans and the outside environment. In contrast, the fungal community was largely derived from the surrounding outdoor environment. Most of the fungi detected indoors originated only from diverse outdoor sources and not from human activity. The profile of bioaerosols in this study provide a deeper insight into airborne microbial diversity needed for developing public health policies regarding the monitoring and management of indoor air quality.
Improved Cultivation of Bacteria from Coastal Saline Habitats and Their Mining for Novel Carotenoids

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Major part of the India is covered by coastal areas which are represented by several moderate saline habitats whose bacterial diversity and community structure is less understood. Coastal saline habitats of Odisha were surveyed for the bacterial diversity and community structures analysis by adopting the bacterial Tag-Encoded FLXTitanium Amplicon Pyrosequencing (bTEFAP) of 16S rRNA gene. Most abundant sequences (>1%) were belong to Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Bacteroidetes and Firmicutes, while less abundant sequences (<1%) were represented by the Actinobacteria, Chlorobi, Deferribacteres, Planctomycetes, Verrucomicrobia and “TM7”. About 20.31% of sequences indicated the existence of eight novel putative bacterial lineages that have potential to be created as altogether new taxa at the phylum level. Based on the presence of unique bacterial resources a cultivation based analysis was conducted from coastal saline samples to cultivate the previously uncultivated members. Three different media (GM1, GM2 and GM3) were optimized for the improved cultivation from a composite sample made from soils collected from Odisha, India. Massively parallel pyrosequencing platform was employed to detect to bacterial members based on 16S rRNA gene. Medium GM1 was able to support the growth of the members of the phylum Firmicutes and Proteobacteria while other two medium (GM2 and GM3) supported the growth of the members of phylum Bacteroidetes, Proteobacteria, Actinobacteria and Firmicutes. In addition, medium GM3 supported the growth of few bacteria (0.83% of the total sequence detected on medium GM3) which were not classified at SILVA database. Medium GM3 supported the growth of diverse bacterial members in comparison to the medium GM1 and GM2 which was reflected by highest phylogenetic diversity index. To exploit the carotenoids producing bacteria from Odisha samples a total of nine bacterial isolates were further selected for study based on their unique pigmentation. Out of nine isolates strains were indicated to be potentially new taxa which were validated as Falsirhodobacter halotolerans, Erythrobacter odishensis and Pontibacter odishensis. Spectral analysis (by HPLC and LC-MS) of pigments of all the validated strains reveals the presence of β-carotene, erythroxanthin, erythroxanthin sulfate, astaxanthin, astaxanthin glucoside, rhodoxanthin along with several unidentified carotenoids which are potentially new carotenoids which could be used as important antioxidants. Hopanoids analysis revealed the presence of diploptene, diplopterol, adenosylhopane and bacteriohopane derivatives (BHD1,2) along with few unidentified hopanoids in the validated strains which could be used as potential taxonomic markers in the differentiation and classification of new taxa names.
Symposium

S24 Research and Business Development of Biomedical Material, Poly-γ-glutamic Acid
Medical and Pharmaceutical Applications of Poly-γ-glutamic Acid

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Poly-γ-glutamic acid (γ-PGA) is a bio-polymer traditionally produced during the fermentation of soy based nutrients by Bacillus subtilis, a naturally occurring microorganism to ferment soy foods known as “natto” and “chungkookjang” in Japan and Korea, respectively. The polymeric γ-PGA, which gives those foods their unique sticky texture, is linked D- and/or L-glutamic acid with γ-peptide bond. We successfully scaled up the production yield of γ-PGA with high-molecular-weight during last 15 years and tried development of new functional applications in cosmetics, pharmaceuticals and functional foods with its physical and biological properties.

As a part of efforts, we extensively analyzed immune response by γ-PGA for functional application as an immune modulator in vitro and in vivo, respectively. First of all, the studies of natural killer (NK) cell-mediated cytotoxicity and interferon-γ (IFN-γ) secretion from splenocytes resulted in that the γ-PGA induced NK-cell mediated cytotoxicity and IFN-γ secretion in mice. In order to take advantage of its wide beneficial properties, development was underway to utilize it in an immune therapy as liquid suspension that could be administered orally. The action of the oral formulation comes from the potential of the main ingredient γ-PGA to stimulate immune cells in the gut mucosal lining and initiate the induction of innate immune system. We found that γ-PGA stimulated immune system through TLR4 receptor and it required both MD2 and CD14 proteins to activate NK cell. Moreover, γ-PGA induced production of IFN-β via IRF3 and IL-12 via NF-κB, indicating that the NK cell activation came from both MyD88-dependent and -independent pathway. To confirm whether both cytokines induce NK cell, we tried to monitor production of IFN-γ in vitro, in vivo and ex vivo, respectively. Remarkably, we confirmed the production of IFN-γ and CD107a in PBMCs from healthy donors that incubated with γ-PGA, indicating that NK cell is activated by γ-PGA and the numbers of NK cell are increased by γ-PGA too. As a next, a single-center, randomized, double-blind and placebo-controlled clinical trial was carried out in order for evaluating the effect on the human immune system after 8-week administration of γ-PGA in healthy volunteers. This study showed the tendency to increase NK-cell mediated cytotoxicity, and the expressions of CD8 and CD56 were also increased by administration of γ-PGA.

On the other hand, the immunological profile of several cytokines was monitored with cervical intraepithelial neoplasia patients, who are infected by Human Papilloma Virus (HPV). The regressed state from CIN 3 to CIN
2, CIN 1 or normal status highly produced IFN-γ rather than progression or stable state patients, implying that activated NK cell affect the destruction of dysplasia and HPV virus infected cell. Taken together, immune therapies based on γ-PGA cures cervical intraepithelial neoplasia states through the activation of innate immunity. The effect of our proprietary immune therapy based on γ-PGA can be explained as follows; 1) activation of innate immunity for the clearance against virus, 2) activation of NK cell number and activity to eliminate viral infected and dysplasia cells.

Phase 2b clinical trials with γ-PGA are underway with CIN1 patient, and the interim analysis demonstrated a statistical significance in this phase 2b clinical trial. In this research we provide an updated overall understanding of the biopharmaceutical and biomedical applications of γ-PGA, and we hope to be facilitated further research on this valuable biomaterial.

[This study was supported by Chung Cheong Leading Industry Promotion Project (R0001832) of the Korean Ministry of Knowledge Economy.]
We previously reported that the oral administration of high molecular mass poly-gamma-glutamate (γ-PGA) induced antitumor immunity but the mechanism underlying this antitumor activity was not understood. We found that application of high molecular mass γ-PGA induced secretion of tumor necrosis factor (TNF)-alpha from the bone-marrow-derived macrophages of wild type (C57BL/6 and C3H/HeN) and Toll-like receptor 2 knockout (TLR2(-/-)) mice, but not those of myeloid differentiation factor 88 knockout (MyD88(-/-)) and TLR4-defective mice (C3H/HeJ). Production of interferon (IFN)-gamma-inducible protein 10 (IP-10) in response to treatment with γ-PGA was almost abolished in C3H/HeJ mice. In contrast to LPS, γ-PGA induced productions of TNF-alpha and IP-10 could not be blocked by polymyxin B. Furthermore, γ-PGA-induced interleukin-12 production was also impaired in immature dendritic cells (iDCs) from MyD88(-/-) and C3H/HeJ mice. Downregulation of MyD88 and TLR4 expression using small interfering RNA (siRNA) significantly inhibited γ-PGA-induced TNF-alpha secretion from the RAW264.7 cells. γ-PGA-mediated intracellular signaling was markedly inhibited in C3H/HeJ cells. The antitumor effect of γ-PGA was completely abrogated in C3H/HeJ mice compared with control mice (C3H/HeN) but significant antitumor effect was generated by the intratumoral administration of C3H/HeN mice-derived iDCs followed by 2,000 kDa γ-PGA in C3H/HeJ. These findings strongly suggest that the antitumor activity of γ-PGA is mediated by TLR4.

Although vaccine adjuvants have been used for almost a century, alum is only adjuvant licensed by the US FDA for human vaccine use. Many adjuvants studied to date have generated inflammatory properties and lack specificity in terms of targeting immune compartments and cell populations. It has been reported that toll-like receptors bind different determinants to trigger unique inflammatory cascade events that has yielded TLR agonist with adjuvant potential. Specific examples of TLR4 stimulation include bacterial derived adjuvant such as monophosphoryl lipid A (MPLA). Because TLR signaling may not be critical for Th2 immune response, TLR agonists show particular promise as adjuvants of cytotoxic T cell activity. Currently, we reported that the innate immune responses induced by the treatment of γ-PGA in macrophages and DCs were mediated by TLR4 using MyD88 knock-out and TLR4 deficient mice. We also found that γ-PGA nanoparticles strongly induced cytokine production, up-regulation of costimulatory molecules, and the enhancement of T cell stimulatory capacity in DCs. γ-PGA chitosan NPs are excellent vaccine carriers capable of delivering antigenic proteins to antigen-presenting
cells and eliciting potent immune responses based on antigen specific cytotoxic T lymphocytes. Ag-mixed with γ-PGA nanoparticles are capable of inducing strong cellular and humoral immune responses. We also investigated the adjuvant effect of γ-PGA nanogel using influenza split vaccine. These finding indicates that γ-PGA nanogel may be a candidate of the future vaccine adjuvant.
The Natural Substance, Poly-γ-glutamic Acid, Induces Antiviral Effects

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Recent studies have addressed natural substances that have the ability to inhibit or prevent viral infections in a safe manner. Poly-γ-glutamic acid (γ-PGA), which was originally discovered from chungkookjang, a traditional Korean fermented soybean food, was evaluated to know whether γ-PGA has anti-influenza virus function through over expressed Myxovirus resistant (Mx) proteins. Ability of γ-PGA to inhibit virus replication, cytokines and expressed Mx gene products induced by γ-PGA were confirmed in murine macrophage cell. Based on these verification, the antiviral effect of γ-PGA was evaluated in a C57BL/6.A2G-Mx mouse model. After γ-PGA treatment, mice were intranasally challenged with highly mouse pathogenic virus. Clinical signs, weight and survival were monitored for 9 days. Moreover, changes in Mx1 mRNA and protein expression levels over time were confirmed by PCR and immunoblotting. The high molecular weight (5,000 kDa) γ-PGA was able to inhibit PR8 and H5N2 influenza A virus replication by induced type I IFN and stimulate Mx1 protein expression in RAW 264.7 cells. Also, γ-PGA showed complete protection against influenza virus challenge in the C57BL/6.A2G-Mx mouse model. When γ-PGA was administered, secretion of type I IFNs increased within a day and it stimulated the expression of Mx gene products at the early stage of virus infection.

These results suggested that HM-γ-PGA induced a potent antiviral state both in vitro and in vivo. Consequently, HM-γ-PGA treatment could inhibit virus replications and enhance clearance of viruses from lung cells and mouse survival against the virus infection through stimulation of Mx1 protein expression mediated by type I IFNs. Likewise, mRNA expression levels of Mx1, IFNs, tumor necrosis factor (TNF)-α, and interferon-stimulated genes (ISGs) were measured from harvested lungs or bone marrow-derived macrophages (BMDM, 2×10^6/well of a 6-well tissue cell culture plate). BMDMs were isolated from leg bones of 7-week-old B6.A2G-Mx1 mouse and then differentiated by growth for 7 days with M-CSF (25 μg/ml, R&D) according to the previous study. BMDMs were treated with media only, 1 mg/ml HM-γ-PGA, 500 U of IFN-β, or 1 MOI of H5N2 virus and harvested at 0, 6, 12, 24, and 36 hpt. Total mRNA was extracted using the RNeasy Mini Kit (Qiagen, USA) from 0.03 g lung tissue harvested for immunoblotting or treated cells in each well of 6-well tissue cell culture plate. The extracted mRNAs were then amplified by RT-PCR. The mRNA level of Mx1, TNF-α, IFN-α and IFN-β increased after HM-γ-PGA treatment of both normal C57BL/6 and B6.A2G-Mx1
mouse strains, and the stimulation in mRNA expression was similar to the levels seen in the IFNs treated animals. Interestingly, mRNA levels remained high in the HM-γ-PGA treated group out to 36 hpt, but levels had decreased in all of the other treatment groups. In addition, the determined mRNA expression levels of several ISGs in B6.A2G-Mx1 BMDMs increased following HM-γ-PGA or IFN-β treatment and HM-γ-PGA treatment yielded a longer lasting stimulation, as describe for the lung tissue. H5N2 virus-treated BMDM showed an early increase and later decrease of mRNA expression. These results suggested that induced IFNs by HM-γ-PGA stimulate the Mx1 gene as well as other ISGs, and this pathway appears to have a crucial role in protecting mice against influenza virus infection in vivo. Moreover, according to the previous study, HM-γ-PGA is engulfed into immune cells by Toll-like receptor 4-mediated endocytosis. After endocytosis, the signal is transduced by MyD88-dependent or independent pathways, and NF-κB and IFNs are produced as a result of the signal cascade. Together with this previous finding, a certain function of HM-γ-PGA to induce type I IFNs has been demonstrated again through this study.

In conclusion, this study has shown an antiviral function of HM-γ-PGA against influenza virus through the stimulation of type I IFN and Mx1 protein both in vitro and in vivo. In another study, the HM-γ-PGA was able to activate the subsequent signals through TLR4/MD2 to result in dimerization of IRF-3, a transcription factor required for IFN gene expression, leading to increases in mRNA levels of the type I IFN-response genes, 2′-5′ OAS and ISG56. Moreover, HM-γ-PGA displayed an antiviral activity against SARS coronavirus and hepatitis C virus in vitro. Therefore, HM-γ-PGA, a safe natural substance, may have potential as a prophylactic treatment against influenza virus and possibly other IFN/Mx-sensitive RNA virus infections.
Clinical Application of A Natural Polypeptide, Poly-\(\gamma\)-glutamic Acid Which Has Immunotherapeutic Efficacy

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Poly-gamma-glutamic acid (\(\gamma\)-PGA) is known as viscous component of Korean chungkookjang and a natural polypeptide consisting of only glutamic acid with gamma-amide linkages. High molecular weight \(\gamma\)-PGA was isolated and mass-produced from \textit{Bacillus subtilis} Chungkookjang. Previously, accumulating evidences indicate that the \(\gamma\)-PGA induces innate immune response including natural killer (NK) cell activation and interferon secretion through toll-like receptor 4 (TLR4). To evaluate the immunotherapeutic effects, we've investigated the effect of \(\gamma\)-PGA on human NK cell activity and antiviral effect. First human study involving 99 healthy volunteers revealed that high-dosage \(\gamma\)-PGA group were significantly higher cytotoxic activities of NK cells than the low dosage and placebo groups at weeks 4 and 8 after the treatment. Furthermore, we studied the effect of \(\gamma\)-PGA on the treatment of vaginal intraepithelial neoplasia (VAIN). A retrospective observational study on \(\gamma\)-PGA therapy for biopsy-proven VAIN suggest that \(\gamma\)-PGA may be helpful for the cytological regression and reduction of viral load in patients with high-risk HPV-positive VAIN. Since previous human studies showed the possibility of drug, we have accomplished a phase I dose escalation study to determine the safety, tolerance and pharmacokinetics of \(\gamma\)-PGA in healthy adult male subjects. The phase I study of oral \(\gamma\)-PGA was conducted in 20 healthy volunteers. There was no any significant difference in abnormal diagnoses or clinically relevant changes in laboratory parameters, vital signs, or other safety parameters. Currently, we have initiated a phase II trial to determine the efficacy and safety of \(\gamma\)-PGA compared with placebo in patients with cervical intraepithelial neoplasia grade 1 (CIN1). Total 200 patients with CIN1 will be administered with \(\gamma\)-PGA or placebo for 4 weeks followed by 8 weeks observation and compare the regression rate of CIN1. So far there was no serious adverse event (SAE) during phase II study. These results indicate that the oral administration of \(\gamma\)-PGA can serve as treatment option that are safe and clinical efficacy via inducing NK cell activity and anti-viral effect.

Acknowledgement

BioLeaders Corporation provided \(\gamma\)-PGA and co-worked for the human clinical study and phase II clinical trial.
The Role of NRF for the Development of Microbiology and Biotechnology of Korea in 2016

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The National Research Foundation of Korea is the major research funding agency, established in 2009. This is the biggest governmental funding agency of Korea which was the merge product of 3 formerly independent foundations. They were the Korea Science and Engineering Foundation (KOSEF), The Korea Research Foundation (KRF), and the Korea Foundation for International Cooperation of Science and Technology (KICOS).

NRF supports various academic research not only in science and technology but also in humanities and social sciences including academic studies, and interdisciplinary academic fields. The funding programs in Directorate for Basic Research will be changed in 2016 with variable amount of grant money based upon researcher’s needs.

Division of Life Sciences is under the Directorate for Basic Research in Science & Engineering which has governmental funding programs as follows.

First, ‘General Researcher Program including Mid-career Researcher Program’ is organized for the increase of the research capacity of universities and research institutes.

Second, ‘Leading Researcher Program’ is organized to discover next-generation scientists and stimulate them to become global leaders and distinguished scientists.

Third, ‘Advanced Research Center Program’ is organized to foster world-class scientists of a group of about 10 scientists and promote the nation’s knowledge competitiveness by supporting excellent scientist group in their specific fields. It has SRC, ERC and MRC programs.

Fourth, Basic Research Lab Program is organized to foster promising research group of 3 in universities by developing professors’ personal laboratories into major/department-level basic research labs.

Even though last 3 years have been very tough years for all the scientists, we sincerely hope our programs will help develop your research further and eventually improve the quality of our lives in general. The details of present and future status of microbiological sciences and biotechnology in Korea with respect to the role of NRF will be discussed in this session.
sRNA Regulates the Anaerobic Induction of FrsA Expression

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Fermentation respiration switch (FrsA) is an enzyme catalyzing pyruvate decarboxylation. Since the cellular level of FrsA was minimal in Vibrio vulnificus cells grown under aerobic conditions, the regulatory mechanism for the anaerobic induction of FrsA was investigated in this study. Transcript quantitation showed no significant difference of frsA mRNA levels in cells grown in the presence or absence of oxygen. This result suggests that regulation at the post-transcription level is important in FrsA expression, leading us to examine the presence of a putative regulatory sRNA. A candidate FrsA-regulating sRNA (Frr), including the sequences complementary to the 5'-UTR of frsA mRNA, was identified in V. vulnificus genome. A northern blot revealed the presence of 350 nucleotide-long sRNA. Its regulatory role was investigated by comparing the cellular contents of FrsA in wild type and its isogenic frr deletion mutant. In the absence of Frr, the negative effect of oxygen on the FrsA level was abolished. This oxygen-dependent repression of FrsA expression by Frr was achieved through the repression of frr expression by Fnr under anaerobic conditions. Thus, this study demonstrates the cellular content of FrsA is minimized during aerobic growth via repression by Frr. This repression, however, is relieved during anaerobic growth via repression of the frr transcription by Fnr, resulting in higher FrsA activity.
Autoinducer-2 Quorum Sensing of Probiotic *Lactobacillus* spp.

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Autoinducer-2 (AI-2) signalling is a universal cell-density dependent quorum sensing found in a wide range of bacteria including probiotic *Lactobacillus* spp. The system has been reported to affect the expression of genes associated with corresponding adaptation to the environment. Furthermore, a recent study (Thompson et al., 2015) investigated that increased AI-2 can modulate the microbiota under conditions of antibiotic-induced dysbiosis. Therefore, it is expected that the AI-2 activity of probiotics may a key factor cause the modulation of the gut microbiota. However, reports on AI-2 activity of probiotics are still scarce.

We profiled the AI-2 characteristics of lactobacilli and observed the change of AI-2 activity of probiotic strains (*Lactobacillus rhamnosus* and *Lactobacillus plantarum* strains) in response to gastrointestinal (GIT) environment. The results showed that the dynamic modulation of AI-2 activity in the strains was induced by the GIT conditions, and was both species- and strain-specific. Although all mechanisms are not known yet, these data may be of support to future mechanistic studies on AI-2 signalling of probiotics and present one way of bacterial interaction and modulation by probiotics within the intestinal ecosystem.

References:


Keywords: Quorum sensing, Autoinducer-2, Probiotics, Gut microbiota

Acknowledgement:

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Glucose Metabolism Affects Trimethylamine N-Oxide Stimulated Cholera Toxin Production

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Vibrio cholerae, a causative agent of pandemic cholera, infects human intestine, which is considered to be anaerobic environment. Production of cholera toxin (CT), a major virulence factor of V. cholerae, is highly induced during anaerobic respiration with trimethylamine N-oxide (TMAO) as an alternative electron acceptor. However, the molecular mechanism of TMAO-stimulated CT production is not fully understood. Herein, we revealed that CT production during anaerobic TMAO respiration was affected by glucose fermentation, another major mode of energy metabolism. When N16961, V. cholerae strain was grown together with glucose, the CT production was markedly reduced. Furthermore, an N16961 Δcrp mutant, devoid of cyclic AMP receptor protein (CRP), became defective in CT production during growth by anaerobic TMAO respiration, further suggesting a role of glucose metabolism in regulating TMAO-mediated CT production. TMAO reductase activity was noticeably decreased when grown together with glucose or by the mutation of crp gene. A CRP binding region was identified in the promoter region of torD gene encoding a structural subunit of TMAO reductase and our gel shift assay further confirmed the binding of purified CRP to the torD promoter sequence. Together, our results suggest that bacterial capability to respire using TMAO is controlled by CRP. This is a demonstration that CT production, an important virulence feature, is critically controlled by bacterial metabolism.

Keywords: Vibrio cholerae, cholera toxin, Trimethylamine N-oxide, glucose metabolism, cyclic AMP receptor protein
Characterization of *Vibrio vulnificus* CabA Essential for Biofilm Development on Abiotic Surfaces and Oysters

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A transcriptome analysis identified *Vibrio vulnificus* cabABC genes which were preferentially expressed in biofilms. The cabA gene was induced by elevated 3',5'-cyclic diguanylic acid (c-di-GMP) and encoded a calcium-binding protein CabA. Comparison of the biofilms produced by the cabA mutant and its parent strain JN111 in microtiter plates demonstrated that CabA contributed to biofilm formation in a calcium-dependent manner under elevated c-di-GMP conditions. Genetic and biochemical analyses revealed that CabA was secreted to the cell exterior through functional CabB and CabC, distributed throughout the biofilm matrix, and produced as the biofilm matured. These results indicated that CabA is a matrix-associated protein required for maturation of biofilms. Microscopic comparison of the structure of biofilms produced by JN111 and the cabA mutant demonstrated that CabA is an extracellular matrix component essential for the development of the mature biofilm structures in flow cells and on oyster shells. Exogenously providing purified CabA restored the biofilm-forming abilities of the cabA mutant when calcium was available. Circular dichroism and size exclusion analyses revealed that calcium binding induces CabA conformational changes which may lead to multimerization. Consequently, the combined results suggested that CabA is a structural protein of the extracellular matrix and multimerizes to a conformation functional in building robust biofilms.
Staphylococcus aureus Uses Its Membrane Vesicles to Inhibit Biofilm Formation by Other Bacterial Species

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Although the outbreak of S. aureus in worldwide was still investigated, there are no clear reasons behind the spreads of this bacterium. Here, we hypothesized that S. aureus might get the superiority among the communications via their membrane vesicles. We demonstrated that S. aureus inhibited biofilm formation by other respiratory tract bacteria when co-cultured. Subsequent analyses using Acinetobacter baumannii as a model found this effect is primarily mediated by membrane vesicles (MVs) from S. aureus. These MVs were found to coat the underlying surfaces and inhibit A. baumannii from adhering to the surface and, thus, reduced the biofilm formed by up to 93%. Evaluating the properties of the MVs-treated surfaces, we found they were more hydrophilic, which makes it difficult for the bacteria to adhere initially. Additional tests found the biofilm antagonism of S. aureus extended to other bacterial species, with 40~70% inhibition. The findings of this study describe a new perspective on interspecies interactions occurring between S. aureus and other microbes via MVs.

Keywords: Staphylococcus aureus, Membrane Vesicles, Acinetobacter baumannii, Anti-Biofilm, Pathogens, Multi-Drug Resistance
First Report of Brown Rot Caused by *Cryptococcus pseudolongus* on Fruit Body of *Lentinula edodes* (shiitake) in Korea

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In 2014 autumn, browning phenomenon appeared on shiitake fruit body’s caps. This phenomenon which is considered to be new disease was observed in mushroom farms located at Gwangju and Muju in Korea. We named the disease tentatively as brown rot. Disease incidence was nearly 20% in two mushroom farms where sawdust media were used for shiitake cultivation. Because some bacteria were reported to cause this phenomenon in other mushroom, and *Ewingella americana* were reported to cause bacterial brown rot and *Hyphozyma*, syrananoph of *Eleutheromyces subulatus*, was reported to cause brown spot on shiitake fruit bodies, we tried to investigate microorganisms isolated from brown rot lesion of shiitake fruit bodies. During an investigation, fungi were isolated. Among the isolates, one group was classified into *Cryptococcus pseudolongus*. Pathogenic test revealed that *C. pseudolongus* caused brown rot on shiitake fruit body but did not on button mushroom. Among the several tested antifungal agents, benzalkonium chloride and benomyl were found to be effective on the inhibition of the growth of *C. pseudolongus*. We have shown that brown rot of shiitake mushroom caused by *C. pseudolongus* is new disease in Korea.
Symposium

YS2 Young Scientist Session 2

International Meeting of the Federation of Korean Microbiological Societies
Investigation of the Interaction Partner of HPr in *Vibrio cholerae*

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*Vibrio cholerae* is known as a human opportunistic pathogen which infects hosts through contaminated water and food. Many bacterial species including *V. cholerae* possess phosphotransferase system (PTS) as a sugar transport system. Sugar PTS consists of sugar-specific enzyme II proteins and two general components, enzyme I and histidine phosphocarrier protein (HPr). PTS components play critical roles in transport of sugar from outside of cells by sequential phosphoryl transfer. Besides of sugar uptake, PTS regulates other function through protein-protein interaction in phosphate-dependent manner. Interactions between PTS proteins and other proteins have been focused on *Escherichia coli* model system which is well studied so far. In contrast, physiological regulation through protein-protein interaction with PTS components is barely known in *V. cholerae*. In this study, new protein-protein interaction between HPr and FruR (Fructose repressor) was found in *V. cholerae*. FruR is known as GalR/LacI-type repressor which regulates expression of genes involved in metabolism and transport of fructose through direct binding to upstream DNA domain of operon coding for fructose-specific PTS. It is assumed that HPr participates in FruR-dependent regulation as a new protein interaction partner.

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**Keywords:** HPr, FruR, Protein-protein interaction
Rsd Stimulates ppGpp Hydrolase Activity of SpoT upon Glucose Starvation in *Escherichia coli*

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Bacteria properly respond to various stresses by modulating the level of ppGpp (guanosine 3’-5’ tetraphosphate), which is called stringent response. In *Escherichia coli*, the level of ppGpp is regulated by RelA and SpoT proteins. RelA catalyzes the synthesis of ppGpp, whereas SpoT catalyzes the synthesis/hydrolysis of ppGpp as a bifunctional enzyme. Notably, the ppGpp hydrolase activity of SpoT is essential to bacterial cell, as high concentration of ppGpp severely inhibits cell growth. However to date, underlying mechanisms to regulate the hydrolase activity of SpoT remain unknown in *E. coli*. To elucidate regulatory mechanisms, we searched for candidates responsible for the stress response. Because stress conditions and ppGpp can influence the transcription level of Rsd, an anti-σ⁷⁰ factor known to complex with dephosphorylated HPr in the presence of glucose, we assumed that Rsd might have stress-responsive physiological roles. The ligand fishing experiment using Rsd in this study identified specific interaction of Rsd with the TGS domain (Threonyl-tRNA synthetase, Obg family of GTPases and SpoT) of SpoT both *in vitro* and *in vivo*. Furthermore, we demonstrated that ppGpp hydrolase activity of SpoT can be activated by Rsd, and that dephosphorylated HPr antagonizes this stimulatory effect of Rsd on SpoT. Based on these data, we suggest that SpoT can be regulated by its direct interaction with Rsd upon glucose starvation, proposing a novel stringent response mechanism in *E. coli*.

**Keywords:** ppGpp, glucose starvation, stringent response
Characterization of the Viable but Nonculturable State in *Ralstonia solanacearum*

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*Ralstonia solanacearum* is a plant pathogenic bacterium causing lethal wilt in various plant species. Viable but nonculturable (VBNC) state of this bacterium and other Gram-negative bacteria considered to be a bacterial survival mechanism. In this study, VBNC state was induced in *R. solanacearum* SL341 strain at low temperature using modified artificial soil microcosm (mASM). There were no distinct differences of total DNA, RNA and protein contents of SL341 strain at two different temperatures. Moreover, the VBNC cells maintained respiration activity in the mASM. Culturability of VBNC cells was recovered by supplementation of catalase. Expression of *omp*, *rpoS*, *dps*, *oxyR* and the 16S rRNA gene were not different from bacterial cells in mASM at two temperatures by RT-qPCR. To get a more in-depth knowledge about the gene expression of *R. solanacearum* in the VBNC state, total RNAs of *R. solanacearum* derived from mASM were linearly amplified and subjected to RNAseq analysis. Transcriptome analysis by RNAseq showed that 254 genes were down-regulated at VBNC state, however, 182 genes were clearly up-regulated. Differentially expressed genes in VBNC state were functionally annotated by COG analysis, showing that genes responsible for the transport, lipid metabolism and coenzyme biosynthesis were highly expressed in VBNC state. Our result suggested that the VBNC cells in mASM induced by low temperature are viable in a physiologically and genetically unique state.
Growth Inhibition of *Cronobacter sakazakii* in Experimentally Contaminated Powdered Infant Formula by Kefir Supernatant

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Kefir is a type of fermented milk containing lactic and acetic acid bacteria and yeast. In this study, we evaluated the antimicrobial activity of kefir supernatant against *Cronobacter sakazakii* in powdered infant formula (PIF). In a spot-on-lawn test, the growth of 20 *C. sakazakii* strains—including 10 clinical and 10 food isolates—was completely inhibited in the presence of kefir supernatant. Significant differences in the diameters of inhibition zones were observed upon treatment with kefir as compared to *Lactobacillus kefiri* and *Candida kefyr* culture supernatants or solutions of lactic and acetic acid and ethyl alcohol in the agar well diffusion test (*P* < 0.05). The addition of 100-μl kefir supernatant to 1 ml of nutrient broth completely inhibited the growth of *C. sakazakii*, as evaluated by spectrophotometry. The antimicrobial activity of kefir supernatant in experimentally contaminated PIF was also tested; we found no viable *C. sakazakii* cells remaining in PIF rehydrated with 30% kefir supernatant solution. These results suggest that kefir, with its well-established safety for oral consumption, can be used as an alternative to antibiotics to control infections caused by *C. sakazakii* in PIF.

**Keywords:** *Cronobacter sakazakii*, powdered infant formula, kefir, antimicrobial activity, microbial control
Roles of Ornithine Lipid in *Pseudomonas aeruginosa* Infection to Host Cells

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In phosphorus-limited condition, bacteria replace some components of membrane lipids with phosphate-free lipids. The ornithine lipids (OLs) are the most representative bacterial phosphate-free lipids. *Pseudomonas aeruginosa* has *olsBA* operon encoding acyltransferases that functions to synthesize OLs. OlsBA works in two steps for the OL biosynthesis, in which OlsB transfers an acyl group to ornithine to make lyso-ornithine lipid and OlsA converts the lyso-ornithine lipid into ornithine lipid by another acyl-group transfer. The *olsBA* overexpression attenuated the virulence of *P. aeruginosa* to animal hosts and affected some virulence-related phenotypes. Since the acyl-homoserine lactone (AHL) production and activity of quorum sensing (QS) regulators were repressed by the *olsBA* overexpression, we suggest that these effects are caused by the reduced activity of QS. The *olsBA*-overexpressing *P. aeruginosa* cells and purified OLs were able to increase calcium release and reduce the expression of iNOS, and COX-2 in animal cells, implying that OLs may directly modulate the inflammation-related physiology of host cells. Another important virulence phenotype, the biofilm formation was also affected by OL in *P. aeruginosa*, in which OLs directly enhanced the biofilm formation of *P. aeruginosa*. In conclusion, the *olsBA* overexpression is likely to attenuate the virulence of *P. aeruginosa* by sequestering the significant portion of the cellular acyl-group pool to the OL synthesis and as a consequence, by reducing the synthesis of AHLS and QS activity. Differently from this, the effects of OLs on the expression of inflammatory factors of host cells and biofilm formation may be directly exerted.
The Role of DesB on Virulence Traits in *Pseudomonas aeruginosa*

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The present study is aimed at investigating whether DesB (an aerobic desaturase) contributes to the pathogenic activities in host cells, such as exotoxin production, hemolysis, cell invasion and intracellular replication. For exotoxin production assay, HeLa cells were exposed to cell-free supernatant of wild type *P. aeruginosa* (WT) or its derived desB mutant, and exotoxins were indirectly quantified by cell viability assay. For invasion and intracellular replication assays, WT or desB mutant was inoculated in HeLa cells, and the efficiencies of invasion and intracellular replication of WT and desB mutant were compared. Hemolysis assay was performed by spotting overnight cultures of WT and mutants on blood-containing agar plate. In order to determine underlying mechanism of DesB on virulence at the molecular level, the transcriptional profiles of WT and desB mutant were compared by microarray analysis and qRT-PCR. desB mutant had different efficiency in exotoxin production, invasion and intracellular replication in cells compared to WT. Furthermore, decrease in hemolysis was observed in desB mutant, but plcH expression had no difference between WT and desB mutant, indicating that reduced hemolysis in desB mutant is not attributed to plcH. The results demonstrate that *P. aeruginosa* DesB have effect on pathogenesis-related behaviors in host cells, including exotoxin production, hemolysis, cell invasion and intracellular replication.
Development of Infectious Clones of a Wild-Type Korean Rabies Virus and Evaluation of Their Pathogenic Potential

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Most reverse genetic (RG) systems for rabies viruses (RVs) have been constructed on the genome background of laboratory-adapted strains. In this study, we developed an RG system using a street KGH strain to investigate the pathogenic potential of different strains. We developed a RG system with the Korean wild type (KGH) strain for the first time. Following the complete genome sequencing of the KGH strain, pKGH infectious clones were constructed using the CMV/T7 promoter, and hammerhead ribozyme (HamRz) and hepatitis delta virus ribozyme (HdvRz) were introduced to allow self-cleavage of the synthesized RNA. We successfully recovered the rescued virus by constructing chimeric RVs in which we replaced a part of the construct with the partial gene from the fixed RC-HL strain. The rescued viruses formed clearer and countable plaques in an immunostaining plaque assay, with a distinct plaque morphology. Furthermore, compared with the parental viruses, the pKGH/RcinsA4 strain containing the KGH strain G protein exhibited a decreased efficiency of cell-to-cell spreading in BHK-21 cells and significantly reduced (100-1000 fold) replication kinetics. However, pKGH/RcinsA4 strain-infected mice revealed 100% morbidity at 11 days post-infection, whereas other chimeric RV strains showed no mortality. Our RG system is a useful tool for studying differences in the cell-to-cell spreading efficiency and replication with respect to the different internalization patterns of street and fixed laboratory-adapted viruses.

Keywords: rabies virus, KGH strain, RC-HL strain, reverse genetic system, street virus, fixed virus

Acknowledgements: This work was supported by an intramural fund (2013-NI52001-00) by research of Korea Centers for Disease Control and Prevention.
Positive Role of Promyelocytic Leukemia Protein in Type I Interferon Response and its Regulation by Human Cytomegalovirus

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Promyelocytic leukemia protein (PML), a major component of PML nuclear bodies (also known as nuclear domain 10), is involved in diverse cellular processes such as cell proliferation, apoptosis, gene regulation, and DNA damage response. PML also acts as a restriction factor that suppresses incoming viral genomes, therefore playing an important role in intrinsic defense. Here, we show that PML positively regulates type I interferon response by promoting transcription of interferon-stimulated genes (ISGs) and that this regulation by PML is counteracted by human cytomegalovirus (HCMV) IE1 protein. Small hairpin RNA-mediated PML knockdown in human fibroblasts reduced ISG induction by treatment of interferon-β or infection with UV-inactivated HCMV. PML was required for accumulation of activated STAT1 and STAT2, interacted with them and HDAC1 and HDAC2, and was associated with ISG promoters after HCMV infection. During HCMV infection, viral IE1 protein interacted with PML, STAT1, STAT2, and HDACs. Analysis of IE1 mutant viruses revealed that, in addition to the STAT2-binding domain, the PML-binding domain of IE1 was necessary for suppression of interferon-β-mediated ISG transcription, and that IE1 inhibited ISG transcription by sequestering interferon-stimulated gene factor 3 (ISGF3) in a manner requiring its binding of PML and STAT2, but not of HDACs. In conclusion, our results demonstrate that PML participates in type I interferon-induced ISG expression by regulating ISGF3, and that this regulation by PML is counteracted by HCMV IE1, highlighting a widely shared viral strategy targeting PML to evade intrinsic and innate defense mechanisms.
Metabolic Reprogramming after Infection: Knowledges from Systems Biological Analysis of *in vivo* Transcriptome and Proteom of *Vibrio vulnificus*

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During infection, host and microbial pathogen do dynamic crosstalks and mutually affect gene expressions. Pathogens robustly change gene expression profiles to adapt to host environments. *Vibrio vulnificus* is a estuarine bacterium causing very rapidly progressing septicemia. To address how the pathogen adapts to host environment to cause disease, we studied global *in vivo* *V. vulnificus* gene expression through transcriptome, proteome, metabolome and flux analyses using an intraperitoneal semipermeable infection model. In comparison with in vitro culture in a rich medium, *V. vulnificus* underwent a genome-wide metabolic reprogramming after infection. Infecting *V. vulnificus* appeared to run microaerobic metabolism with shutting off the TCA cycle and having acetate and formate effluxed. Carbon and nitrogen metabolic pathways were reorganized to maximize siderophore production through upregulation of chorismate production. Energy was primarily produced through glycolysis and functional activation of membrane ATP synthase complex. Gluconeogenesis was inhibited. Fatty acid metabolism did not appear to contribute significantly to ATP production. Nucleotides were synthesized primarily through the salvage pathway. And we proved our hypothesis on metabolic reprogramming by applying molecular genetic experiments. In summary, we found a genome-wide ‘metabolism reprogramming’ after infection by adopting systems biological approach to multi-omics datasets.

**Keywords:** *Vibrio vulnificus*, Metabolism reprogramming, Systems biology, Transcriptome, Proteome
A Novel Natural Compound Alleviates Severe Sepsis

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The incidence of sepsis has steadily increased during the last few decades. However, the development of targeted therapy has been hampered because bacteria trigger the activation of multiple pro-inflammatory pathways. Here, we have investigated the effects of SNU-WR1, a novel compound derived from natural products, on mouse models of sepsis. SNU-WR1 has shown to protect mice against sepsis induced by LPS or CLP (cecal ligation and puncture). We show that SNU-WR1 expands CD11b\textsuperscript{+}Ly6g\textsuperscript{−}Ly6c\textsuperscript{int} MDSC-like cells (MDLC) in mice and protects mice against sepsis. SNU-WR1 treatment normalizes blood pressure and inflammatory cytokine levels, protects against SNU-WR1 administration also reduced blood pro-inflammatory cytokines level, such as TNF-\textgreek{a}, IL-1\textbeta, IL-6, MCP-1, and IFN-\gamma. SNU-WR1 treatment normalizes blood pressure and inflammatory cytokine levels, protects against acute kidney and liver damages in septic mice. In conclusion, SNU-WR1 mitigated the development of severe sepsis by modulating immune system.
Hepatitis B Virus X protein Stabilizes Myc Oncoprotein by Inhibiting Ubiquitination of Myc via Binding to Myc and Contributes to Oncogenesis

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The stability of Myc is regulated by ubiquitination, especially, via either SCFSkp2 or SCFɛbw7 ubiquitin E3 ligases. An earlier study has shown that hepatitis B virus X protein (HBx) enhances Myc stability. However, the underlying mechanism by which HBx enhances Myc stability and the impact of HBx-mediated Myc stabilization on viral oncogenesis remained to be elucidated. Here, we demonstrated that HBx stabilizes Myc by inhibiting Myc ubiquitination via interaction with Myc. In particular, our results showed that the inhibition of Myc ubiquitination by HBx occurs via Skp2, implicating that HBx inhibits the SCFSkp2 ubiquitin E3 ligase-mediated Myc ubiquitination. By mutagenesis, an HBx variant with mutated VFVL residues (Myc-binding region) failed to not only interact with Myc but also suppress Myc ubiquitination. Importantly, the VFVL mutant lost the ability to transforming cells, implying that the HBx-Myc interaction is critical for viral oncogenesis. In conclusion, our results showed that HBx stabilizes Myc by inhibiting SCFSkp2 ubiquitin E3 ligase-mediated Myc ubiquitination, and that the HBx-mediated Myc stabilization contributes to viral oncogenesis.

Keywords: cullin RING E3 liagse, HBx, hepatitis B virus, Myc, Skp2
Symposium

YS4 Young Scientist Session 4
Metabolic Engineering of *Escherichia coli* for the Production of Industrial Platform Chemicals

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In this study, a novel metabolic pathway was designed for the production of industrial platform chemicals including fumaric, 3-aminopropionic, and 3-hydroxypropionic acid.

Firstly, rational metabolic engineering together with flux optimization were performed for the development of an *E. coli* strain capable of efficiently producing fumaric acid. Using a fumaric acid producing *E. coli* strain as a host, the *C. glutamicum* panD gene (encoding L-aspartate-α-decarboxylase) was overexpressed and the native promoter of the *aspA* gene was replaced with the strong trc promoter, which allowed efficient production of 3-aminopropionic acid. Additionally, metabolic pathway was extended by introducing beta alanine pyruvate transaminase and malonic semialdehyde reductase to produce 3-hydroxypropionic acid from 3-aminopropionic acid. Finally, fed-batch fermentations were performed for mass production of target products using developed strains in this study. (This work was supported by Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A2026556) funded by the Ministry of Education, Science and Technology.)
Technique of Fine-Tuned Knockdown Using Small Regulatory RNA and Its Application in Metabolic Engineering for Production of Putrescine from *Escherichia coli*

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Putrescine, 1,4-diaminobutane, is an important chemical which is used as a monomer of nylon in chemical industry. Biosynthesis of putrescine from renewable biomass is able to be a good substitute of petroleum based putrescine production from an environmental point of view. There was a trial of development of putrescine producing *Escherichia coli* strain through construction of biosynthesis pathway using gene knockout and overexpression. We expected that productivity of this strain is able to be increased by use of recently developed knockdown method, small regulatory RNA system. Using this system we repressed competitive branch pathways and increased flux to putrescine. Additionally, culture condition was modified to support the increased putrescine productivity by adding higher amount of nitrogen source and dissolved oxygen. The fed-batch cultivation of finally engineered strain and modified culture condition resulted in dramatically increase of productivity and yield. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012-C1AAA001-2012M1A2A2026556); the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea]

**Keywords:** metabolic engineering, sRNA, small RNA, putrescine
Autoinducer-2 Mediated Quorum Sensing Influences on Bacterial Growth under Stress Conditions

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Quorum sensing is a cell density dependent signalling. Bacteria use small molecules, autoinducers, to communicate by quorum sensing. LuxS mediated autoinducer-2 (AI-2) is an inter-species bacteria signal that is especially used by many pathogenic bacteria, both gram-positive and gram-negative, to regulate their virulence expression. From this concept, a number of studies have suggested that AI-2 inhibition is a key factor of new antimicrobial agents. Recent studies suggest that the AI-2 system is also involved in stress response. We observed the growth of Enterohemorrhagic Escherichia coli O17:H7 (EHEC) and its luxS-deficient strain under specific stress conditions. We found there are significant growth differences in NaCl and bile salt conditions. Interestingly, the luxS-deficient EHEC strain showed strong resistance to osmotic stress compared to the wild type of EHEC. We observed specific virulence gene expressions on the microarray. Moreover, the same pattern was observed by the qRT PCR data. These results suggest that AI-2 mediated quorum sensing has a direct influence on, not only bacterial growth rate but also, virulence expression under the specific condition. Therefore, the environmental factors should be considered in order to provide AI-2 quorum quenching strategies.
Antibiotic Effect of Violacein and Purpose of its Production in Nature

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In nature, violacein is one of antibiotics made by various kinds of bacteria. It is well known anticancer, antibacterial antiprotozoal effect. The violacein is well known to inhibit gram positive bacteria growth. 5mg/L of violacein inhibit the growth of multi drug resistant Staphylococcus aureus which extracted from patient, much higher concentration of violacein shown bacteria-cidal effect. But lone questionable point is that violacein is hydrophobic material which is not diffused in water environment. Thus, in nature, even gram positive bacteria specifically weak by exposure of violacein, violacein can not be possible to effective to eliminate surrounding rivals. Nevertheless this hydrophobicity problem, co-cultured Staphylococcus aureus is 99.9% killed with violacein production strains. That means violacein producing strain has some method to spread violacein surrounding environment even if it is hydrophobic. And also production of violacein can block predatory protozoan survival and reproduction thus violacein give big advantage to its producing strain to compete their rival and protect the strain threaten from predatory protozoans.

Keywords: Violacein, Deoxyviolacein, Antibiotics, Co-Culture
Development of Novel SRP Machinery-Engineered *E. coli* Mutant for the Secretary Production of Antibodies and Therapeutic Proteins

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Signal recognition particle (SRP)-dependent secretion pathway of *E. coli* is an analogue of eukaryotic SRP pathway, which is featured by co-translational translocation. Even though this pathway offers great benefit of preventing cytoplasmic aggregation of proteins before secretion, limited capacity was major hurdle for utilizing the pathway as an alternative of recombinant protein secretion. To overcome this problem, we developed a novel *E. coli* mutant, of which SRP machinery was dramatically increased. For the engineering of *E. coli* strain, we firstly designed a new periplasmic fluorescent reporter system by using a special fluorescent dye, FlAsH-EDT₂. With this reporter system, we screened transposon-generated *E. coli* mutant library through the several rounds of FACS, after then a novel *E. coli* strain, of which SRP machinery was fairly improved, was isolated. It was identified as single gene-deficient strain, and the general effect of this mutation for SRP pathway-mediated protein secretion was demonstrated by different kinds of model proteins. In addition, very high production yields of human full-length IgG and GPCR mutant were achieved in the fed-batch cultivations with the isolated *E. coli* mutant.
Luncheon Seminar

Research and Publication Ethics in Science and Scientific Writing

International Meeting of the Federation of Korean Microbiological Societies
How To Publish a Scientific Paper

Yong-Sun Bahn

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Publish a paper is an essential process for scientists to share their scientific achievements with the colleagues in their field as well as the public. More practically, it is critical for those who aim to get a job, promotion, and to obtain funding from their government and other sources, as it is well reflected by saying “Publish or Perish”. This talk is mainly designed for graduate students and young scientists, who have just started their academic career, to prepare how to publish a scientific paper. I will present how the scientific paper should be structured and what is the normal journal process for submission, peer-review, and editorial decision. In addition, I will also discuss about some of key indexes, including impact factor, Eighenfactor score, and h-index, which young scientists should be familiar with during their career development.
The retraction rate of published papers has increased recently. Scientific papers are retracted for many reasons including misconducts or honest errors. The recent rise in retraction rate is partly due to the growing awareness of research misconduct in scientific communities. The journals have made a greater effort than ever to police scientific and publication misconducts. The availability of tools for detecting text similarities such as Cross-Check made it easier to detect possible text plagiarism. Case studies in plagiarism, however, revealed that some scientific researchers had not been fully aware that copying words and/or sentences from other papers may constitute a serious misconduct. They misunderstood that, as far as they performed their own research and collected novel data, some of the texts might as well be borrowed from other papers. In this session, I will emphasize the importance of using one’s own expressions in writing a science paper. A paper has to report authors’ original work in their own words. Other people’s data and their words can only be utilized when they are explicitly and appropriately mentioned. The proper ways for citing and paraphrasing other people’s work will be discussed. It is crucial to understand that the results of a research are accepted as objective scientific knowledge only when it is performed and reported in an ethical manner. (The presentation will be given in Korean.)
Poster Session
**A. Systematics and Evolution**

### A-1

**Some Unrecorded Species Psathyrella ammophilla, Marasmiellus mesosporus and Tulostoma fimbriatum var. campestre from Taean Coastal(Tacanaeum) National Park in Korea**

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*Psathyrella ammophilla, Marasmiellus mesosporus and Tulostoma fimbriatum var. campestre* are proposed as a new species in Korea. They are growing singly or in small clumps near beach grass feeding saprotrophically on the decaying roots or living stem. In Korea, a report of macrofungi from coastal sand dune habitat is quiet rare. Collected species are described macro- and microscopically, and the most important features are illustrated.

Keywords: Unrecorded species, Saprotroph, *Psathyrella ammophilla*, *Marasmiellus mesosporus*, *Tulostoma fimbriatum*

### A-2

**New Records of Three Sarcoscypha Species (Sarcoscyphaceae, Ascomycota) in South Korea**

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Biological and Genetic Resources Assessment Division, National Institute of Biological Resources

The genus *Sarcoscypha* (Fr.) Boud, characterized by a bright scarlet and cup-shaped apothecium, is ascomyceteous fungi in the family *Sarcoscyphaceae*. To date, more than 50 *Sarcoscypha* species have been reported worldwide with 2 species (*Sarcoscypha coccinea*, *Sarcoscypha occidentalis*) in South Korea. The important taxonomic characters for distinguishing species in *Sarcoscypha* pertain to smooth ascospores with truncated end and the presence of the mucilaginous envelope surrounding the ascospore after it is ejected. However, the species are difficult to distinguish from one another because these characters are obtained when the organism is both alive and mature. In the present study, two new *Sarcoscypha* species and an unrecorded species were confirmed on the basis of internal transcribed spacer rDNA sequence analyses and micro-morphological features. A detailed taxonomical description and illustrations for new *Sarcoscypha* species are provided.

Keywords: Sarcoscyphaceae, Phylogenetic analysis, Taxonomy

### A-3

**Colbella collisoli sp. nov., Isolated from Lava Forest Soil**

Keun Chul Lee1, Kyoung Kyu Kim1, Jong-Shik Kim2, Jae-Shin Kim2, Suk-Hyang Ko1, Seang-Hoon Yang1, and Jung-Sook Lee1*

1KRBIB, 2GIMB, 3World Heritage and Mt. Hallasan Research Institute, 4UST

A novel bacterial strain NKM-5T was isolated from soil of lava forest, Nokkome Oreum, Jeju, Republic of Korea. Cells of strain NKM-5T were Gram-staining-positive, motile, endospore-forming, rod-shaped strain and oxidase- and catalase-positive. It contained anteiso-C15:0 and iso-C15:0 as the major fatty acids, menaquinone-7 (MK-7) as the predominant isoprenoid quinone, phosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, lysyl-phosphatidylglycerol, an unidentified phospholipid and three unidentified aminophospholipids as the polar lipids, and meso-DAP as the diagnostic diamino acid in the cell-wall peptidoglycan. The DNA G+C content was 48.3 mol%. Phylogenetic analysis, based on 16S rDNA gene sequencing, showed that strain NKM-5T was most closely related to *Colbella lupini* RLA4U3B2 (96.9 % sequence similarity) and fell into a clade in the genus *Colbella*. On the basis of phylogenetic, chemotaxonomic and phenotypic data, strain NKM-5T represents a novel species in the genus *Colbella*, for which the name *Colbella collisoli* sp. nov. is proposed, with strain NKM-5T (= KCTC 33634T = CECT 8805T) as the type strain. This work was supported by Mid-Career Researcher Program through NRF grant funded by the Ministry of Science, ICT and Future Planning (MSIP) of the Republic of Korea and a grant from the KRBIB Research Initiative Program.

Keywords: Firmicutes, *Colbella collisoli*, taxonomy, lava forest, Nokkome Oreum

### A-4

**Revision of the Genus Arthonia (Arthoniaceae) in Korea**

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This study is aimed to revise all *Arthonia* species in North East Asia by various analyses in morphology, chemistry and molecular phylogenetics. This research will be the first approach concentrating on *Arthonia* and its family in Korea, and will produce baseline data of diverse *Arthonia* species, and profiles of lichen substances and nuclear rDNA and RNA sequences from them. To efficiently collect corticolous *Arthonia* species, the relationship between the corticolous *Arthonia* species and substrate plants was analyzed. The result showed the corticolous species have distinct substrate preference to five major plant genera such as *Quercus*, *Fraxinus*, *Glycyrrhiza*, *Coriaria* and *Alnus*. Following the substrate-specific analysis, field surveys in 2015 produced overall 60 specimens of *Arthonia* species and major substrates of them are *Fraxinus rynchophylla* and *F. sieboldiana*. All 60 specimens are different with previous Korean *Arthonia* species and possible new records or new species, categorized into 7 groups such as Apatetica, Granosa, Pinastri, Punctiformis, Rhiodis, Small 2-septation, and 4-septation. Most species in *Arthonia* are highly phorophyte-specific and more dominant in tropical and subtropical zones. The substrate-specific and geography-based field survey will assure baseline data of diverse *Arthonia* species and this will produce better results in the next laboratory analyses.

Keywords: *Arthonia*, Korea, Lichenized fungi, Revisonal study, Taxonomy
A Bio-control Efficacy of Bacteria against *Fusarium Oxysporum* f. sp. *Conglutinans*

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*Fusarium oxysporum* is found in many types of soil and in countries around the world. And *F. oxysporum* causes Fusarium wilt. Fusarium wilt is a common vascular wilt fungal disease. Fusarium wilt generally caused such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off. One of the most important diseases of brassicas in the warm regions of the world is *Fusarium oxysporum* it is caused by the vascular wilt pathogen, *F. oxysporum* f. sp. *conglutinans*

The production of antifungal substances by bacteria has long been recognized and this knowledge is entering practical life through the use of bacterial antagonists to protect crops against their fungal enemies. Isolated bacteria witch have antifungal activity against *Fusarium oxysporum* f. sp. *conglutinans* from agriculture soil. Isolated total 878 bacteria and screened with dual culture method. 31 bacteria show inhibition zone and according to reproducibility, 17 strain selected as candidate. And then spread *Fusarium oxysporum* f. sp. *conglutinans* suspension on PDA and inoculate 17 strains which showed reproducibility. Finally 4 strains showed efficiency and one of them showed 1.3cm of inhibition zone. Future work is identify the most efficiency strain and identify antifungal substance of efficiency strain.

Keywords: *Fusarium Oxysporum*, Antifungal activity, Brassica, Wil disease

A Bacterium Representing Novel Species in the Genus *Pontibacter*, Isolated from Seawater of Gwangyang Bay

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A Gram-negative, non-motile, aerobic and light pink pigmented bacterium, designated strain KYW1030 was isolated from the seawater of Gwangyang bay, Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain KYW1030T formed a distinct lineage within the genus *Pontibacter* and was most closely related to *Pontibacter akesuensis* KCTC 12367T (95.09 %) and *Pontibacter actiniarum* KCTC 12758 T (97.87 % sequence similarity) and *Pontibacter akesuensis* KCTC 12367T (95.09 %). Strain KYW1030T grew at 4-40 °C (optimally at 30 °C) and 1-5% of NaCl (optimally at 1% NaCl). Cells grew on LB, MA, R2A, TSA and NA. Catalase-positive and oxidase-negative. The predominant fatty acids are summed feature 4 (C16:0), iso (anteiso) B (or) C17, anteiso (anteiso) Briso I), iso-C15:0 and iso-C17:0 3-OH. KYW1030T were proposed as a new species of the genus *Pontibacter*.

This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea. It was also supported by the CK(university for Creative Korea)-1.

Keywords: *Pontibacter*, seawater

Taxonomic Description of *Humibacter* sp. nov., Isolated from Soil

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A novel actinobacterial strain, R1-20T was isolated in the process of analyzing the microorganism diversity of the soil that was habitat of white heron in Daedeon, Republic of Korea. The isolate was a non-motile, Gram-positive, and short rod. The colonies were white, dull, convex, and entire margined during the early stages of growth, but gradually became yellow. The analysis of 16S rRNA gene sequence indicated that the strain belongs to the genus *Humibacter* of the family *Microbacteriaceae*, and the sequence similarity was 97.16% with *Humibacter aterii*, and 96.44% with *Humibacter albus*. The G+C content of R1-20 T was 65.5%, and major respiratory isoprenoid quinones were MK-11, MK-12 and MK-10. The acyl group of peptide glycan was acetyl type, and the diagnostic diarnmonobutyric acid (DAB). Glutamic acid, alanine and glycine were also present in the cell wall. The major fatty acids of strain R1-20T were anteiso-C17:0, iso-C16:0, and anteiso-C15:0.

On the basis of the phylogenetic and chemotaxonomic analysis, strain R1-20T (KCTC 318606T = JCM 31015 T) is considered a novel species of *Humibacter*, for which the name *Humibacter* sp. nov. is proposed.

Keywords: Actinobacteria, Microbacteriaceae, *Humibacter* soil

Genome-wide SNP Analysis Associated with Origin and Fruitbody Color in *Flammulina velutipes*

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In this study a total of 20 strains of *F. velutipes* were sequenced and analyzed to identify the genomic regions related to origin and fruitbody color. NGS data was yielded by using Illumina HiSeq platform. Short reads were filtered by quality score and read length were mapped on the reference genome (KACC42780). Polymorphic SNPs of *F. velutipes* strains representing the phylogenetic segregation of white- and brown-fruitbody forming groups were compared. As previously reported, white is recessive to brown in fruitbody color. The white strains produced 131,874 SNPs to be aa type or Homozygous compared. As previously reported, white is recessive to brown in fruitbody color. The white strains produced 131,874 SNPs to be aa type or Homozygous compared. As previously reported, white is recessive to brown in fruitbody color. The white strains produced 131,874 SNPs to be aa type or Homozygous compared.
A-9

**Micromonospora fulva** sp. nov., Isolated from Forest Soil

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A novel actinobacterium, designated strain UDF-1T, was isolated from forest soil in Chungnam, South Korea. Its taxonomic position was investigated through a polyphasic approach. Strain UDF-1T is non-motile and formed a branched brownish orange substrate mycelium and none aerial hyphae which differentiated into spherical to oval appeared to be rough. Comparative 16S rRNA gene sequence analysis showed that strain UDF-1T belonged to the genus *Micromonospora*, showing the highest sequence similarity to *Micromonospora paludicola* NEAU-CX1T (99.18%), *Micromonospora maasaeensis* NEAU-MES19T (99.03%), *Micromonospora endolithica* DSM 44398T (98.73%) and *Micromonospora maitumotensae* IMISNU 22005T (98.73%). However, low DNA-DNA relatedness (< 30 %) and many physiological and molecular properties differentiated the isolate from those previously described type strains. The cell wall contained meso-diaminopimelic acid and the whole-cell sugars were arabinose and xylose. The major polar lipids were phosphatidylinositol, diphasphatidylglycerol and phosphatidylethanolamine. The major cellular fatty acids were iso-C15:1 0, anteiso-C17:0 3-iso-C17:0 and C17:0 and the predominant menaquinones were MK-10 (H1) and MK-10 (H1). The genomic DNA G+C content was 73.1 mol%. On the basis of the data presented, strain UDF-1T is suggested to represent a novel species of the genus *Micromonospora*, for which the name *Micromonospora fulva* sp. nov. is proposed.

Keywords: Gene typing, Xanthomonas, Multilocus sequence analysis

A-10

**Advanced Gene Typing Analysis of Xanthomonas Isolated from Korean Pepper**

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Xanthomonas species can cause bacterial spots and blights of leaves, stems, and fruits on a wide variety of plant species. Bacterial leaf spot has caused significant crop losses over the years. This species have been known to include X. oryzae pv. oryzae, X. campestris pv. pisi, and X. oryzae pv. citrulli. Many DNA-based techniques have been developed, including the analysis of the 16S rRNA genes in order to differentiate and classify the Xanthomonas species. We focused on difference between four housekeeping genes and two avirulence genes for analysis of Xanthomonas isolates for multilocus sequence analysis. Twenty-eight Xanthomonas were collected from various regions in Korean pepper fields. DNAs of partial Xanthomonas strains were amplified by PCR using four housekeeping genes (dnaK, ftsA, gyrB and rpoD) and analysed for phylogenetic tree. These Xanthomonas strains were grouped into *X. oryzae*. An experiment with two avirulence genes (avrBs2 and avrBs3) is encoding under going. These results of analysis might provide another approach for identification of the Xanthomonas species isolates.

Keywords: Gene typing, Xanthomonas, Multilocus sequence analysis

A-11

**Effects of Coffee and a Traditional Chinese Herbal Medicine on the Growth of Two Gat Lactobacilli from Koreans and Europeans**

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The gut microbiota has enormous impact on human physiology and metabolism. Various studies showed that diet, geographical origin, and ethnic background play a crucial role in the diversification of the gut microbiota. In this study, effects of Huwangyuanhaedok-tang (HHT) and coffee on the survival rates of two Lactobacillus acidophilus strains isolated from guts of Koreans and Europeans, respectively, were examined. Both compounds are known to have antimicrobial activities and have been consumed differentially in East Asia and in West for a long time. The Korean strain was found to have higher resistance against HHT, while the European strain showed higher resistance against decaffeinated coffee. It was difficult to compare their survival rates in the presence of coffee due to considerable antimicrobial effect of caffeine on both strains. Lower resistance of the European strain against HHT can be attributed to our consumption of herbal medicines in the Western culture. This indicates that the European gut microbiota had less development resistance against antimicrobial properties of HHT. Higher resistance of the European strain against decaffeinated coffee suggests that other that bioactive components besides caffeine in coffee might be involved in antimicrobial activity which induced resistance of the European strain to decaffeinated coffee.

Keywords: Gut microbiota, Diversification, Lactobacillus acidophilus, Antimicrobial activity, Probiotics

A-12

**Rhodanobacter panaegriseus** sp. nov., isolated from Ginseng Rhizosphere

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Department of Microbial & Nano Materials, Mokwon University

In the course of screening for new bioactive compounds, two-hundred-two strains were isolated and 26 strains were selected for further studies which had formed clear zone around colonies on medium containing protein, cellulose, starch and chitin as a sole carbon sources. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolates belonged to six major taxa: *gamma-proteobacteria* (7 isolates), *beta-proteobacteria* (6 isolates), *gamma-proteobacteria* (6 isolates), *Bacteroides* (3 isolates), *Actinobacteria* (3 isolates) and Firmicutes (1 isolate). In the taxonomical characterization of *gamma-proteobacteria*, designated strain CR164T, was the closely related to *Rhodanobacter casi* JCM 16242T (98.3%), *R. thiocyanatoxydans* KCTC 12771T (98.3%), *R. gironisdenitrans* KCTC 12823T (98.4%), *R. limaniclatus* KCTC 12596T (98.1%), *R. denitrificans* JCM 17641T (97.9%) and *R. fusae* KCTC 12068T (97.6%) and *R. xianggansui* KCTC 23100T (97.1%). DNA-DNA relatedness with closely related strains was lower than 60%. The growth of strain CR164T was observed at 10–37 °C. The pH range for growth was 5.0–9.0 and the strain CR164T grew at salinities of 0.1 % (w/v) NaCl. The predominant isoprenoid quinone was ubiquinone-8 and the major cellular fatty acids were iso-C15:0 3-iso-C17:0 and iso-C15:0 3-iso-C16:0. On the basis of polyphasic analysis, strain CR164T represents a novel species of the genus *Rhodanobacter* for which the name *Rhodanobacter panaegriseus* sp. nov. is proposed.

Keywords: *Rhodanobacter panaegriseus*, *gamma-proteobacteria*
**A-13**

**Endoradicisella ginsengi gen. nov., sp. nov., a Slow Growing Bacterium Isolated from Inside of Ginseng Root**

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A noble bacterium, designated strain 4NR53T was isolated from inside of ginseng root. Based on the 16S rRNA gene sequence analysis, strain 4NR53T was shown to belong to the family Bradyrhizobiaceae and showed closest phylogenetic similarity to Pseudoburkholderia taiwanensis CC-BB4T (94.72%), Varivacter gonginoides HE970589T (93.31%), Bradyrhizobium japonicum USDA 6T (93.05%) and Alphapius felis ATCC 5360T (92.7%). Cells of strain 4NR53T were Gram-stain-negative, non-spore-forming rod bacterium and motile by one flagellum. The strain was oxidase and catalase-positive. Growth was observed at salinities of 0-1% (w/v) NaCl, at pH 5.0-11.0 (optimal growth at pH 7.0) and at temperatures of 20-37 °C (optimal growth at 28 °C). The predominant ubiquinone was Q-10 and the major cellular fatty acids were C18:1 with the major fatty acids being C14:0 and summed feature 3 (C15:0 3OH and/or iso-C17:0 3OH). The type strain is 4NR53T (KACC 17632T =NBRC 109815T).

**Keywords:** Endoradicisella ginsengi, inside of ginseng root, a slow growing bacterium

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**A-14**

**Sphingobium polysaccharaeus sp. nov., an Exopolysaccharide-producing Bacterium Isolated from the Rhizosphere of Angelica sinensis**

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Thirty-five strains of exopolysaccharide-producing bacteria were isolated from rhizosphere of Angelica sinensis. Among them, a strain Tan-25T produced the highest amount of EPS in 2% glucose medium. The growth of the bacteria was totally inhibited in 10-1NB but was supported in 10-2NB. The strain Tan-16T produced optimal growth at 28°C, pH 9.0 and salinities of 0 % (w/v) NaCl. It was notable that the growth of the isolates was sensitive to the salinity of 0.1% NaCl. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain Tan-16T belonged to the genus Flavobacterium, showing the closest phylogenetic similarity to F. frigidimarum KUC-1T (98.03 %), F. heimbrem DSM 12611T (98.0 %), F. pectinivorans DSM 6368T (97.8 %) and F. hiedsatis DSM 2067T (97.4%). However, the results of DNA-DNA hybridization and physiological and biochemical tests showed that strain Tan-16T could be differentiated from its closest relatives. The predominant menaquinone was MK-6 and the major cellular fatty acids were iso-C15:0 and summed feature 3 (C16:1 7c and/or iso-C15:0 2-OH). On the basis of polyphasic analysis from this study, strain Tan-16T represents a novel species of the genus Flavobacterium for which the name Flavobacterium halomollis sp. nov. is proposed.

**Keywords:** Flavobacterium halomollis, halo sensitive bacterium

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**A-15**

**Flavobacterium halomollis sp. nov., halo-sensitive bacterium isolated from rhizosphere soil of Angelica sinensis**

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The number of colonies on 100-fold dilution of nutrient agar (100-1 NA) was increased with incubation time following colony formation curves (CFUs) during the isolation of bacteria from rhizosphere soil of Angelica sinensis. According to CFU, we obtained 27 isolates from CFU-II (about 288 to 1200 hr) period. Two isolates, designated strain Tan-16T and Tan-111, were investigated on the growth characteristics on diluted NB. The growth of the bacteria was totally inhibited in 10-2NB but was supported in 10-3NB. The strain Tan-16T showed optimal growth at 28°C, pH 9.0 and salinities of 0 % (w/v) NaCl. It was notable that the growth of the isolates was sensitive to the salinity of 0.1% NaCl. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain Tan-16T belonged to the genus Flavobacterium, showing the closest phylogenetic similarity to F. frigidimarum KUC-1T (98.03 %), F. heimbrem DSM 12611T (98.0 %), F. pectinivorans DSM 6368T (97.8 %) and F. hiedsatis DSM 2067T (97.4%). However, the results of DNA-DNA hybridization and physiological and biochemical tests showed that strain Tan-16T could be differentiated from its closest relatives. The predominant menaquinone was MK-6 and the major cellular fatty acids were iso-C15:0 and summed feature 3 (C16:1 7c and/or iso-C15:0 2-OH). On the basis of polyphasic analysis from this study, strain Tan-16T represents a novel species of the genus Flavobacterium for which the name Flavobacterium halomollis sp. nov. is proposed.

**Keywords:** Flavobacterium halomollis, halo sensitive bacterium

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**A-16**

**Burkholderia humisilvae sp. nov., Burkholderia solisilvae sp. nov. and Burkholderia rhizosphaerae sp. nov., Isolated from Forest Soil and Rhizosphere Soil**

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Two strains, Y-12T and Y-47T, and a strain, WR43T were isolated from forest soil of a mountain and rhizosphere soil at Daejeon in Korea, respectively. The three strains grew between 10 and 55 °C (optimal growth at 28-30 °C), between pH 3.0 and 8.0 (optimal growth at pH 6.0) and at salinities of 0-4.0 % (w/v) NaCl growing optimally with 0 % (w/v) NaCl. On the basis of 16S rRNA gene sequence analysis, three strains were found to belong to the genus Burkholderia, showing closest phylogenetic similarity to Burkholderia diazotrophica WY461T (97.2-97.7 %) and inter-species similarities ranged from 98.3 % to 98.7 %. Additionally, these strains formed a distinct group in phylogenetic trees based on the housekeeping genes recA and gyrB. The predominant ubiquinone was Q-8 and the major fatty acids were C15:0 3OH and C17:0 cyclo, and the DNA G + C content of novel isolates were 61.6 – 64.4 mol%. DNA-DNA relatedness among three strains and the closest species of the genus Burkholderia was less than 50 %. On the basis of 16S rRNA recA and gyrB gene sequence similarities, chemotaxonomic and phenotypic data, the three strains represent novel species within the genus Burkholderia, for which the names Burkholderia humisilvae sp. nov. (type strain, Y-12T), Burkholderia solisilvae sp. nov. (Y-47T) and Burkholderia rhizosphaerae sp. nov. (WR43T) are proposed.

**Keywords:** Burkholderia sp. nov., forest soil, rhizosphere soil
Halomonas sp. nov., a Halophilic Bacterium Isolated from the Sediment of a Solar Saltern Pond

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Twenty halophilic bacterial strains were isolated from sediment of solar saltern pond by salinity on 15% on marine agar (MA). Eight halophilic bacteria growing at the salinity on 20% (w/v) NaCl on MA. A halophilic strain CPS111 was represented a noble species was grown at the MA with salinity on 20% (w/v) NaCl. Cells Gram-staining-negative, aerobic, non-spore-forming rods. Strain CPS111 was catalase-and oxidase-positive and reduced nitrate to nitrite. It grew at salinities of 0-20% (w/v) NaCl (optimum, 9 % NaCl), at 10-40°C (optimum, 37°C), at pH 5.0-9.0 (optimum, pH 7.0-8.0). On the basis of 16S rRNA gene sequence analysis, strain CPS111 was shown to belong to the genus Halomonas, showing closest phylogenetic similarity to Halomonas pantalipodii LMG 24455T (98.5 %), Halomonas venetiana DSM 15911T (98.4 %), Halomonas campaniensis DSM 15293T (98.1 %). The major fatty acids were C14:0 and C16:1ω7c, C16:0 3-OH, C17:0 cyclo-C19:0 cyclo C16:0, summed feature 3 (C16:0ω7c/cis-C10:0 2-OH) and the predominant respiratory quinone was Q-9. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, aminophospholipid and phospholipid. The DNA G + C content of this novel isolate was 65.2 mol%. On the basis of evidence from this taxonomic study using a polyphasic approach, strain CPS111 represents a novel species of the for the name Halomonas sp. nov. is proposed. The type strain is CPS111 (KCTC 42557T, = NBRC 110636).

Keywords: Halomonas, halophilic bacterium, sediment, solar saltern pond

Chryseobacterium stabulisuum sp. nov. Isolated from Air in a Pig Farm

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The taxonomic status of a novel Gram-negative and deep yellow-pigmented bacterium (strain PBT33-4) isolated from air in a pig farm was determined. Phylogenetic analysis of 16S rRNA gene sequence revealed that the strain PBT33-4T was a member of the family Flavobacteriaceae phylogenetically related to genus Chryseobacterium. The 16S rRNA gene sequence of strain PBT33-4T shared 97.3% and 97.1% sequence identities with type strains of C. halifex KACC 14174T and C. hominis KACC 14168T, respectively. It shared less than 97% sequence identities with other species in the genus Chryseobacterium and its DNA–DNA relatedness with C. halifex KACC 14174T and C. hominis KACC 14168T resulted in values below 70%. The DNA G+C content and predominant fatty acids of strain PBT33-4T were 43.4 mol% and iso-C15:0. Summed feature 9, iso-C17:0 3-OH and anteiso-C15:0. The phylogenetic evidence and results of phenotypic analyses showed that this isolate should be classified as a member of a novel species in the genus Chryseobacterium, for which a name of Chryseobacterium stabulisuum sp. nov. is proposed. The type strain is PBT33-4T (KCTC 42557T).

Keywords: Air, Chryseobacterium, Flavobacteriaceae, Pig Farm

New Lichen-Forming and Lichenicolous Fungi from South Korea

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Our knowledge on the biodiversity of lichen-forming and lichenicolous fungi of the Korean Peninsula has increased considerably in the last decade. More than 1,100 taxa have been published up to now. Among them about 120 species were described as new for science from territory of South Korea (i.e. about 10% of total number of species). Proximately about half of them were described during last 10 years (2005-2015 years). Fifteen new for science species of lichen-forming and lichenicolous fungi, i.e. Arthonia cavernicolis S. Y. Kon.1, L. Lokos et J.-S. Hur, Caloplaca chiajsiensis S. Y. Kon.1, L. Lokos et J.-S. Hur, Lichenochorus mairevetzicarida S. Y. Kon.1, L. Lokos et J.-S. Hur, and Roselloa cornuana S. Y. Kon.1, et J.-S. Hur as well as species of the genera Fistigecia, Helfelia, Amandinea, Buellia, Halcidactia, Lecanactis, Lecanoria, Marvellina, Melanophlebia are described from South Korea during 2015 (Kondratyuk et al. 2015 a,b).

Keywords: Lichen-forming fungi, Lichenicolous fungi, New species, New records, South Korea

Whole Genome Analysis of Hantaan Virus from Rodents Captured in Gangwon Province, Republic of Korea

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Hantaviruses, bunyaviridae, are negative sense RNA viruses containing Large (L), Medium (M), and Small (S) segments. Hantaan virus (HTNV) exists in Apodemus agrarius and is an etiology of Hemorrhagic Fever with Renal Syndrome (HFRS) demonstrating a mortality rate of 4-15% in Asia and Europe. In 2013, appearance of a dead HFRS-patient from the Korean Army in Gangwon leads to investigate the epidemiology of HTNV in the region as well as Gyeonggi. The tripartite genomes of 6 HTNV strains was performed by RT-PCR. Phylogenetic analyses showed all segments of 3 HTNV strains from Cheorwon formed a cluster with those from Pocheon in Gyeonggi. The tripartite genomes of 6 HTNV strains from Yanggu were an independent group from the strains in Gyeonggi. However, M and S segments of 6 HTNV strains from Hwacheon formed a cluster with the strains from Gangwon whereas L segment of them formed a cluster with those in Gyeonggi suggesting a possible genetic reassortment. In conclusion, this study demonstrates HTNV strains from Gangwon form geographic clusters showing the genetic diversity of HTNV whole genome sequences in the ROC. The phylogeographic analysis may provide a way to establish molecular diagnosis and epidemiological study for HFRS patients in the endemic area.

Keywords: Hantaan virus, Phylogenetic tree, Genetic diversity
**A-21**

*Bacillus paralicheniformis* sp. nov., Isolated from Fermented Soybean Paste

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An isolate of a Gram-positive, facultatively anaerobic, motile, rod-shaped, endospore forming bacterium was recovered from soybean fermented paste. Phylogenetic analysis of the 16S rRNA gene indicated that the strain was most closely related to *Bacillus sonorovis* KCTC-13918T (99.5 %) and *Bacillus licheniformis* DSM 13T (99.4 %). The predominant cellular fatty acids were anteiso-C₁₅ : 0 (37.7 %) and iso-C₁₅ : 0 (31.5 %). The predominant menaquinone was MK-7. The cell-wall peptidoglycan contained meso-diaminopimelic acid. A draft genome of the strain was completed and used for phylogenetic analysis. A phylogenetic analysis of all published genomes of species in the *Bacillus* licheniformis group revealed that strains belonging to *B. licheniformis* clustered into distinct groups, with group 1 consisting of *B. licheniformis* DSM 13T and 11 other strains and group 2 consisting of KJ-16T and 4 other strains. The DNA G+C content was 45.9 mol%. Strain KJ-16T and another strain from group 2 were subsequently characterized using a polyphasic taxonomic approach and compared to strains from group 1 and another closely related *Bacillus* species. Based on the consensus of phylogenetic and phenotypic analyses, we conclude that this strain represents a novel species within the genus *Bacillus*, for which the name *Bacillus paralicheniformis* sp. nov. is proposed, with type strain KJ-16T (=KACC 18426T = NRRL B-65293T). Supported by grants from National Academy of Agricultural Science.

Keywords: *Bacillus paralicheniformis*, genome, taxonomy

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**A-22**

*Friedmanniella aerolata* sp. nov., Isolated from Air

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A bacterium, strain 7515T-26⁸, was isolated from an air sample collected in Taean region, Republic of Korea. The isolate was aerobic, Gram-stain-positive, non-flagellated cocci, growing in the temperature, pH and NaCl ranges of 10-33 °C, pH 5.0-9.0 and 0-2 % (w/v). It shared the high sequence similarities with *Friedmanniella lacustris* EL-17A (97.6 %), *Friedmanniella haicki* FA2 (96.9 %) and *Friedmanniella luteola* FA1T (96.9%), showing high sequence similarities of 96.5-97.6 % with members of the genus *Friedmanniella*. The phylogenetic trees showed strain 7515T-26⁸ and members of the genus *Friedmanniella* formed a compact cluster separable from other genera. The isolate contained anteiso-C₁₅ : 0, iso-C₁₄ : 0 and iso-C₁₅ : 0 as the major cellular fatty acids, and MK-9(H₂) as the predominant isoprenoidquinone. Polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids and one unknown lipid, and G+C content was 73.1 mol%. The peptidoglycan type was A3L. It showed less than 70 % DNA-DNA hybridization values with *Friedmanniella lacustris* KACC 17306T (94.9 %) and *DSM 27139T*. Supported by grants from National Academy of Agricultural Science.

Keywords: *Friedmanniella aerolata*, 16S rRNA sequence, taxonomy

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**A-23**

*Flavitalea soli* sp. nov. Isolated from Soil

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A Gram-stain-negative, rod-shaped, non-flagellated, motile, yellow-pigmented, aerobic bacterium, designated strain KIS20-3⁷, was isolated from a soil sample of Baengnyeong Island in Orjin county, Republic of Korea. The isolate showed the highest 16S rRNA sequence similarities with *Flavitalea populii* HY-508T (94.5 %), *Niastella populii* THYL-44T (94.2 %) and *Flavitalea gansuensis* JCN-23T (93.7 %). Neighbor-joining tree based on 16S rRNA gene sequences showed strain KIS20-3⁷ formed one sub-cluster with members of the genus *Flavitalea*, and the sub-cluster was closely related with members of the genus *Niastella, Segitibacter* and *Parasegitibacter* within the family *Chitinophagaceae*. The major fatty acids of the strain KIS20-3⁷ was iso-C₁₅ : 0, iso-C₁₄ : 0 and iso-C₁₅ : 0, G, and the predominant isoprenoid quinone was menaquinone 7. The polar lipids were large amounts of phosphatidylethanolamine and one unknown polar lipid, and moderate or small amounts of four unknown amino phospholipids, two unknown aminolipids, three unknown lipids and one unknown phospholpid. The G+C content of the DNA of strain KIS20-3⁷ was 55.7 mol%. On the basis of the results of the polyphasic characterization presented in this study, it is concluded that strain KIS20-3⁷ represents a novel species of the genus *Flavitalea*, for which the name *Flavitalea soli* sp. nov. is proposed. The type strain is KIS20-3⁷ (= KACC 17319T = JCM 19937T). Supported by grants from National Academy of Agricultural Science.

Keywords: *Flavitalea soli*, 16S rRNA sequence, taxonomy

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**A-24**

Molecular Diversity of Injin Virus, A Shrew-borne Hantavirus, in Gangwon Province, Republic of Korea

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Hantaviruses, *hantavirus*, are negative sense tripartite RNA viruses causing Hemorrhagic Fever with Renal Syndrome (HFRS) and Hantavirus Pulmonary Syndrome (HPS) in humans. Injin virus (MINV) is a shrew-borne hantavirus identified from *Crocidura lasiura* in the Republic of Korea (ROK). The genomic analysis of MINV is required for understanding the molecular evolution of hantavirus. A total of 60 shrews was collected in Gangwon province, ROK during 2012-2014. Partial L, M and S segment sequences of MINV from anti-MINV IgG-sero-positive shrews were sequenced. The sequence of MINV from anti-MINV IgG-sero-negative shrews were identified from 4 of 57 (7.0 %). Phylogenetic analyses of MINV L, M and S segment revealed the difference of <13%, <11%, <12% at the nucleotide level and <4.3%, <2.7%, <1.5% at the amino acid sequence, respectively. As a result, the partial sequence of MINV strains in Gangwon province are phylogenetically divergent. MINV strains from the east side of Gangwon province form an independent cluster and those from the west side show a close relationship with the strains in Gyeonggi province. The phylogenetic analyses of MINV strains may provide a broader insight into the genetic diversity of shrew-borne hantaviruses.

Keywords: Hantaviruses, Injin Virus, Shrew, Phylogenetic analysis, RNA viruses
Previously Uncultured Marine Bacteria Linked to Novel Alkaloid Production
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Low nutrient media and long incubation times facilitated the cultivation of over 20 taxonomically diverse Gram-negative marine bacteria within the phyla Bacteroidetes and Proteobacteria. These strains comprise as many as three new families and include members of clades that had only been observed using culture independent techniques. Chemical studies of the type strains representing two new families within the order Cytophagales led to the isolation of nine new alkaloid secondary metabolites that can be grouped into four distinct structure classes, including azepinones, aziridines, quinolones and pyrazinones. Several of these compounds possess antibacterial properties and appear, on structural grounds, to be produced by amino acid-based biosynthetic pathways. Our results demonstrate that relatively simple cultivation techniques can lead to the isolation of new bacterial taxa that are capable of the production of alkaloid secondary metabolites with antibacterial activities. These findings support continued investment in cultivation techniques as a method for natural product discovery.

Keywords: Gram-negative, Uncultured marine bacteria, alkaloid secondary metabolites

In vitro and In vivo Activities of LCB10-0200 a Novel Cephalosporin against Clinical Isolate Bacteria
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Handong global university

LCB10-0200, a novel siderophore-conjugated cephalosporin, has potent antibacterial activity against clinical isolates of gram-negative bacteria especially multi-drug-resistant pseudomonas aeruginosa (MDRPA). In vitro activity of LCB10-0200 was compared with that of ceftazidime, ceftriaxone and ciprofloxacin. Among the tested agents, LCB10-0200 showed the most potent antibacterial activity against clinical isolated gram-negative bacteria and also beta-lactamase producing bacteria. LCB10-0200 was 2-16 times more active than others. Time-kill curve of LCB10-0200 was analyzed at concentrations of 0.5×, 1×, 2×, 4× and 8× MIC against P. aeruginosa strain. LCB10-0200, at concentration of 8× MIC, had bactericidal activity during 24 h. In vivo activity of LCB10-0200 against systemic infection caused by susceptible and resistant P. aeruginosa strain in mice was also more effective than that of ceftazidime and doripenem.

Keywords: new cephalosporin, antimicrobial activity, mdr pseudomonas aeruginosa

Isolation and Identification of Wild Yeasts from Soil of Chungcheongnam-do in Korea
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This study has focused on isolation of wild yeasts from mountains or field soils and elucidation of yeast diversity in natural environments. Several kinds of yeasts were isolated from various soils of Chungcheongnam-do in Korea and identified by comparison of nucleotide sequences for PCR-amplified D1/D2 region of 26S rDNA using BLAST. Totally ninety-seven strains of twenty species from 307 soil samples in 9 sites including Daejeon city were isolated, and Cryptococcus podzolicus (11 strains), Debaryomyces hansenii (6 strains) and Trichosporon asahii (6 strains) were dominant species.

Keywords: Chungcheongnam-do, Isolation, Soil, Wild yeasts

Identification and Classification of Falsi aeromonas sp. Strain ARI Isolated from a Fresh Water Stream
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A gram-negative, facultatively aerobic, motile, rod shaped bacterium, strain ARI was isolated from a water sample of a fresh water stream in Jeonju, South Korea. Colonies of strain ARI on Mueller-Hinton agar were translucent, off white coloured and convex with entire margin. The predominant cellular fatty acids of strain ARI were C16:1ω5c/C16:1ω7c (37.4%) and C16:1ω6 (35.8%). Identification by EzTaxon blast analysis revealed that strain ARI showed 96.78% 16S rDNA gene sequence similarity with Aeromonas sharmana GPTSA-6T. In the phylogenetic tree constructed by neighbour joining programme of MEGA6 software, strain ARI clustered with the members of the genus Aeromonas but formed a separate clade along with Aeromonas sharmana GPTSA-6T. Based on the phenotypic and genotypic characteristics, we propose that strains ARI and GPTSA-6T should be described as members of novel genus for which the name “Falsi aeromonas” is suggested.

Keywords: Aeromonas, Falsi aeromonas, classification
Current-generation by Genus Bowmanella and Bowmanella dokdonensis sp. nov., Isolated from the Seawater of Dokdo, Korea

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A Gram-negative, motile, and rod-shaped bacterial strain, UDC354T, was isolated from seawater of Dokdo, Korea. The strain UDC354T grew optimally at 30-37°C, in the presence of 2% (w/v) NaCl, and at pH 8. The organism also grew in the absence of NaCl. It was characterized chemotaxonomically as containing the Q-8 and Q-10 isoprenoid ubiquinones, and the major fatty acids C16:0 (23.48%), C14:0 and/or C12:0 2-0H (17.40%), and C16:0 7c (14.38%) (~12% of total fatty acid content). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. The DNA G+C content was 54.06 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that UDC354T falls within the genus Bowmanella of the Alteromonadaceae family. It is closely related to B. pacifica W3-3A1 (94.64%) and B. denitrificans BD1T (94.49%). This is the first study reporting the generation of electrical current by a strain characterized to the genus Bowmanella. Based on the phenotypic properties and phylogenetic distinctiveness, strain UDC354T was classified to the genus Bowmanella as the type strain of a novel species, for which the name Bowmanella dokdonensis sp. nov. has been proposed. B. pacifica W3-3A1 and B. denitrificans BD1T is capable of generating current in MFC chambers.

Keywords: Bowmanella dokdonensis, seawater, exoelectrogen, microbial fuel cell, Bowmanella

Poster Session

The Species Identity of Phellinus linteus (Sanghuang) Extensively Used in Korea

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Sanghuang is a polypore belonging to the family Hymenochaetaceae of Hymenochaetales in Basidiomycota. It has gained particular attention in Korea as an alternative to traditional cancer treatments such as chemo- and radio-therapies. As a natural immune booster and a cancer suppressor, sanghuang has been intensively studied for the past decades. The scientific name, Phellinus linteus (Berkeley & M.A. Curtis) Teng, has been commonly used to refer the sanghuang mushroom. However, the species identity of sanghuang has been repeatedly doubted due to the ambiguity of its circumscription and the inadequacy of morphological distinctions within allied species. As the species concept of sanghuang was newly established by recent molecular phylogenetic studies, it is urgently required to clarify the taxonomic positions of sanghuang strains extensively utilized in Korea. In this study we conducted a phylogenetic analysis of 74 strains belonging to P. linteus-linteus complex based on the ITS rDNA sequences. The resulting trees showed that the parental stains of sanghuang varieties formally registered in KSVS (Korea Seed & Variety Service), viz. ASI 26046 (Corea sanghuang), 26115 (HK 1-ho), and 26114 (Boolro), are grouped with Sanghuangporus sanghuang (Sheng H. Wu, T. Hatt. & Y.C. Dai) Sheng H. Wu, I.W. Zhou & Y.C. Dai instead of P. linteus. Keywords: Phellinus linteus, Phylogeny, Sanghuangporus sanghuang, sanghuang mushroom

Poster Session

Community Assessment of Endophytic Fungi Isolated from Abies koreana, an Endemic Species of Korea

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Endophytic fungi are microorganisms inhabiting living plant tissues without causing apparent harm to the host. They are drawing increasing attention due to their ability to produce various bioactive compounds as well as their effects on host growth and resistance to biotic and abiotic stresses. This study was conducted to investigate community diversity of endophytic fungi from Abies koreana which is an endemic species in Korea. We collected 34 plant samples from the 9 sites of South Korea. One hundred twenty four fungal isolates, 1-12 from each samples, were grouped into 50 MOTUs based on ITS sequences, were obtained. Most of fungi were belonged to Ascomycota (119 isolates) encompassing four classes (Dothideomycetes, Eurotiales, Leotiales, Sordariales) while 5 remaining isolates were Basidiomycota which is all configured as Agaricomycetes. Fungi belong to genus Xylaria, Alternaria and Diaporthe, of which diversity will further be analyzed, had the highest abundance. There was no apparent relations between fungal specificity and sampling sites. Rhizosphaera kalkhoffii, a causing agent of needle cast disease of fir trees were also isolated. These data will be valuable resources to understand the endophytic microbial diversity of A. koreana.

Keywords: Endophytic fungi, Abies koreana, Genetic diversity

Poster Session

Identification and Characterization of Antifungal Metabolites Produced by Bacillus sp. Against Phytopathogens

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Plant diseases caused by pathogenic microorganism such as fungi cause major economic damage every year and destroy crop yields that could feed millions of people. Soil microbes from the rhizosphere of phytopathogen affected sites can develop strategies to treat the outbreak of crop pests. Since, soil microbes are a rich source of bioactive compounds, constituting proteins, and lipopeptides elucidated as potential antibiotics. Strain 4-3 was isolated from ginseng cultivated soil and identified as a biocontrol agent, in application of ginseng field. The lipopeptide was isolated from culture filtrate by acid precipitation method. This is the first report of lipopeptide fengycin identified as an antifungal agent against Cylindrocarpon destructans. The production of the metabolite was observed from 24 h extending upto 96 h of culture and gradually decreased over the period. In vitro analysis of antagonism with radial plate assay showed ~50% inhibition to the strains of Cylindrocarpon destructans and Rhizoctonia solani. The mass spectrometry analysis determined the compound was highly similar to homologous of fengycin gene sequence the strain was found belonging to Cylindrocarpon genus. The lipopeptide fengycin can exhibit potent antifungal activity against many soil borne fungal pathogens and can be used as a biocontrol agent, in application of ginseng field.

Keywords: Bacillus, Phytopathogens, Cylindrocarpon, Antifungal Activity, lipopeptides
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Polyethyleneimine (PEI), a weakly basic aliphatic polymer is known to permeabilize bacterial membrane and as such is used in a wide range of formulations ranging from washing agents to microbialid compositions. Bacterial bioreporters used in many fields are limited in their ability to detect certain molecules such as large polar molecules due to their outer membrane structure which act as a barrier to many substances. In this study PEI which due to its permeabilization effect on bacterial membrane thus overcoming the membrane barrier which is a limit of bacterial bioreporters, was used to improve on the sensitivity of bacterial bioreporter harboring the recA::luxCDABE transcriptional fusion which responds to the chemotherapy drug mitomycin C. The two forms of PEI (linear and branched) used in this study showed a 3-fold and 2-fold increase in induction respectively of the bioreporter’s response when used singly but gave a 4-fold increase when mixed together at much lower concentrations, about 5-times and 40-times lower the optimal concentrations used for each respectively. EDTA, a well known permeabilizer used in a wide range of fields was also evaluated.

Keywords: Polyethyleneimine, Chemotherapeutic drug, Bacteria biorreporter

A-34  Contribution to the Lichen Flora of Vietnam, with Ten New Record Species
Luu Dong, Soon Ok Oh, Jung-Shin Park, Beeyoung-Gun Lee, Jung-Jai Woo, and Jae-Seoun Hur*
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Vietnam is a medium sized country, a little attention has been paid to the lichen flora of this country, and totally almost 300 species were reported until 2014. We made a survey from 2010 to 2014, and collected lichens for the research in order to evaluate lichen species diversity in this area. The following ten species were discovered as new records: Cladonia cornuta (L.) Hoffm., C. diaphana (Fée) Vain., C. mucicincta Hoffm., Everniastrum fragile Sipman, Hauromyenia africanaum (J. Steiner) C.W. Dodge, Heteroderma obtusata (Nył.) Trevis., Leptogium sessile Vain., Lobaria isidophora Yoshim., Physciidae australasica Kalb & Eliax, Pseudocyphellaria crocata (L.) Vain.

Keywords: Lichen, New record, Vietnam

A-35  Lichen Taxonomy Study of Pertusaria (Pertusariaceae, Ascomycota) in South Korea
Jung-Shin Park and Jae-Seoun Hur*
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Crustose lichen Pertusaria is worldwide species (hemispheres, from subarctic to tropical regions) and reported around 350 species. Korean Pertusaria species were studied only form Jeju Island. This present study firstly included 363 species collected from the southern part of Korea peninsula. In this study, we found out 23 species including 19 known species and 4 species new to South Korea (P. leiocarpella, P. leioplacca, P. leucorosa var. violascens and P. multipunctoides) based on the morphological and chemical analyses, and nrDNA ITS sequences. The result suggested that the Korean species can be identified by type of fertile verrucae (apothecia scattered or centrally located), presence or absence of soredia, and lichen substances such as xanthone, depsone and depside. Main lineage of Korean Pertusaria belongs to sorediate group such as P. opithalnica, P. salubrina and P. sorediata. Fertile verrucae with scattered apothecia species of Pertusaria laeviganda and P. subobductans are the most common species in Korea.

Keywords: Lichen, New records, Pertusaria, South Korea, Taxonomy

A-36  Sediminibacterium aquarii sp. nov. Isolated from Sediment of a Fishbowl
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An aerobic, Gram negative, orange-pigmented novel bacterial strain, designated AAS1, was isolated from sediment of a fishbowl. Phylogenetic analysis based on 16S rDNA gene sequence revealed that strain AAS1 belongs to the genus Sediminibacterium of family Chitinophagaceae, exhibiting the highest level of sequence similarity with S. goheungense HME7863 (96.6% sequence similarity). The major fatty acids of strain AAS1 were iso-C15:0 3-OH and iso-C17:0 3-OH (~5% of the total fatty acids). The DNA G+C content and major respiratory quinone were 44.7 mol% and MK-7. On the basis of polyphasic taxonomy a consensus approach to bacterial systematics, strain AAS1 is considered to be a novel species of the genus Sediminibacterium, for which the name Sediminibacterium aquarii sp. nov. is proposed. The type strain is AAS1 (=KACC 18509T = KCTC 42806T).

Keywords: environment, novel bacteria, sediment, Sediminibacterium
**A-37**

**Mycelial Growth and Microscopic Morphological Characteristics of Poria cocos**

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The cultivation of Poria cocos on tree-stumps and logs, was first described at the beginning of the 13th century. Poria cocos has been used for medicinal purposes to treat physical and mental recuperation, promote diuresis, forgetfulness and physical weakening. The artificial cultivation techniques of P. cocos was reported in China in 1957. The basic study on morphological characteristics and artificial cultivation method of Poria cocos with pine tree log buried under ground were initiated by Rural Development Administration. Poria cocos, and is usually in the form of spherical or potato-shaped, the diameter of 15 ~ 30cm, object weight is about 300 ~ 1,000g. Collecting 17 kinds of strains characterized was performed and field trials, five days in a thermostat at 25 °C were cultured on PDA medium degree of mycelial growth, five kinds of strains including ASL 13007 were grown 61 ~ 70mm, review the mycelium culture characteristic pH 4 and 5, the growth was good. Poria cocos fruiting body of the form is initially white in a honeycomb shape, gradually changed into a dark yellow color, the hole has a polygonal shape, and the diameter of the hole is 0.5 ~ 1mm, about 2 ~ 5mm depth. In the form of spores are oblong or cylindrical shape, the size is 2.3 ~ 3.0 × 5.2 ~ 6.0 μm.

Keywords: Characteristics, Microscopic, Mycelial, Poria cocos

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**A-38**

**Pelocola oligotropha gen. nov., sp. no., Isolated from a Shallow Stream Sediment**

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A novel, gram-negative, rod-shaped and aerobic bacterium, designated An3-4T, were isolated from sediments of shallow stream, Cheonan, South Korea. The growth of strain An3-4T was observed at 15-40°C (optimum 25-30°C) and pH 6.5-7.5 (optimum 6.5). Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain An3-4T belonged to the family Zoogloea and distinguished from members of the genera Paracraurococcus, Thauera, Ligilignobacterium, Zoogloea. The DNA G+C content was 66.0 mol%, and OO was the major respiratory quinone. The major cellular fatty acids were C16:1ω5c (13.0%), C16:0 (17.3%) and Summed feature5 (48.5%), contained C16:1ω7c and/or C16:1ω6c. On the basis of phylogenetic and chemotaxonomic data, strain An3-4T represents a novel species of a new genus within the family Zoogloea, for which the name Pelocola oligotropha gen. nov., sp. no. is proposed. The type strain of Pelocola oligotropha is An3-4T (=KACC 18518T).

Keywords: Gram negative, Sediment, Zoogloea family, Pelocola oligotropha

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**A-39**

**A Noble Agar-hydrolyzing Bacterium Vibrio sp. S4 Isolated from Seawater of Jeju Island, Republic of Korea**

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Agarose is composed of repetitive units of alternatively arranged β-D-galactose and 3,6-anhydro-α-L-galactose. Agarases are glycoside hydrolase (GHs) that catalyze the hydrolysis of agar and are classified into α- and β-agarases according to the cleavage pattern. α-agarases and β-agarases hydrolyze α-1,3 linkages and β-1,4 linkages in agarose, respectively. For an efficient use of agarose biomass in various biotechnological fields, we have attempted to isolate and characterize agarolytic strains. Sample of coastal sea water of Jeju Island was collected and plated on ASW-YP medium and incubated at 40°C for 24 h. Agar-hydrolyzing colonies were selected and identified by 16S rRNA gene sequence analysis. From these bacteria, one marine bacterium identified as a novel species belonging to genus Vibrio, based on morphological and physiological characteristics and 16S rRNA gene sequence analysis. The isolate was gram negative bacteria and forms smooth rounded and beige-colored colony. The isolate produces extracellular agarase which is able to degrade agarose into (neo)agarotetraose and (neo)agarohexaose. [supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR2015292021)]

Keywords:

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**A-40**

**Dankookia rubra gen. nov., sp. no., Isolated from Sediment of a Shallow Stream, Korea**

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A Gram-negative, non-motile and aerobic bacterium, designated strain WS-10T, was isolated from sediment of a shallow stream, Korea. The optimum range of temperature and pH for growth were 20-40°C and pH 6.0-8.0, respectively. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain WS-10T was most closely related to members of the genera Paracraurococcus (95.3%) and Belnapia (95.3%) but formed a distinct lineage in the family Acetobacteraceae. The DNA G+C content of strain WS-10T was 66.0 mol%. The major fatty acids were summed feature 8, summed feature 3, C16:0 and C16:1 2-OH. On the basis of phenotypic characteristics and phylogenetic analysis, strain WS-10T represents a novel species of a new genus in the family Acetobacteraceae, for which the name Dankookia rubra gen. nov., sp. no. is proposed. The type strain of WS-10T is KF309177 (=KCTC 42449T=JCM 96002T).

Keywords: new genus, novel species
A-41

**Dermabacter vaginalis** sp. nov. Isolated from the Korean Vaginal Sample

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A novel, Gram-stain-positive, facultative anaerobic, non-motile and short rod shaped bacterium, strain AD1-86 was isolated from a human vaginal sample. The colonies of the new isolate were convex, circular, white in color and 1-2 mm diameter after 3 days incubation on DSMZ 92 medium at 37°C. Based on the 16S rRNA gene sequence similarity, the new isolate was most closely related to *Dermabacter hominis* DSM 7083T and Helcocobacillus massiliensis 6401990T (98.34 and 96.25 % similarity, respectively). Strain AD1-86 grew optimally at 37°C, at pH 7.0 and in the presence of 0.5 % (w/v) NaCl. Catalase-positive and oxidase-negative. The major cellular fatty acids (>10%) were C16:0 anteiso (43.97%), C17:0 anteiso (26.09%) and C16:0 iso (13.43%). The cell-wall hydrolysates contained galactose as a major sugar. The principal menaquinone is MK-9 with MK-8 and MK-7 also present. The G+C content of the genomic DNA was 62.59 mol%. On the basis of polyphasic chemotaxonomic data and phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate belonged to the genus *Dermabacter*, with the closest relatives being *Dermabacter dongtangensis* KCTC 22672T (97.12 % sequence similarity), *Dermabacter troitsensis* KCTC 12303T (97.12 %) and *Dermabacter xinjiangensis* KCTC 42431T (97.03 %). Cells grow on NA, PCA, TSA and R2A, but do not on MA. Growth occurs with 0-3 % (w/v) NaCl (optimum, 0-1 %), 4-37°C (optimum, 25°C) and pH 6.0-9.0 (optimum, 7.0). Oxidase-negative and catalase-positive. The predominant fatty acids were C16:1ω7c (43.79%) and C16:0 (28.43%). The major respiratory quinone was ubiquinone 10 (UK-10) and DNA G+C content of the strain was 63 mol%. Strain WW3T represents a novel species of the genus *Dermabacter*, for which the name *Dermabacter vaginalis* sp. nov. is proposed. Supported by a grant from Ministry of Health & Welfare, Republic of Korea (HI14C 03680200).

Keywords: Dermabacter vaginalis

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A-42

**Agarolytic Novel Bacterial Strain KA6, Isolated from Seawater of Gwangyang Bay**

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A Gram-stain-negative, rod shaped, aerobic, agarolytic and orange pigmented bacterium, designated strain KA6T, was isolated from seawater (Gwangyang bay, Republic of Korea). Growth occurs at 10-30°C (optimum, 25-30°C), at pH 7-8 (optimum, pH 7) and with 2-8 % (w/v) sea salts (optimum, 3-5 %). Flexirubin-type pigments are absent. Catalase- and oxidase-negative. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain KA6T formed a distinct lineage within the genus *Polaribacter*. The predominant fatty acids are iso-C15:0 3-OH (23.6 %), iso-C16:0 3-OH (9.9 %), C16:0 (9.8 %) and anteiso-C15:0 (7.8 %). The major menaquinone is menaquinone 6 (MK-6). The DNA G+C content of the strain was 63 mol%. Strain WW3T represents a novel species of the genus *Polaribacter*, for which the name *Polaribacter curvus* sp. nov. is proposed. Supported by Ministry of Health & Welfare, Republic of Korea [KCTC 32271T].

Keywords: *Polaribacter curvus* strain KA6T

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A-43

**Altererythrobacter wooponensis** sp. nov., Isolated from Freshwater of Woopo Wetland in Korea

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A non-motile, Gram-stain-negative, ovoid-rod shaped, yellow pigmented bacterium, designated WW3T, was isolated from the freshwater of Woopo wetland, Republic of Korea (35.33N 128.25E). Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate belonged to the genus *Altererythrobacter*, with the closest relatives being *Altererythrobacter dongtangensis* KCTC 22672T (97.12 % sequence similarity), *Altererythrobacter troitsensis* KCTC 12303T (97.12 %) and *Altererythrobacter xinjiangensis* KCTC 42431T (97.03 %). Cells grow on NA, PCA, TSA and R2A, but do not on MA. Growth occurs with 0-3 % (w/v) NaCl (optimum, 0-1 %), 4-37°C (optimum, 25°C) and pH 6.0-9.0 (optimum, 7.0). Oxidase-negative and catalase-positive. The predominant fatty acids were C16:1ω7c, C16:1ω6c and Summed feature 8 (C18:1ω7c and/or C18:1ω6c). The major respiratory quinone was ubiquinone 10 (UK-10) and DNA G+C content of the strain was 63 mol%. Strain WW3T represents a novel species of the genus *Altererythrobacter*, for which the name *Altererythrobacter wooponensis* sp. nov. is proposed.

This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea. It was also supported by the CK(university for Creative Korea)-I.

Keywords: *Altererythrobacter*, wetland freshwater

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A-44

**Plantibacterium curvus** gen. nov., sp. nov. Isolated from Surface Sterilized Root of *Artemisia princeps*

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A novel Gram-stain-negative, aerobic, non-spore-forming bacterium designated strain UR 7-11T was isolated from surface sterilized root of *Mugwort* (*Artemisia princeps*). Phylogenetic analysis of 16S rRNA gene sequences revealed that strain UR 7-11T belonged to the family *Rhodospirillaceae*, being closest to *Lachnobacter aquatilis* LTC-2T (93.14%) and *Elsnera litoralis* Dia-1T (93.08%). Cells of strain UR 7-11T were slightly curved to straight rods and motile by bipolar-polytrichous flagella. Growth occurred at 15-37°C (optimum temp. 25-30°C) and pH 6-8 (optimum pH 7). The major isoprenoid quinone of the novel strain was Q-10 and the G+C content was 64.09 mol%. DNA G+C content of the strain was 63 mol%. Strain WW3T represents a novel species of the genus *Plantibacterium*, for which the name *Plantibacterium curvus* gen. nov., sp. nov. is proposed, with strain UR 7-11T (=KCTC 42057T =JCM 30459T) as the type strain of the type species.

Keywords: Endophytic bacteria, *Rhodospirillaceae*

www.fkms.kr | 237
Arbovirus Surveillance in Mosquitoes Collected from Airports, Sea Ports and Animal Quarantine Stations during 2014 in Republic of Korea
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This study investigates the distribution of mosquitoes and presence of arboviruses that cause encephalitis in humans and horses, which were collected as part of the mosquito monitoring program at airports and sea ports in 2014. A total of 65,269 mosquitoes, representing 18 species and 7 genera were captured and the most frequently collected species was Culex pipiens (72.34%, n=47,214). A total of 9,809 mosquitoes were pooled to 771 samples and screened for West Nile virus (WNV), and Eastern, Western and Venezuelan Equine Encephalitis Viruses (EEEV, WEEV and VEEV) by reverse transcription polymerase chain reaction. All of the 9,809 mosquitoes tested were negative. In Korea, no outbreaks of WNV, EEE, WEE and VEE have never been reported and our study continues to support the view that Korea is free from these diseases. However as climate change continues to raise the risk of introduction of these arboviruses into the country, continued vector monitoring will be needed to identify areas of risk and quickly detect new introductions.

Keywords: Mosquitoes, Arbovirus, RT-PCR

Isolation and Molecular Identification of Endophytic Fungi from Arctic Plants
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The leaves of arctic plants such as Salix polaris, Bisotorta vivipara, Silene acuclus were collected from the Spitsbergen Island of the arctic region. One species, Salix polaris is a woody plants and other two species are herbaceous plants. Totally, 34 strains were isolated from the leaves and we try to group them by morphotype and analyze using DNA marker (ITS1F-ITS4). Among them, 10 strains are not able to locate into classification system of fungi and some of them also are difficult to exact identification of species level so we think to need more taxonomical study to elucidate its entity. Except unidentified strains, the 26% of fungal isolates were belongs to Eurotiomycetes, 8.6% isolates Leotiomycetes, 39.4% isolates Dothideomycetes, 17.4% isolates Sordariomycetes and 8.6% isolates Agaricomycetes. In this poster presentation, we first report endophytic fungi of arctic region and try to analyze its entity and function of secondary metabolite.

Keywords: Arctic region, Endophytic fungi, Salix polaris, Bisotorta vivipara, Silene acuclus

More New Records of Pyrenocarpous Lichens from South Korea
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Lichenological research of pyrenocarpous lichens were continued in 2015. Some more of rare and overlooked microlichens of pyrenocarpous group (Verrucariaceae, Pyrenulales, Porinales and some genera of Ostropales) were explored at several valleys of Mt. Halla in Jeju Island. The field trip has brought new records of 14 species of Verrucariaceae, of which 6 are reported for the first time from South Korea (Agnominia globulifera, A. repelata, Thelidium japonicum, T. phlotum, T. radiatum, Verrucaria margaacea and V. simplex) and 5 pyrenocarpous from other genera for the first time from South Korea (anisomerdium japonicum, A. robustum, Anthracothecium macrosorum, Porina leptideas and Strigula aquatica). Some of collected species were known from Europe or Japan only (Agnominia globulifera, A. repelata, Thelidium japonicum, T. phlotum, T. radiatum, Verrucaria margaacea and V. simplex) and some of them with very rare distribution and pure known (anisomerdium japonicum, A. robustum, Anthracothecium macrosorum and Strigula aquatica). The total number of pyrenocarpous lichens of South Korea has increased to 87 species.

Keywords: Lichen, New records, Pyrenocarpous, South Korea, Taxonomy

Phylogenetic Analysis of Russula Section Foetentinae (Russulales, Basidiomycota) in Korea
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Russula section Foetentinae (Melzer & Zvara) Singer is characterized by the articulated and branched hairs cuticle and brownish cap color. Approximately twenty species are found worldwide, with six species have been reported in Korea. In this study, we re-evaluate the inventory of Russula section Foetentinae through morphological data and phylogenetic analysis of internal transcribed spacer sequences. Phylogenetic analysis recovered nine species, where only four (Russula cereolens, R. grata, R. senex, R. sororia) corresponded to the previously known Korean species. two species (R. ingratis, R. pectinatoides) are new records to Korea, while three are undescribed species. Our data show that the size and Q value of basidiospores are important key of morphological identification. We described the morphology and taxonomic information of these new species with two unrecorded species in Korea.

Keywords: Russula section Foetentinae, inventory, ITS, new species, unrecorded species
Two new Lycoperdon Species Collected from Korea
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Thirty four Korean Lycoperdon specimens were examined with ITS sequences data. The result of phylogenetic analysis indicated that our Korean specimens were divided into nine different species. To confirm the taxonomic position of these species, we conducted the morphological investigation intensively. Among them, we found two new species and one new to Korea: Lycoperdon sp. 1, Lycoperdon sp. 2 and L. ericaeum. Lycoperdon sp. 1 is closely related to L. ericaeum in ITS, but they could be distinguished by morphological characteristics, especially shape of basidioconsp and diameter of eucapilitial threads. Lycoperdon sp. 2 are quite similar to European and American L. perlatum based on their morphological description. However, Lycoperdon sp. 2 is clearly separated from European and American L. perlatum in ITS tree, and somewhat differ between them in macro- and micro-scope characteristics. Here, we taxonomically described four Lycoperdon species and discussed the Korean Lycoperdon species.

Keywords: Agaricaeaceae, gasteroid, morphology, phylogeny, taxonomy

Morphological, Physiological and Molecular Characters of Brown Yeast-like Fungus Producing Melanin from the Antarctic
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The dark brown yeast-like fungus producing melanin from the Antarctic, Argentina Galindez Island, previously named as Nadsoniella nigra, was especially studied after morphological, physiological and molecular characters. It is characterized by very slow growth in culture on all nutrient media but showing better development of ‘colony-like formations’ on Saburo agar medium, potato sections, as well as in the liquid media with peptone water. It is also showing a phylogenetic affiliation to the family Exophialaceae. The dark brown yeast-like fungus producing melanin from the Antarctic, Argentina Galindez Island, previously named as Nadsoniella nigra, was especially studied after morphological, physiological and molecular characters. It is characterized by very slow growth in culture on all nutrient media but showing better development of ‘colony-like formations’ on Saburo agar medium, potato sections, as well as in the liquid media with peptone water. It is also showing a phylogenetic affiliation to the family Exophialaceae.

Keywords: Antarctic, Meripilaceae, Ascomycota: Current Status

Three Gene Phylogeny of the Teloschistaceae (Lecanoromycetes, Ascocytota): Current Status
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The taxonomy of the family Teloschistaceae has been dramatically exchanged on the basis of the molecular phylogenetic data during last three years. Totally more than 75 genera were proposed for four subfamilies of the Teloschistaceae. Within our study in 2015 one new subfamily and 10 new genera are proposed on the basis of phylogenetic analysis based on ITS and LSU nr DNA and 12S SSU mtDNA sequences. The new genera are proposed for the Asian species Caloplaca zorovifertorium, for the Eastern Asian Caloplaca bogliniana, for the South Korean Caloplaca astrosclerocoma, for the Australian Caloplaca browniolate group, for the South American species Marchantia connubialis, for the Antarctic Caloplaca micheloniensis group, for the Australian Caloplaca vilinayi group, for the Australian species Caloplaca erythrosticta, for the European and North American Caloplaca demissa and for the Antarctic Caloplaca digitata group. Phylogenetic trees and status of new taxa are discussed and illustrated.

Keywords: Genera, New subfamily, Phylogenetic analysis, Teloschistaceae

Aquamarinum salinum gen. nov., sp. nov., and Aquamarinum pelagicum sp. nov., a Novel Marine Bacterium of the Phylum Bacteroidetes Isolated from Seawater
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Two Gram-stain-negative, strictly aerobic, non-motile, scarlet-coloured, rod-shaped bacteria, designated IMCC2672T and IMCC11233T were isolated from seawater samples collected from solar saltern and the East Sea in Korea. Two strains shared 96.7 % of 16S rRNA gene sequences similarity and 46.1-54.1 % of DNA-DNA relatedness value, which indicated that two strains are different genomic species of each other. Optimal growth conditions of two strains were observed at 25-30 °C and pH 7.0-7.5 with 2.0-2.5 % (w/v) NaCl. The DNA G+C content of strains was 42.2 and 40.2 mol %, respectively. The major respiratory quinone of strains was menaquinone-6 (MK-6) and predominant cellular fatty acids were iso-C15:0 3-OH and iso-C15:0 3-OH. The major polar lipids were phosphatidylethanolamine, unidentified aminolipids and other lipids. Phylogenetic analyses based on 16S rRNA gene sequence revealed that strain IMCC2672T and IMCC11233T were most closely related to Onorweekiahypomongkongensis UST20020801T (93.08 and 93.29 % similarity, respectively). On the basic of the phylogenetic analyses and several phenotypic characteristics, strain IMCC2672T and IMCC11233T are considered to represent a novel genus and species within the family Cryomorphaceae; for which the name Aquamarinum salinum gen. nov., sp. nov., and Aquamarinum pelagicum sp. nov., are proposed.

Keywords: Bacteroidetes, Seawater, solar saltern
A-53

Comparative Characterization of Amylolytic Yeast Saccharomycopsis fibuligera and Its Sister Species in Genus Saccharomycopsis

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Yeasts of the genus Saccharomycopsis, belonging to the phylum Ascomycota, have drawn increasing attention due to their unique physiological characters useful for various biotechnological applications, such as Saccharomyces ferment filtrate as a component of cosmetics and high hydrolytic activity for bioiodemediaation. Here we report comparative characterization of Saccharomycopsis fibuligera, the major amylolytic yeast found worldwide in indigenous food fermentation using rice and cassava, and its sister species. The comparative analysis of ITS sequences of eight Saccharomycopsis species revealed their identities ranging from 60% to 78%. Interestingly, only S. fibuligera and S. vini propagated as multipolar budding and mycelial formation when cultivated in YPD liquid medium, indicating considerable variation in the extent of hyphal growth among members of the Saccharomycopsis clade. The yeast-like growth of S. fibuligera was induced under G stranded limited culture condition, and the hyphal growth was almost completely inhibited in the presence of antizyme A. These observations suggest that the morphological shift of Saccharomycopsis species is dependent on environmental conditions, such as nutrient and energy-limited conditions. It is also noticeable that all of the Saccharomycopsis species tested in this study showed lack of growth on sulfate as S-source, in agreement with the generalization of predation and sulfate uptake deficiency across the genus Saccharomycopsis.

Keywords: yeast, Saccharomycopsis, ferment, ITS sequence

A-54

Candidate of New Entoloma Species (Entolomataceae, Basidiomycota) in Korea

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During a researches of Korean mushroom flora in 2013, we collected four specimens (KA13-0159, KA13-0197, KA13-0264 and KA13-0402) of collybioid mushrooms. Based on morphological characteristics and molecular identification (internal transcribe spacer (ITS) and partial large subunit of ribosomal RNA (nLSU)), our specimens were not matched and closely related species.

Keywords: Entoloma, phylogeny, morphology, Korea National Arboretum, taxonomy, collybioid

A-55

Characterization of Two Bacteriocins Produced by Staphylococcus Species to Study Potential Applications for Food Safety

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Staphylococcus aureus is recognized as important pathogen in foodborne disease and has high rates of antimicrobial resistance. 394 staphylococci isolated from foods and leaf vegetables have been tested antimicrobial activity against gram-positive bacteria including S. aureus. Two isolates, Staphylococcus pasteurii RSP1 and Staphylococcus epidermidis S199, among the 394 staphylococci were selected which possessed antimicrobial activity. Purification of bacteriocins was achieved using optimized three-stage procedure comprising precipitation with XAD-16 resin (for S. pasteurii RSP1) or ammonium sulfate (for S. epidermidis S199), dialysis, and reverse-phase HPLC. Purified bacteriocins of two producers were stable at wide range pH and showed sensitive to proteolytic enzymes. But two types of bacteriocins are exhibited different characteristics. Bacteriocin of S. pasteurii RSP1 was extremely heat stable at up to 100°C and showed strong activity against specific Staphylococcus species, while bacteriocin of S. epidermidis S199 was sensitive to heat and has broad spectrum activities. The combination of two different bacteriocins could be potential application for food safety to control of gram positive foodborne pathogenic bacteria.

Keywords: bacteriocin, MRSA, foodborne pathogens, food safety, Staphylococcus aureus

A-56

A New Species of Aspergillus Section Cremei Isolated from a Forest Soil in Korea

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A new fungal species belonging to Aspergillus section Cremei was isolated from a soil sample collected in Gunsan, Korea. The strain grew fast on MEA, the colony was initial white light and then changed to white cotton, reaching 37-40 mm in diameter at 25°C after 7 days of incubation. The isolate was confirmed as a new species based on the morphology and sequence analysis of combined genes including rDNA ITS, RNA polymerase II second largest subunit, β-tubulin and calmodulin. The strain represented a low ITS sequence similarity of 95% (493/520 bp) with Aspergillus infilatus (GenBank accession No.JN617710). The conidiophores of our isolate were hyaline, smooth and thin-walled, varying greatly in length, measured 3.1-4.3 μm in diameter and had similar structures to described A. infilatus, including metulae and phialides. However, our strain was different from number of metulae and phialides such as conidiophores consisted of 5 to 12 mostly strongly diverging metulae bearing phialides; Phialides developed successively and occurred in small clusters of 2 to 6, slightly diverging. Especially, the conidia of our isolate were globose to subglobose, reaching 2.6-3.5 μm in diameter and larger than previously described A. infilatus species 0.9-1.0 μm.

Keywords: Aspergillus infilatus, Morphological characteristics, Molecular analysis, Soil
**Multi-gene Analysis Reveals New Species within the Ilyonectria radicicola Species Complex from Korean Panax ginseng**

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Keywords: genetic analysis, ginseng, Ilyonectria radicicola, reclassification, root rot

A soil-borne pathogenic fungus, *Ilyonectria radicicola* (Cylindrocarpon destructans) species complex causing ginseng root rot is the major pathogen on ginseng. Recent research was renamed the *Nectria/Nectonia radicicola* complex as *Ilyonectria radicicola* complex after analyzing its morphological characteristics and multi-gene relatedness, and reclassified the fungi in this complex into 15 species under the genus of *Ilyonectria* based on their morphological characteristics and genetic diversity. In order to identify species in the complex, we analyzed the genetic diversity of *I. radicicola* isolates obtained from Korean ginseng (*Panax ginseng*) roots. Genetic analysis of the partial nuclear ribosomal RNA-Internal Transcribed Spacer (rRNA-ITS), β-tubulin, histone H3 and translation elongation factor 1-α genes were used to support for the morphological features among ginseng isolates associated with root rot disease symptom. In result, we found that the ginseng isolates in Korea should be reclassified into several species such as *I. minor-paranicis*, *I. lirioides*, *I. cyclaminicola*, *I. robusta* and *I. venezuelensis* within *Ilyonectria* species complex.

**Domibacillus ruralsis sp. nov. Isolated from Air Near the Garlic Field**

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A novel Gam-positive bacteriain strain designated GTB21-2T was isolated from air near the garlic field. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain GTB21-2T belonged to the genus *Domibacillus* in the phylum *Firmicutes*. The highest 16S rRNA gene sequences similarities were found to the sequences of *Domibacillus robustoginse* DSM 25058T (97.4%) and *Domibacillus indica* DSM 28032T (96.4%). DNA-DNA relatedness between strain GTB21-2T and *Domibacillus roboginse* DSM 25058T was less than 70%. Strain other *D. linteus* and other phylogenetically related type strains were characterized based on various polyphasic approaches including physiological characterization, DNA G+C content, quinone and fatty acid composition.

On the basis of these results, strain GTB21-2T represents a novel species of the genus *Domibacillus*, for which the name *Domibacillus ruralsis* sp. nov. is proposed. The type strain is GTB21-2T (=KCTC 33660T).

Keywords: Air, Domibacillus, Firmicutes

**Pancibacterplanktonicus sp. nov., A Novel Bacterium Isolated from Daechong Reservoir, Korea**

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Gram-negative, aerobic, rod-shaped bacterium, designated strain Chu3T was isolated from the Chung, Korea.Comparison of 16S rDNA sequences showed that strain Chu3T was most similar to *Pancibacter toxinivorans* 2C20T (=KCTC 42569T) with similarity of 97.75%. Strain Chu3T was clustered distantly with other type strains of fast mycelial growth rate from oak and mulberry logs in Korea. However, fruiting body morphology of *P. linteus* and other phylogenetically related type strains were characterized based on various polyphasic approaches including physiological characterization, DNA G+C content, quinone and fatty acid composition.

Myological Characteristics and Artificial Cultivation and of a Novel Phellinus linteus KACC 93057P strain

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Sanghoongmushroom, Phellinus linteus (PL) is a mushroom species belonging to the *Hymenochaetaeae*, Basidiomycetes. PL has been studied extensively for its extraordinary capacity of suppressing cancer or enhancing body immunity. The mycelial materials of PL have mainly been used as research samples worldwide because fruiting bodies was difficult to be artificially cultivated. Alternatively, *P. hainui* (variety, “fungus”) have been cultivated in Korea. However, fruiting body morphology of *P. hainui* is clearly different to that of PL. We found Pellinellulose. KACC 93057P suffering features of fast mycelial growth rateform *Phellinus isolates* collected in Korea. The optimum temperature for the mycelial growth was at 25-30°C and growth was optimal at pH 5.7. The mycelial growth of *P. linteus* was faster than *P. hainui*, other *P. linteus* isolates and other *Phellinus* sp. The fruiting bodies were successfully produced by artificial cultivation on oak and mulberry logs in a year. The fruiting bodies showed typical morphology of *P. linteus*. The pore shape in basidiocarpis circular with 5-7 per mm, hyphal system is dimity, and basidiospores are ellipsoid. Internal transcribed spacer (ITS)-rDNA sequence analysis showed a homology over 98% with those of reference isolates of *P. linteus*. Furthermore, antioxidant and immune activation efficacy of the fruiting bodies were evaluated by comparing with those of *Phellinus* spp.

Keywords: *Phellinus linteus*, Novel strain KACC 93057P, Fruiting bodies, Morphology, Artificial cultivation

www.fkms.kr | 241
A-61
Possibility of Race Identification for Korean Plasmodiophora brassicae Isolates through RAPD Analysis
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Kiwoon Baek 1,2, Ahyoung Choi1,3, Eu Jin Chung 3, Hyangmi Kim3, and
Leucothrix arctica
and Hong Gi Kim*
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Clubroot caused by Plasmodiophora brassicae is a disease characterized
by gall formation on roots of cruciferous crops. P. brassicae was well
known to have physiological races. Accurate identification of race using
single spore inoculation is very difficult due to life cycle and pathological
characteristics of this pathogen. The characteristics of genetic variation
of P. brassicae isolates in Korea were analyzed through genetic diversity
analysis. Used galls were collected at Asan and identified for races by
using Williams’ clubroot differential(WCD) cultivar set, and B2 that was
a collected gall from Baekbang second region in Asan coexisted with four
races(race 1, 4, 9, 11). In order to conduct RAPD analysis, primers
(URP1-12, REP, ERIC primer) were used and out of them URP2, 3,
6, 7, 8, REP, ERIC primer gave their effect. The result of experiment
showed that primers(URP2, 7, 8, REP, ERIC) as well as primer 3 and
6 also have possibility of each race identification through the difference
of agarose gel band patterns. As it indicated that isolates of Korean P.
brassicae showed polymorphisms according to the different races, we were
able to expect possibility to classify each race of the pathogen based on
the RAPD analysis.
Keywords: Plasmodiophora brassicae, Kimch cabbage, RAPD analysis,
Race, Genetic variation

A-62
Leucothrix arctica sp. nov. Isolated from Arctic Ocean
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Biological Resources
Gram-staining-negative, motile by polar flagella, aerobic and
rod-shaped bacterium, designated strain IMCC9719T, was isolated from
a seawater sample collected from the Arctic Ocean. Optimal growth of
strain IMCC9719T was observed at 15 °C, pH 7.5-8.0 and in the
presence of 2.0 % NaCl. Strain IMCC9719T formed a robust phylogenetic clade with members of the genus Leucothrix. The 16S
rRNA gene sequence similarity showed that strain IMCC9719T was
most closely related to Leucothrix pacifica XH122T (97.4 % similarity)
and Leucothrix mucor DSM 2157T (95.1 %). The DNA G+C content
of strain IMCC9719T was 46.3 mol%. The major fatty acids were
C18:1 α9c and/or C18:0 ω6c (43.4 %) and C18:1ω7c and/or C18:0ω9c (19.3 %).
Strain IMCC9719T contained ubiquinone-8 (Q-8) as the respiratory
quinone. The major polar lipids were phosphatidylethanolamine,
phosphatidylglycerol and diphosphatidylglycerol. On the basis of
taxonomic data collected in this study, it was concluded that strain
IMCC9719T represented a novel species of the genus Leucothrix,
for which the name Leucothrix arctica sp. nov. is proposed with the type
strain IMCC9719T.
Keywords: Arctic ocean, genus Leucothrix, Leucothrix arctica sp. nov.

A-63
A New Endophytic Species from Abies firma Leaf in Korea
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An endophytic fungal isolate EML-AS5 has been isolated from Abies
firma leaf in Korea. Colony of strain grew slowly on PDA, reaching
26-30 mm diameter at 25°C after 7 days of incubation. The colony was
greenish glaucous in the center with a white margin. Conidia measured
25.3-34.3 (av. 29.7) x 3.2-5.1 (av. 4.4) μm, were fusiform, rounded at
both ends, mostly 3-septate. BLASTn search of the rDNA ITS and 28S
rDNA sequences via NCBI indicated that the EML-AS5 isolate was
closest to Bartalmania sp. P7E2 (GenBank accession no. JN207255) and
Truncatella restionacearum (GenBank accession no. DQ278929) with
identity values of 96.5% (474/491 bp) and 99.0% (831/839 bp),
respectively. However, the strain was different from a described
Truncatella species, Truncatella species, Truncatella restionacearum in shape. Especially, apical
appendages were shorter, and measured 4.3-6.5 (av. 5.4) μm. Conidia
have only an apical appendage. In the phylogenetic tree, the strain
formed a separate branch showing that the strain EML-AS5 is new
Truncatella species.
Keywords: Pleosporales, Morphology, ITS, 28S

A-64
Syzygites sp. nov. from Contaminated Mushroom in Korea
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A zygomycete fungal isolate EML-FCM1 has been isolated from
contaminated mushroom tissue in Korea. The strain grew fast on PDA,
and covered the 90 mm petri after 3 days at 25°C, the colony was initial
grey and then changed to black. Sporangiospores were globose to
irregularly subglobe, ovoid, and measured 13.0-20.5 μm wide ×
114.2-214.8 μm in diameter. Based on the sequence analysis
of the internal transcribed (ITS) regions and morphological characteristics,
the EML-FCM1 isolate was identified as a new Syzygites species,
representing low ITS sequence similarity of 96.2% (483/502 bp) and
91.8% (464/505 bp) with Syzygites megalocarpus (GenBank accession no.
FJ481032 and JN206369) via NCBI BLASTn search, respectively.
Morphologically, the isolate differed from those of other related species
by having smaller sporangia (range, 45.4-126.9 μm), and columella were
divided in shape. Our study showed that the strain is a new fungal species of
Syzygites.
Keywords: Mucoraceae, Morphology, Mushroom, ITS
Characterization and Identification of Methane Oxidizing Bacteria Isolated in Yellow Loesses from Jeollanam-do, Korea

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Two methanotrophic bacteria were isolated from yellow loesses in Jeollanam-do province, Korea. Cells were Gram-negative, aerobic, non-motile and motile, rod and short rods with appendages. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the first strain formed a tight phylogenetic lineage with Methylomonas clara with a value of 99.5% and the second strain was related most closely to Prosthecomicrobium hirschi with 99.8%. 16S rRNA gene sequence similarity. In addition, tests were performed for making them differentiate from other strains and for identifying their various characteristics such as carbon source, nitrogen source, temperature, pH and NaCl tolerance. On the basis of this analysis, we came across that Methylomonas sp. can utilize sole carbon source as methane, ethanol, methanol, glucose, monomethylamine, acetate, malate, α-,γ-xylene and Prosthecomicrobium sp. can utilize methane, malate, succinate, γ-xylene. Furthermore, Methylomonas sp. can use KNO3, monomethylamine, urea, formamide, yeast extract, γ-alanine, cysteine, γ-glutamine and Prosthecomicrobium sp. can employ KNO3, monomethylamine, urea, formamide, yeast extract, γ-alanine, γ-glutamine as nitrogen sources. Growth was observed for both strains at 25-45°C (optimum, 30°C) and pH 4-8 (optimum, pH 7) and NaCl tolerance up to 1-4% (w/v).

Keywords: Methane oxidizing bacteria, Methanotroph, Methylomonas sp., Prosthecomicrobium sp.

Runella palustris sp. nov., Isolated from Wetland Freshwater

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A novel Gram-staining negative and rod-shaped bacterial strain, designated strain HMF3829T, was isolated from wetland freshwater in Korea. A phylogenetic tree based on 16S RNA gene sequences showed that strain HMF3829T formed a lineage within the genus Runella. Strain HMF3829T was closely related to Runella stiltformis (95.22% sequence similarity), R. limosa (94.9%), R. zaceae (94.2%) and R. defluvii (94.0%). The major fatty acids of strain HMF3829T were iso-C15:0 (26.8%), summed feature 3 (comprising C16:0 and/or C18:1ω6c; 22.9%), C10:0,ω6c (11.5%) and iso-C17:0 3-OH (10.5%). DNA G+C content of strain HMF3829T was 45.7 mol%. On the basis of the evidence presented in this study, strain HMF3829T represents a novel species of the genus Runella, for which the name Runella palustris sp. nov. is proposed. The type strain HMF3829T (= KCTC 42850T = CECT -ing).

Keywords: 16S rRNA gene, wetland, freshwater, Runella

Emticicia paludis sp. nov., Isolated from Wetland Freshwater

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A novel bacterium, designated HMF3850T, was isolated from wetland freshwater sample in Gyeong-an wetland, Republic of Korea. Cells were Gram-staining-negative, facultative anaerobic, curved rod, non-motile, oxidative- and catalase-positive. Growth was observed at pH 7.0-8.0 and at 10-30 °C on R2A agar. Comparative analysis of 16S rRNA gene sequences revealed that strain HMF3850T is a member of the genus Runella. Strain HMF3850T was closely related to Runella stiltformis (94.7%), R. limosa (95.0%), R. defluvii (94.0%) and R. zaceae (95.9%). The major fatty acids were C16:0 (59.0%), summed feature 8 (comprising C16:1ω7c and/or C18:1ω6c; 11.5%) and C16:1ω6c (11.5%). DNA G+C content of strain HMF3850T was 45.7 mol%. The predominant respiratory quinone was Q-8. The DNA G+C content was 37.4 mol%. On the basis of the evidence presented in this study, strain HMF3850T represents a novel species of the genus Emticicia, for which the name Emticicia paludis sp. nov. is proposed. The type strain is HMF3850T (= KCTC -ing = CECT -ing).

Keywords: 16S rRNA gene, wetland, freshwater

A-65

Planktotalea arctica sp. nov., Isolated from Artic

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A Gram-staining-negative, non-motile, non-pigment, catalase- and oxidase-positive, aerobic and rod-shape bacterium, designated ICOC9565T was isolated from coastal seawater of the Arctic. Optimal growth of strain ICOC9565T was observed at 20°C, pH 7.0 and in the presence of 2% (w/v) NaCl. Phylogenetic analysis base on 16S rRNA gene sequences revealed that strain ICOC9565T belong to the genus Planktotalea, forming a robust clade with member of the genus. Strain ICOC9565 was most closely related to Planktotalea frisia (97.9% similarity). Average nucleotide identity value between Strain ICOC9565 and P. frisia SH6-1T was 79.6% and genome-to-genome distance was 21.0% on average. The G+C content of the DNA of strain ICOC9565 was 53.8%. The major fatty acids were C16:1ω7c and/or C16:1ω6c (30.3%) and C18:1ω7c 11-methyl (6.7%). The major respiratory isoprenoid quinone was ubiquinone Q-10 and the polar lipids were phosphatidylglycerol and phosphatidylcholine. On the basis of phylogenetic analysis and genetic data, strain ICOC9565 (KACC 18009T = NBRRC 110393T) represents a novel species of the genus Planktotalea, for which the name Planktotalea arctica sp. nov.

Keywords: Arctic Ocean, Genus Planktotalea, Planktotalea arctica sp. nov.
Evaluation of Water Quality of Hot Spring Water for Agricultural Use by Legionella-Incidence Risk

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With increasing trend of using indoor or water-curtain cultivation, ground water, especially hot spring water, are often used as heat source for indoor climate control. In this study, safety of hot spring water by estimating correlation with presence/absence of Legionella, a non-fecal opportunistic pathogen with heat-tolerance. Microbiological data in 7 studies that surveyed Legionella in hot spring waters were subjected to meta-analyses with odds ratio (OR) as effect size. Presence/absence of Legionella were significantly correlated to coliform levels [OR=3.1 (1.5-6.4, 95% CI), P=0.002]. Legionella presence was also correlated with heterotrophic plate count [HPC; 4.0(2.2-7.2), P=0.001] and water temperature [4.3(1.4-13.6), P=0.011] when temperature ranged <40°C. Therefore, bacterial standing crops in hot spring waters appear to be determined by water temperature, with a positive correlation. According to this relationship, re-circulated used of hot spring water for temperature control in greenhouse or indoor plant cultivation facility will generate health risk by Legionella abundance correlated with water temperature.

Keywords: water curtain greenhouse, indoor cultivation, health risk

Macilaginibacter ginsengisoli sp. nov. Isolated from a Ginseng-cultivated Soil

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A dark-pink-colored bacterial strain, B4Y-8T, was isolated from a soil cultivated with ginseng. The 16S rRNA gene sequence of this strain showed the highest sequence similarity with Mucilaginibacter litoreus BR-18T (96.8%), Mucilaginibacter latimaris BR-3T (96.6%), and Mucilaginibacter defluvii A5T (96.2%) among the type strains. The strain B4Y-8T was a strictly aerobic, Gram-negative, non-motile, short-rod-shaped bacterium producing a large amount of extracellular polymeric substance. The strain grew at 10-35 °C (optimum, 25 °C), at a pH of 3.0-11.0 (optimum, pH 7.0), and in the presence of 0-1% (w/v) NaCl (optimum, 0%). The DNA G+C content of strain B4Y-8T was 49.0 mol%. It contained menaquinone 7 (MK-7) as the major isoprenoid quinone, and summed feature 3 (C16:0 3- and C18:0 3- cyclo) and iso-C15:0 2-oct. The strain B4Y-8T was phylogenetically closely related to the genus Mucilaginibacter. Based on those results, it was considered that anamorph of Mucilaginibacter, for which the name Macilaginibacter ginsengisoli sp. nov. is proposed. The type strain is B4Y-8T (=KACC 18152T = JCM 30759T).

Keywords: Macilaginibacter, strain B4Y-8, novel species, polyphasic taxonomy, soil

Hydrogenophaga sp. LPB00072T, a Novel Species Candidate within the Family Comamonadaceae

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A Gram-reaction-negative, aerobic, motile, rod-shaped and yellow-pigmented bacterial strain LPB00072T, was isolated from a Crassostrea gigas in Yellow Sea, Korea. The phylogenetic analysis based on 16S rRNA gene sequence placed the test strain within the genus Hydrogenophaga, a member of the family Comamonadaceae. The highest sequence similarity was observed with Hydrogenophaga tautropis (97.6 %) and followed by H. pullerii (97.1 %). Other species in the genus Hydrogenophaga showed less than 97 % similarity. Though strain LPB00072 showed high sequence similarity with the two Hydrogenophaga species, it did not cluster with any of the relatives. Rather, it formed a distinct phyletic clade within the radiation of the genus Hydrogenophaga. Optimal growth occurred in the presence of 2% (w/v) NaCl at 26°C and at pH 6.5. Oxidase- and catalase-activities were positive. In API 20E strips, acid were produced from 4-nitrophenyl-β-D-galactopyranoside, D-glucose, D-mannitol, and malic acid. The phenotypic characteristics distinguished strain LPB00072T from the close neighbors. The genome sequencing is under processing to determine the genomic relatedness among the test strain and reference strains. The taxonomic position of strain LPB00072T is still under investigation with the strong possibility that the test strain is a novel species of the genus Hydrogenophaga.

Keywords: Comamonadaceae, Hydrogenophaga, ANI, taxonomy, marine invertebrate

A Different Viewpoint of the Anamorph Stage of Mycosphaerella nawae Based on Phylogenetic and Morphological Characteristics

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Mycosphaerella nawae causes circular leaf spot which is destructive fungal agent on persimmon, has been reported in Korea, Japan and Spain. Up to date, it has been reported that anamorph stage of M. nawae is belonged to Ramularia spp. with its morphological characteristic and there were no studies about phylogenetic relationship with allied species. To consider the appropriate taxonomic placement of M. nawae, we analyzed phylogenetical relationship with allied species and morphological characteristics using light and digital microscope. For the phylogenetic analysis, molecular marker genes were examined to understand a phylogenetic relationship with allied species of M. nawae. As the result, it was shown that M. nawae isolates were very closely related to Phaeopleospora spp. while distinguishable with Ramularia spp. In microscopic observation, pseudothecia were observed on the front while pycnidia were observed from reverse side of over wintered diseased leaves and diseased leaves, respectively. According to previous reports, production of pycnidia is the main characteristics of Phaeopleospora spp. Based on those results, it was considered that anamorph of M. nawae is belonged to Phaeopleospora spp. and the anamorph of M. nawae need to be changed to Phaeopleospora kaki.

Keywords: Mycosphaerella nawae, Anamorph stage, Morphological characteristics, Phylogenetic analysis, Phaeopleospora spp.
**B-1**

Detection of *Pseudomonas otitidis* E42 Gene for Polyethylene Degradation
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Mesophiles degrading polyethylene (PE) were isolated from a cultivated land soil of Goyang City, Gyeonggi-do, where plastic wastes had been heaped up. The isolated strain was identified as *Pseudomonas otitidis* E42 through morphological properties examined by scanning electron microscopy, biochemical properties ascertained by API 20NE, 20E Kits and 16S rRNA sequence analysis. The activity of the isolated toward PE degradation was assessed by measuring the amount of CO₂ evolved from a sterilized compost inoculated with the isolated strain after loading with low molecular weight PE (LMWPE) whose molecular weight was in the range of 17,000–23,700 at 37°C for 80 days according to KS M3100-1,2002; MOD ISO14855,1999. *P. otitidis* E42 degraded not only low molecular weight PE but also PE with relatively high molecular weight. In order to examine the genes encoding protein comprising alkane hydroxylase system in *P. otitidis* E42, 1134bp alkB, 181bp rubA1, 184bp parB, and 1074bp rubB were detected by performing PCR using each gene’s specific primer.

Keywords: Biodegradation, *Pseudomonas otitidis*, polyethylene, alkane hydroxylase system

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**B-2**

A Single ORF Unique to Pathogenic Strains and ORFs Necessary but Not Sufficient for the Virulence of *Vibrio vulnificus* as Determined by Comparative ORFeome Subtraction
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In order to identify ORFs associated with the pathogenicity of *Vibrio vulnificus*, 12 *V. vulnificus* strains obtained from clinical sources and from environmental sources were subjected to mouse lethality assay and comparative genomic analysis. Three clinical strains and two environmental strains were determined to be pathogenic (P) and seven environmental strains to be nonpathogenic (N). Subtraction of the core ORFeome constructed for P strains (P-CORE) using sequential RBH analyses by pan-ORFeome of N strains resulted in a single ORF, which was orthologous to ORFs annotated with hypothetical protein in our analyses by pan-ORFeome of N strains resulted in a single ORF, which was orthologous to ORFs annotated with hypothetical protein in our reference genomes. BLASTP and TBLASTN searches against GenBank database showed that this ORF (tentatively designated as parBP) is a homolog of parB, which encodes bacterial chromosome partitioning protein. On the other hand, we obtained 84 ORFs commonly present in P strains as well as ‘some’ of N strains from the subtraction of P-CORE by a core ORFeome of selected N strains, and these P strain-directed (not absolutely specific) ORFs were considered as ORFs necessary but not sufficient (NBNS) for the pathogenicity. Approximately 40% of P-directed ORFs belonged to functional categories of transcription, signal transduction network (STN), and ion transport/metabolism (ITM).

Keywords: *Vibrio vulnificus*, Genome

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**B-3**

A Meta Analysis of Ruminal Archaeal Diversity
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The objective of this study was to conduct a meta-analysis of 16S rRNA gene sequences of ruminal archaea that are publicly available in the Ribosomal Database Project. A total of 8623 sequences were retrieved from the Ribosomal Database Project (Release 11, Update 3). The 8620 of the 8623 sequences were classified to 3 phyla, 5 classes, 6 orders, 9 families and 12 genera, while the remaining 3 sequences could not be classified to a known phylum. *Phylum Euryarchaeota* accounted for 99.8% of the total archaeal sequences where genus *Methanobrevibacter* was the first predominant genus and accounted for 63.2% of the total archaeal sequences, followed by *Methanomassiliicoccus* (9.8%), *Methanobacterium* (7.7%), *Methanocorpusculum* (1.2%), *Methanococcus* (0.4%), *Methanobrevibacter* (0.1%) and *Methanomassiliicoccus* (0.1%). The 7544 sequences that were trimmed to the V2 to V3 region were clustered into 493 OTUs at 97% sequence similarity. This study will help guide future studies for further analyses of ruminal methanogens.

Keywords: 16S rRNA gene, meta-analysis, ruminal archaea, Ribosomal Database Project, methanogens

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**B-4**

Genotypic Diversity and Population Structure of *Vibrio vulnificus* Strains Isolated in Taiwan and Korea as Determined by Multilocus Sequence Typing
Hye-Jin Kim and Jae-Chang Cho*
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The genetic diversity and population structure of *Vibrio vulnificus* isolates from Korea and Taiwan were investigated using PCR-based assays targeting putative virulence-related genes and MLST. The majority (~50%) of strains had virulent genotypes for all loci tested. Although significant (p<0.05) inter-relationships among the genotypes were observed, the association between genotype and strain source was not significant, indicating that genotypic characteristics alone are not sufficient to predict the isolation source or the virulence of a given *V. vulnificus* strain and vice versa. Two major monophyletic groups (lineages A and B) were observed, lineage A had six STs that were exclusively environmental, whereas lineage B had STs from both environmental and clinical sources. Virulent and nonvirulent genotypes predominated in the MLST lineages B and A, respectively. In addition, *V. vulnificus* was shown to be in linkage disequilibrium (p<0.05), although two different recombination tests detected significant evidence of recombination. Tajima’s D test also indicated that *V. vulnificus* might be comprised of recently sub-divided lineages. These results suggested that the two lineages revealed by MLST correspond to two distinct ecotypes of *V. vulnificus*.

Keywords: *Vibrio vulnificus*, Diversity
**B-5**

**Bacterial Community Changes during Enrichment Cultivation of Oil-Polluted Soil Loaded with Low-Molecular-Weight Polyethylene**

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A beach soil sample was inoculated to a basal medium containing low-molecular-weight polyethylene as a sole carbon source. Total DNA was extracted from the medium after 90 days of enrichment culture. Alkane mono-oxygenase gene (alkB) was measured quantitatively from the extracted total DNA using quantitative real-time PCR to examine variation of microbial community as a result of the PE enrichment culture. As the PE enrichment culture time went on, the [alkB]/[16S rRNA] ratio increased confirming that the strains possessing alkB increased in number among the total microbial community in the medium. The sequence analysis was performed to examine the variation and diversity of the microbial community through 16S rRNA library. Acidovorax and 3 other genera, which were incapable of alkB gene expression, were detected in the medium before the enrichment culture but were not detected after the enrichment culture. In addition, the population of Arthrobacter, Curtobacterium, Gordonia, Rhodococcus, which were reported to be capable of alkB gene expression, became more numerous after the enrichment culture.

Keywords: Metagenome, Bacterial community, Polyethylene

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**B-6**

**Comparisons of ATP Analytic Methods for Measuring Total Active Biomass in Granular Activated Carbon Filters in Water Treatment Plants**

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K-water

The total active biomass of GAC (Granular Activated Carbon) is one of important factors in determining the backwashing and replacement period of the GAC in water treatment plants. Although a culture based method, such as a heterotrophic plate count agar, is used to measure the total active biomass in GAC, a method that measures the total active biomass indirectly through measuring the amount of ATP has also been used. A method that directly measures the total active biomass at a site may represent more active data than that of measuring it at a laboratory. For investigating whether a portable luminometer can be used to directly measure the amount of ATP, the reproducibility and accuracy in a bench top luminometer are compared. The comparison between the bench top and the portable luminometer shows a concentration dependent result for the amount of rATP up to 20 nM. However, the portable luminometer does not represent the concentration dependent result at the ATP concentration more than 20 nM. The result by the bench top luminometer shows a higher value of the rATP with an average of 421 ± 34 times. Although the result of the portable luminometer is reliable for the amount of ATP below about 20 nM, it is difficult to trust the result at the concentration more than 20 nM. It is not possible to apply the portable luminometer for considering the amount of ATP included in GAC and is necessary to develop an additional device to measure the highly concentrated ATP.

Keywords: Granular activated carbon, Total active biomass, Luminometer, ATP

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**B-7**

**Microbial Treatment of Food Waste**

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All the problems with food waste in our society are well known like its technologies to treat. There are many kinds of excellent technologies, even though technology upgrade is very useful, more than enough. In this study we tried to isolate microbial cells from variable food waste in different ages. The studies on the isolated microorganisms were carried. Microorganisms were identified and their growth curves in batch cultivation were also analyzed. And several kinds of food waste were treated with microbial cells in variable ways.

Keywords: food waste, microorganism

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**B-8**

**Comparison of Rhizobacterial Communities in Pepper Greenhouse under Different Cropping Systems**

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The aim of this study was comparison of rhizobacterial communities in pepper greenhouse under a paddy-upland (rice-pepper) rotational system (PURS) and repeated cropping system (RCS) and investigated adverse effects of high salinity on soil properties. Soil properties were analyzed including electrical conductivity (EC), pH, total nitrogen, and organic matter content and so on. Composition of rhizobacterial communities was established through culture-based and culture-independent methods using pyrosequencing. In addition, all culturable bacteria isolated from each sample were studied for their traits related to plant-growth promotion. The EC was 5.41 dS/m for RCS and 2.11 dS/m for PURS, respectively. Based on the culture-based method, the diversity of rhizobacterial communities and bacterial characteristics were significantly more abundant under the PURS than under RCS. The pyrosequencing data also revealed that the richness and diversity of rhizobacterial communities were greater under the PURS than under RCS. Spearman’s correlation coefficient showed that the relative abundance of dominant phyla was positively or negatively correlated with soil properties.

Keywords: Rhizobacterial communities, Pyrosequencing, Cropping system, 16s rRNA, Salt accumulation
Occurrence of the Novel Human Norovirus GI.17 in Coastal Stream Water of South Korea, 2015
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Human noroviruses (hNoV) are a main causative agent of viral acute gastroenteritis in the world. This positive-sense RNA virus, especially in relation to the genogroup II.4 (GI.4) pandemic variants, has brought many important issues in public health over the last decades. To insight environmental prevalence of the GI in South Korea, we collected environmental water samples from coastal streams and neighboring waste water treatment plant (WWTP) of North Jeolla Province in March 2015. On the basis of capsid region C analysis, four different genotypes (GI.4, GI.13, GI.17, and GI.21) were detected with the highest prevalence of GI.17. Additional sequence analyses for ORF1-ORF2 junction and ORF2 from the water samples revealed that the GI.17 sequences from North Jeolla Province were closely related with the novel GI.17 Guangdong/Kawasaki strains, the main causative genotype of 2014-2015 hNoV outbreak in China and Japan. These results suggest that the novel GI.17 has recently replacing the GI.4 pandemic variants in South Korea as in the cases of China and Japan. Therefore, an in-depth surveillance for the novel GI.17 prevalence in environmental water and clinical cases should be carried out to prepare a potential threat of a new pandemic including South Korea.

Keywords: novel human norovirus GI.17, coastal stream water

Investigation of Diversity of Extremely Halophilic Archaea from Solar Salts
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Extremely halophilic archaea, called haloarchaea are adapted to hypersaline environments, although high salinity is toxic to most cells. They have usually red or pink pigmented colonies and require at least 1.5 M NaCl for growth and grow optimally in NaCl concentrations of 2.6 M or higher. In this study, the haloarcheal diversity was analyzed from different kinds of non-purified solar salt samples using culture-independent and -dependent approaches. Metagenomic analysis based on the next-generation sequencing showed that dominant phylotypes in the samples belong to the genera Haloarcula, Haloburrum, Halobacterium, Halogenica and Natronomonas. About 670 haloarchaea strains were isolated from the sample and most of the identified genera were Haloburrum, Halobacterium, Haloarcula and Halokaminza. In addition, novel strains, designated CBA1103T, CBA1105T and CBA1107T, were characterized phylogenetically and phenotypically. Based on the polyphasic taxonomic analyses, the names Halobellum rifutum, Halapricum salinum and Haloarcula rubra were proposed as a novel species, respectively. Genome sequences of the novel three strains were also analyzed and their functional gene information will be of importance for the haloarchaeal researches and industries with extremozymes produced from the extremophiles.

Keywords: microbial diversity, salt

Development of Algicidal Reactor with Bio/ceramics Membrane for Controlling Harmful Algal Blooms (HABs)
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Harmful algal blooms (HABs) have made massive economic losses and marine environmental disturbances in Korea. Normally, lees was scattered to control HABs in Korea, but it arises secondary contamination problems. Although there are many papers and patents about algicidal bacteria and chemicals in Korea, cause of its efficiency and safety, it has not been applied on practical site before. Therefore, high effective and eco-friendly method to control the HABs is urgently required to be developed. We screened more than 1,000 bacterial strains killing Cochlodiniu polymorphides which is a major kind of HABs in Korea, about 100 strains showed >30% algicidal activity. From the screening result, we choses strain 2R1 which showed high algicidal activity against C. polymorphides. To supply and preserve algicidal bacteria continuously, we developed algicidal reactor with bio-ceramics fusion membrane that consist of algicidal bacteria and multi-pore ceramics. The new multi-pore ceramic materials that we made had 10.30% porosity, 7.40% absorption rate, 1.46 specific gravity. Using this system, the algicidal activity was more than 90%. As a result, this system will be promising material for HABs removal.

Keywords: Algicidal bacteria, Algicidal reactor, Harmful algal blooms

Effect of Three Crops Cultivation on DHA (dehydrogenase) Activity in Highland Areas
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To achieve sustainable agriculture, development of effective soil management system is the most important part. Soil management system such as different crop cultivation affects on soil microorganisms activities. Dehydrogenase activity (DHA) analysis is an effective indirect measurement for soil microorganism activity and the higher value of DHA indicates the more microorganism density in the soil. This research was conducted to determine factors affect on DHA in three different crops, soybean(Glycine max), potato(Solaum tuberosum) and Kimchi cabbage(Brassica rapa). DHA was analyzed in pots and field cultivation of three crops at least once every 2 or 3 weeks. Soil texture of field and pot was sandy loam and sandy, respectively. Pot soil has lower organic matter content than field soil on overall plant growth period. DHA value of pot cultivation was lower than that of soil because of low nutrient holding ability in sandy soil of pot. According to crop effect on DHA, there was no significant relationship between crops and DHA in the field cultivation but soybean has significantly higher DHA (p<0.05) than other crops in pot cultivation. In the field cultivation, various microorganisms might play a role as buffer activity. Simple microorganisms composition in the pot could results in more strong effects on DHA in pot cultivation.

Keywords: Dehydrogenase activity, Soil microorganism
**B-13**

**Combined Application of Cyanobacteria with Soil Fixing Chemicals for Rapid Induction of Biological Soil Crust Formation**

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This study was conducted to rapidly stabilize the initial stage of artificial cyanobacterial soil crust using combined treatments of cyanobacteria with soil fixing chemicals. Poly(vinyl alcohol) and TKS chemicals were examined under laboratory conditions. TKS7 showed noticeable aggregate stability among the examined chemicals. Combined application of cyanobacteria with different concentrations of TKS7 (CT1-CT5) also showed high aggregate stability with MWD (mean weight diameter) values of 0.58-0.69 mm relative to single application of TKS7 (0.18-0.40 mm). For the field trial, SAP (sorbactoplant polymer) was also applied as a water-holding material and nutrient supplement. Application of cyanobacteria with SAP and TKS7 (CST) remarkably improved soil stability and biological activity in the field at 4 months after induction. These results suggest that combined application of cyanobacteria with soil fixing agent rapidly induced biological soil crust (BSC) formation in the field within a few months, and the soil properties and biological activities of the induced BSC reached 55-88% of that of natural cyanobacterial crust aged approximately 20 years. The novel method presented herein makes it feasible to artificially induce BSC formation in a very short term with improved stabilization of cyanobacterial soil crust properties during the initial stages of BSC formation.

Keywords: Anti-desertification, Biological soil crust, Soil stabilization

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**B-14**

**Combination of Conventional RT-PCR and Quantitative PCR for Human Norovirus Source Tracking in Environmental Waters**

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Human noroviruses (hNoVs) are an important causative agent of acute gastroenteritis throughout the world. Identifying major point pollution source of hNoV in environmental waters, therefore becomes critical to prevent primary transmission via fecal-oral route. Conventional RT-PCR technique have been popular for ordinary surveillance for hNoV identification in water samples and clinical specimens as well but the limited nature of quantifying contaminant load often precludes its usefulness in hNoV source tracking. In this study, we attempted for finding main sources of hNoV influx to estuaries. Water samples were collected from various nodes of stream where multiple water branches from different point pollution sources are united. Genogroup-specific conventional RT-PCR identified multiple genotypes of hNoV in these water samples. Then, the samples shown to be hNoV positive were subjected to quantitative RT-PCR (qPCR) analysis. Overlapping the results of conventional RT-PCR and qPCR complemented each other and revealed successfully which point pollution source was mainly responsible for the viral influx to estuaries. As a result, unpurified sewages of domestic and elderly nursing homes were identified releasing about 10^2-10^4 higher load of hNoV than industrial sewage or agricultural water. Therefore, combination of conventional RT-PCR and qPCR could be a quite useful alternative for locating the main contamination sources in hNoV surveillance.

Keywords: Norovirus source tracking

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**B-15**

**Monitoring of Culicoides in Korea in 2014**

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Many Arboviral livestock diseases of major importance are transmitted by culicoides species of biting midges, including Bluetongue, African Horse sickness, Epizootic Hemorrhagic Disease, etc. Climate change is expected to increase the activity of insect vectors resulting in the increased risk of new introduction of foreign diseases. For these reasons, a monitoring program for culicoides species was conducted at sea ports, airports, quarantine stations and the other inland sites in 2014. Mosquito magnet traps, black light traps and New Jersey traps were used for monitoring of culicoides species at 20 sites, which were composed of 2 airports, 3 sea ports, 3 animal quarantine stations, 4 horse stables, 2 cattle farms and 6 military camps. The traps were operated continuously from April to December 2014 and the specimens were collected once every 2 weeks. Specimens were identified based on the morphological characters proposed by Ree (2003). A total of 852 specimens, representing 11 species were captured and the most frequently collected species were as follows: Culicoides tatinus, Culicoides arakanus, Culicoides homostoma, Culicoides circumpalpis, Culicoides nipponensis and Culicoides japonicus, etc. The culicoides samples collected by this study will be further tested for the presence of causative viral agents of various livestock diseases such as the Bluetongue virus.

Keywords: Culicoides, Monitoring, Arbovirus

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**B-16**

**Purification and Detection of Alternaria Mycotoxins Using HPLC and LC-MS/MS**

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Alternaria species can contaminate different cereals and result in mycotoxin contamination. Using blotter method, 37 isolates of small-spored Alternaria species were isolated from grain seeds including barley, wheat and corn. Ten isolates were tested for in vitro production of 5 major mycotoxins including alternariol (AOH), alternariol monomethyl ether (AME), alternarin (ALT), alternariol-1 (ATX-1), and tenuazonic acid (TeA) based on LC-MS/MS analysis. Each isolate was grown on 100 g of autoclaved rice kernels at 25°C for 2 weeks. Alternaria cultures were extracted with methanol, purified by using solvent partition, thin-layer chromatography, high performance liquid chromatography (HPLC). Mycotoxins were analyzed by LC-MS/MS with electrospray positive ionization mode. Out of 11 isolates tested, all isolates produced AOH and AME, and only seven isolates of EML-BLD1-4, EML-BLD1-8, EML-BLD1-12, EML-BLD1-15, EML-BLD1-18, EML-BLD1-2 and EML-BLD1-5 produced TeA although quantities of the mycotoxins produced by different isolates were quite variable.

Keywords: Alternaria, Mycotoxin, Seedborne, LC-MS/MS
A Proenvironmental Removal and Reuse Process of Nitrogen and Phosphate

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In the agriculture nitrate and phosphate are important for plant nutrition. With this study the recycling of these minerals are stimulated with domestic natural resources. Swine manure and livestock wastewater can be treated with a chemical simple process and at the same time the environmental problems also can be reduced.

The phosphorus and nitrogen components in the artificial manure are precipitated to $\text{MgH}_2\text{PO}_4\cdot\text{H}_2\text{O}$ or $\text{MgNH}_4\text{PO}_4\cdot6\text{H}_2\text{O}$. Struvite precipitation is known as a removal process of nitrogen and phosphorus with higher efficiency. In this study, firstly, we tried to optimize the whole precipitation process. Secondly we tried to produce an environment-friendly slow release fertilizer, which contains nitrogen, phosphorus and magnesium for plant growth.

Keywords: nitrate, phosphate, Struvite

B-18

Morphological Observation of Saccharomyces cerevisiae KCTC 7296 under Sub-MIC with Amphotericin-B Antibiotics

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Amphotericin-B is one of antifungal antibiotics and We determined Minimum Inhibitory Concentration (MIC) against amphotericin-B for Saccharomyces cerevisiae, which is eukaryotic microorganism. MIC was about 25mg/ml against amphotericin-B. We tried to investigate the morphology of $S.\text{cerevisiae}$ below the concentration of MIC (sub-MIC). We observed the morphology and division patterns of $S.\text{cerevisiae}$ yeast under sub-MIC by using light microscope and scanning electron microscope. Much more daughter cells of $S.\text{cerevisiae}$ were observed under sub-MIC than normal state of concentration.

Keywords: Saccharomyces cerevisiae, sub-MIC, amphotericin-B

Plant Growth-Promoting Bacteria Bacillus sp. CeR16-2, Possessing Drought Stress Tolerance in Kale Plant

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Bacillus strain could be good resources that can be used as potential biofertilizers for improving crop cultivation, owing to their plant growth-promoting and tolerance for high temperature, drought stress and so on. Strain CeR16-2 was isolated from rhizophore soil of celery and identified as a strain of Bacillus methylotrophicus by 16S rRNA gene analysis. B. methylotrophicus CeR16-2 displayed broad-spectrum suppression of plant pathogens like Botrytis cinerea and Sclerotinia sclerotiorum. We surveyed and analyzed the kale seedling-growth after CeR16-2 treatment for its effectiveness as a biofertilizer. As a result, shoot fresh weight was 6.91 g treated with CeR16-2, which showed an increase of about 4 times compared to control (1.68 g) treated with water. When kale seedlings were under drought stress for 7 and 14 days, kale seedlings treated with CeR16-2 presented tolerance for drought stress while kale seedlings treated with other treatments and water showed in very little drought tolerance.

Keywords: plant growth-promoting, Bacillus sp., drought stress tolerance, biofertilizer

A Novel Zinc Dependent Metalloprotease from Pseudoalteromonas sp. Strain SI1A

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The Antarctic Ross Sea forms one of the most unique and diverse biological habitats mainly due to seasonal sea ice formation and a variation of solar radiation. Pseudoalteromonas sp. strain SI1A was isolated from the internal organ of starfish, which is one of benthos residing on the bottom of the Ross Sea. SI1A is suspected to be symbiotic relationship with the benthos and shows psychrotrophic characteristics. Optimal growth temperature of SI1A is 25°C~30°C, but it shows significant growth at 5°C. By using the supernatant of 24 hour cultured SI1A, SDS-PAGE and extracellular protease activity staining were carried out. One protein bands that has caseinase activity at size of approximately 26kDa was transferred onto the PVDF membrane for N-terminal amino acid sequence. The sequence was analyzed by Protein Blot and shown to include ZnMec superfamily domain. As this extracellular protease of SI1A contains zinc ion as cofactor, this gene is called as $\text{ecz}$ (extracellular zinc protease). Molecular modeling was performed to characterize the EcPZ. Interestingly, the structure of EcPZ protein of SI1A is most similar to that of a snake venom, adamalysin. Adamalysin is cell surface transmembrane protein in cell adhesion, proteolytic process and capability of shedding a multitude of proteins from the surface of the cell. EcPZ was expressed as a maltose binding protein(MBP)-fusion protein expression vector system using the Escherichia coli BL21(DE3).

Keywords: psychrotroph, extracellular protease, adamalysin, Antarctic, Pseudoalteromonas

B. Environment and Ecology
B-21  Developing an Eco-Friendly Quality Compost Using Spent Coffee Grounds, Biochar and Beneficial Microorganisms

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The conversion process of coffee beans into coffee drinks results in spent coffee grounds as a by-product, leading to one of the most important solid-wastes in the city lives. A proper reuse technology could render the grounds waste into a quality compost as a valuable commercial product on top of environmental clean up. In this study the spent coffee grounds were mixed with other reusable wastes including chicken manure, sludge from wastewater in food processing company and biochar derived from the spent coffee grounds. The C/N ratio of the composting mixture (spent coffee grounds, 85%) was 42.7. Six kinds of composting reactors were set up depending upon kinds of amendments such as biochar, white rot fungi, plant growth promoting bacteria and beneficial microorganisms. An aerobic composting was performed for 4 weeks before treating the amendments, and then the amendments were made and left for maturation of the composts for 2 weeks. Hot water extractions from the composted materials were subjected to germination test. All of the manufactured composts showed a higher germination index (92.6-133.9) than the control (95.7), indicating a completed composting. Moreover, the six kinds of composts will be tested for their efficacy of crop growth and quality standards of the compost products. This study will be eventually led to a commercial development of a quality compost by recycling the spent coffee grounds and other solid wastes.

Keywords: Coffee grounds, Biochar, compost, Beneficial Microorganism

B-22  Eco-Friendly Odor Control and Remediation of a Contaminated Urban Stream Using Beneficial Microorganism (BM)

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Dongcheun, one of the representative streams in urban area, is a down stream that is connected to Hogyecheon, Bujunchun, Junpochun, Danggamchun and Gayachun as its upstream. Hogyecheon has been mostly covered with concrete structures for decades, causing sewage pollution from the upstream, overflow of the downstream region and other serious pollution that gave rise to many civil complaints from the residents nearby. In this study, we analysed 3 stations, including control station for water quality and malodor changes of Hogyecheon after applying the microbial augmentation (BM-2) for a few months including the rainy season. An activity of the augmented bacterial agent on the station (Middle) was initially expected to be relatively higher and thus show lower levels of COD than the upstream site (Chuhhae). However, it turned out that the removal effect of COD was not clear probably due to a flooding in the rainy season and too much incoming pollutants to remove considering the amount of microbial agent augmented into the stream. However, in the case of efficiency of odor control after 2 months of BM-2 treatment, the average control effect at the station (Middle) was about 65% indicating the microbial activity in reduction of malodor in the polluted stream. It was concluded that the bioaugmentation technology was effective in the malodor control and could be applied to other similar streams and improve their water qualities.

Keywords: Beneficial microorganisms, Urban stream, Contaminated stream, Odor control, Stream remediation

B-23  Analysis of Gut Bacterial Diversity and Exploration of Cellulose-Degrading Bacteria in Xylophagous Insects

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In this study, in order to prospect for potential biotechnological applications in lignocelluloses degradation, gut bacterial communities in xylophagous insects were analyzed using the pyrosequencing of 16S rRNA genes. The result showed that operational taxonomic units (OTUs), species richness and diversity index were higher in the hindgut than in the midgut of all insect samples analyzed. The dominant phyla or classes were Firmicutes (54.0%), Bacteroidetes (14.5%), γ-Proteobacteria (12.3%) in all xylophagous insects except for Rhinotermitidae. The principal coordinate analysis (PCoA) showed that the bacterial community structure mostly clustered according to phylogeny of hosts rather than their habitats. In our study, the two CMC-degrading isolates which showed the highest enzyme activity were most closely related to Bacillus toyonensis BCT-7112\(^{2}\) and Lactococcus lactis subsp. hordiae NCDO 2181\(^{2}\), respectively. Cellulolytic enzyme activity analysis showed that β-1,4-glucosidase, β-1,4-endoglucanase and β-1,4-xylanase were higher in the hindgut of Cerambycidae. The results demonstrate that xylophagous insect guts harbor diverse gut bacteria, including valuable cellulolytic bacteria, which could be used for various biotechnological applications.

Keywords: xylophagous insect, cellulolytic bacteria, pyrosequencing, lignocellulose degradation

B-24  A Study on the Detective Method and Distribution of Aeromonas Genera in Water Supplies

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The Detective Method and Distribution of Aeromonas genera in Water Supplies was investigated using US EPA 1605 method. We collected monthly from raw water samples and finished water samples supplied 3 Water Treatment Plants in Busan Metropolitan City. According to the results of survey from February 2013 to December 2013, Aeromonas in raw water was highly distributed the september and lowly distributed the February. Quantitative enumeration of Aeromonas per 500 mL of the raw water was highly distributed the september and lowly distributed the February. Aeromonas species were verified highly detected Maeri and Heidong region. No Aeromonas were found in any finished water samples. Total Aeromonas numbers were highly correlated with turbidity, water temperature and total coliform in this area. Aeromonas genus grown in medium were identified as Aeromonas salmonicida (48.5%), A. caviae (8.5%), A. schubertii (4.2%), A. sobria (2.5%), A. ichthyoxenica (2.1%) and A. hydrophila (0.8%) species. Particularly Aeromonas salmonicida was dominant in raw water. Detected species of Maeri, Maeri and Heidong region were 5, 6 and 3 genera, respectively, biodiversity of species was verified highly Aeromonas than the other region. In order to effectively manage through the Aeromonas Biofilms within a pipe, nutrition for growth was prevented and reduced the inflow of pipe. The suppress of the regrowth is need to periodic cleaning and the appropriate concentration of residual chlorination in biofilms.

Keywords: Water Treatment Plants, Aeromonas, Aeromonas salmonicida, Biofilms
Eco-Friendly Marine Sediment as Material with Nitrogen and Phosphorus Removal Efficiency Bacteria Activated Sludge SBR Waste Seawater Treatment System
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Major factor for local red tide is eutrophication of the sea by nitrogen and phosphorus. Effluent flowing in the sea from fish farm contains much nitrogen and phosphorus comparatively to normal seawater. General wastewater biological treatment system can not treat high salt wastewater effectively. To treat high salt wastewater, marine sediment as eco-friendly material, nitrogen and phosphorus removal efficiency bacteria activated sludge SBR system was studied.

Keywords: marine sediment, biological treatment, SBR system, removal efficiency bacteria

Survey of Macrofungal Diversity in Forest Genetic Resources Reserve of Mt. Gariwang for Recent 5 Years (2011-2015)
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Site-specific survey of macrofungi was conducted in forest genetic resources reserve (FGRR) of Mt. Gariwang from 2011 to 2015. The aim of this study was to investigate the diversity of macrofungal species present in the surveyed area. The species found in FGRR were recorded by photographs. The GPS location and forest vegetation were also recorded in the field. The recorded species were functionally grouped into three types based on the ecology, edibility and medicinal property. A total of 105 species (38 families 70 genera) were analyzed to obtain the information about macrofungal diversity. The recorded species consisted of ectomycorrhizal (ECM) species (36%), saprotrophic species (57%) and others (7%). The most common ECM fungi found in FGRR were Armillariaceae and Russulaceae, followed by Boletaceae. Mycenaceae was the most common family of saprotrophic fungi found in FGRR, followed by Strophariaceae, and Polyporaceae. Forty percent of total species was edible mushrooms. The number of inedible mushrooms was similar to that of edible mushrooms. The mushrooms with medicinal properties were selected by reviewing the related articles and references. The distribution of medicinal mushrooms (30%) was lower than that of non-medicinal mushrooms (70%).

Keywords: Macrofungal diversity, Site-specific survey

Bio-Resource Values of Some Macrofungi Found in Forest Genetic Resources Reserve of Mt. Gariwang
Sung-Min Jeon and Kang-Hyeon Ka*

We have continually surveyed the macrofungal flora in forest genetic resources reserve (FGRR) of Mt. Gariwang since 2011. In this study, the potential bio-resource values of some macrofungi recorded through the site-specific survey were investigated by reviewing the many articles dealing with the physiological properties and applications of mushrooms. It was found that more than 30 species including Hericium erinaceus have potential medicinal values. The medicinal properties of the investigated macrofungi were varied depending on species, including anti-tumor or anti-cancer, anti-inflammatory, antimicrobial, anti-oxidative and improvement of cardiovascular or immune function. Some species found in FGRR have potential another values as biological resources. It was reported that the bioactive compounds from Alectoria aurantia, Chlorociboria aeruginosa, Lentinula edodes, Phylloporus bietas and Suillus grevillei have antimicrobial activities against phytopathogens. Ectomycorrhizal fungi, Thelephora palustris has been used for afforestation. Panellus stipticus with bioluminescence capability have potential use as biomarker for toxicant detection. Pigment-producing fungi such as Cortinarius sanguineus (blood-red webcap) or Chlorociboria aeruginosa (blue-green cup fungus) have been used as natural dyes in wood or fabric-related industries.

Keywords: Bio-resource, Macrofungi

Cultural and Molecular Analysis of Bacterial Community Structures in Soils for Environmental Risk Assessment with Genetically Modified Soybean and Red Pepper
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The influences of transgenic plants on soil microbial communities were evaluated by using cultivation and molecular methods. The field plot consisted of four subplots planted with genetically modified soybean (Glycine max L. Merr, introduction of osmotic pressure inducible gene (ASIL-6)), non-genetically modified soybean (Glycine max L. Merr), genetically modified red pepper (Capsicum annuum L., introduction of herbicide resistance gene (bar)), and non-genetically modified red pepper (Capsicum annuum L.). The microbial dynamics (bacteria, actinomycetes, and fungi) measured by cultivation methods were quite similar among the four subplots throughout the experiments. However, the population size of Rhizobium, nitrogen-fixing bacteria associated with legume plants, in soybean soils was larger than that of Rhizobium in red pepper soils by a factor of ten. Analysis with real-time PCR DGGE (denaturing gradient gel electrophoresis) method of 16S rRNA genes showed that the bacterial community structures were very similar to each crops in a given month, indicating that there were no significant differences in bacterial communities between GM and non-GM, but there was difference between soybean and red pepper.

Keywords: Bacterial community, Genetically modified crop, Microbial dynamics
**B-30**

**Evaluation of the Coliphages as Fecal Indicator and Markers to Investigate Fecal Contamination in Shellfish Culturing Areas of South Korea**

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Inanimate surface can be contaminated from contact with contaminated human hands or water with noroviruses. By comparing swab materials and buffer solutions for the viral recovery from contaminated surfaces, we determined the most efficient NoV sampling method from various inanimate surfaces. We evaluated three swab materials (cotton, synthetic sponge) and three buffer solutions (PBS, beef extract and PBS with tween 80 solution) to recover murine noroviruses (MuNoV) from three different fertile surfaces (wood, plastic, stainless). We inoculated MuNoV onto tested cotton (10 mm²), and each coupon swab was swabbed and analyzed by real-time PCR and plaque assay to evaluate the recovery of MuNoV. Among them, PBS and cotton material showed the highest recovery than other combinations; over 80% recovery efficiency was shown on plastic and stainless surfaces. Real-time PCR and plaque assay results showed similar recovery patterns, but plaque assay showed lower recovery efficiency compared with real-time PCR. Also, we evaluated the recovery efficiency of human noroviruses (HuNoV) on three swab materials using PBS-cotton swab combination to confirm the usefulness of HuNoV recovery from tested surfaces. This method was able to recover more than 80% of inoculated HuNoV from plastic and stainless surfaces. Therefore, the sampling method with PBS and cotton swab could be very useful tools for recovering NoV from various types of inanimate surface.

Keywords: Norovirus, Sampling methods, Recovery efficiency

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**B-31**

**Invasion of Phytolacca americana Affects to Arbuscular Mycorrhizal Fungal Community**

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The invasion of exotic plants affects to not only ground environment but also subterranean ecosystems. There are many researches to explain how environment has changed due to invasion of exotic plants. But that is not well known how the community of arbuscular mycorrhizal fungi (AMF), what is very important symbiont to root of plants was affected by exotic plant invasion. We investigated how the exotic plant, Phytolacca americana, affected on AMF community. Phytolacca americana is a harmful non-indigenous plant on ecosystem designated by the Ministry of the Environment in Korea. We sampled two kind of indigenous plants (Setaria viridis, Persicaria blumei) and Phytolacca americana with rhizosphere soils from three sites in Cheongju, Chungcheongbuk-do, Korea. We extracted 18S rDNA from collected root of plants, and compared AMF communities in three kinds of plant roots using DNA cloning method. Consequently, it is observed that AMF communities in root of non-indigenous plants had significant difference compared to AMF communities in root of indigenous plants. Our results show that AMF community was changed by the invasion of Phytolacca americana.

Keywords: Environment, Exotic Plant Invasion, Arbuscular Mycorrhizal Fungi

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**B-32**

**Evaluation of Population Changes through Analyzing the Fungal ITS on Soil of the Treatment of Red Clay Processed Nano Material**

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Control of plant pathogen using chemical pesticides causes multiple problems due to accumulation of toxic chemicals in soil therefore it needs to develop environmental friendly materials. The red clay has been known to be effective plant growth and control antimicrobial activity through its absorption properties. Red clay processed nano material (RCPM) was obtained by development focus on antimicrobial efficacy of red clay. In study, we applied RCPM to field soil during 3 months for determine in vivo inhibitory effect against pathogenic fungi. Plant pathogens, Fusarium oxysporum and Rhizoctonia solani that cause multiple diseases, were decreased significantly by treatment with RCPM. We defined specific antifungal effect of the treatment of RCPM by analyzing the population changes of pathogenic fungi. In conclusion, RCPM showed strong antifungal activity and may be applicable as environmental friendly material in biological control of plant pathogenic fungi. [This research was supported by Technology Development Program (812001-3) for Agriculture and Forestry, Ministry for Agriculture, Korea.]

Keywords: population change, fungal ITS, plant pathogen, antifungal activity
**B-33**

**Study of Genotyping of Wild Mushroom *Lentinula edodes* in Mt. Jungwang and Mt. Gariwang in Korea**

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*Lentinula edodes*, edible wild mushroom, is distributed in many places of mountainous regions of the Korean peninsula. Wild oak mushrooms were collected from grown on *Quercus mongolica* at an elevation of more than 1,000 m of Mt. Jungwang and Mt. Gariwang in Gangwon province.

We examined ten oak mushrooms strains to confirm genetic similarity between strain on the bases of analysis of microsatellite markers (Led A2, Led A8, Led B2, Led B6 and Led D6) registered in National Center for biotechnology Information (NCBI) and dual cultures on potato dextrose agar (PDA) for two months at 25°C. The observed heterozygosity over all microsatellites ranged from 0.00 and 0.52 among 5 microsatellite markers and polymorphism information content (PIC) values of Led A2, Led A8, Led B2, Led B6, and Led D6 were 0.00, 0.88, 0.69, 0.45 and 0.43, respectively. The average of total values of all markers came out 0.49 as result. confrontation line between strain formed at almost all combinations, but a few combinations did not clearly. In conclusion, oak mushroom population of Mt. Jungwang and Mt. Gariwang had some mixed gene pools among them. But we need to more study to find genetic similarity and variation among them.

Keywords: *Lentinula edodes*, Microsatellite, Dual clature, Heterozygosity, Confrontation line

**B-34**

**Janthinobacterium lividum** Violacine Production and Vesicle Which Contain Violacine to Dissolve It in Aqueous Phase to Counter Their Rivals

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Violacine is one of Antimicrobial pigment produced by various natural bacteria such as *J. lividum*, *C. violaceum*, *Duganella* sp., *Pseudoduganella* sp., *Collimonas* sp. It inhibit gram positive bacterial growth and kill protozoans which consume bacteria in nature. Which give very advantageous point compete to their rival and counter to their predator. Even this very powerful advantage, however, violacine and its derivatives are too hydrophobic to dissolve aqueous phase. So there is one question following behind: how natural violacine production strain use this too hydrophobic material for their survival advantage. This study chase how violacine producing strain use their violacine to kill their rival. The method to dissolve this hydrophobic material is us lipid bi-membrane moreover use the vesicle. Vesicle is produced by many different bacteria species, violacine producing strain use this vesicle to dissolve violacine in to water. *J. lividum* is one of violacine producer, around 30% of violacine is found 0.22μm filtered supernatant. And 100kDa filter totally blocks violacine materials. The *J. lividum* violacine vesicle shown 50nm size in bioTEM image. Therefore, violacine producing strain produce vesicle to make useful violacine states.

Keywords: *J. lividum*, Violacine, Vesicle, *S. aureus*

**B-35**

**Struvite Formation and Immobilization of Microbial Cells**

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A well-known process to recover nitrogen and phosphorus components from artificial swine manure was studied. With the struvite formation the recycling these mineral components can be more efficient and useful for environment. Struvite also called as Guanite or Magnesium Ammonium Phosphate (MAP), Mg2+ : NH4+ : PO4 (3-) and is a crystal that has been combined in the same molar ratio. With addition of bentonite and hydrogels during struvite formation the removal efficiency of total nitrogen and phosphorus could be increased. The whole process with immobilization of microbial cells were also studied.

Keywords: struvite, bentonite, hydrogel

**B-36**

**Effect of Copper-Resistant Rhizobacteria on Tomato Plant under Copper Stress and Expression Pattern of Metal Stress-Related Genes in Tomato**

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Copper is vitally required for growth of plant, but the accumulation of excess copper in soil may cause adverse impact on plant growth. The purpose of this study was investigating the effects on tomato plant by application of some rhizobacteria that can confer copper resistance to plants. Isolated strains *Pseudomonas veronii* MS1 and *P. migulae* MS2 showed 7.13 and 6.43 μmol α-ketobutyrate/mg/h of ACC deaminase activity, respectively, that can reduce the level of stress hormone ethylene in plants. They also produced 0.13 and 0.26 mM of siderophore, respectively, that is metal-chelating agent. In addition, they showed 67.8 and 81.0% of biosorption ability for Cu in 20 mg l-1 Cu solution. In a pot test for tomato growth under Cu stress for 4 weeks, ACC synthase genes, *ACS4* and *ACS8* and ACC oxidase genes, *ACO1* and *ACO4* were strongly expressed in Cu stressed tomato, whereas significantly reduced in tomato treated with strains MS1 and MS2. Also, a gene encoding a metal binding protein metallothionein, *MT2* showed similar expression pattern with above result. Copper can catalyze the formation of harmful free radicals, which may cause oxidative stress. Removal activity of DPPH radical and antioxidant capacity of MS1 and MS2 increased up to 83 and 78%, respectively at 24 hours. Oxidative stress marker, malondialdehyde was produced at lower level in inoculated tomato than the control, which indicated that the tomato inoculated with rhizobacteria showed copper resistance.

Keywords: Cu-resistant bacteria, plant growth promotion, metal stress, PGPR
**B-37**

Antifungal Activity of *Streptomyces vellosus* HR29 and Its Multiple Mechanisms against Several Plant-Pathogenic Fungi

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Many strains of soil actinomycetes were isolated and their antifungal activities and involved mechanisms were investigated. Among over 400 isolates of actinomycetes, *Streptomyces vellosus* HR29 was selected as a potential antagonist to control several plant-pathogenic fungi. *S. vellosus* HR29 inhibited growth of *Fusarium oxysporum* f. sp. *rhapani*, *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* by 28.4, 34.7, 38.5 and 17.2%, respectively compared to the control after 7-day incubation on PDA plate. Isolate HR391 also inhibited growth of those fungi. Strains HR29, HR391 showed 4.65 and 0.46 μmol/min/mg of chitinase activity and 21.28 and 0.83 μmol/min/mg of β-1,3 glucanase activity, respectively. *S. vellosus* HR29 also secreted some antimicrobial peptides (AMPs). When the AMP was added at 50 μg/ml, it suppressed growth of *F. oxysporum* f. sp. *rhapani*, *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *lycopersici* by 59.1, 53.4 and 55.7%, respectively. In addition, thin layer chromatography of culture supernatant of HR29 suggested production of antifungal lipopeptides iturin A, fengycin and surfactin as secondary metabolites. Among them, iturin A which was produced at 9.49 ± 1.4 mg/L inhibited significantly fungal growth in a bioassay. These results suggest that *S. vellosus* HR29 may be utilized as an environment-friendly biocontrol agent against some important plant-pathogenic fungi.

**Keywords:** antifungal activity, *Streptomyces vellosus*, plant-pathogenic fungi, antimicrobial peptide, biocontrol agent

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**B-38**

PAH (Polycyclic Aromatic Hydrocarbon) Degrading Endolichenic Bacteria Isolated from Chinese Desert Lichens

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Endolichenic bacteria have been recently studied for their diversity, but very little has been known on their functions in terms of lichen symbiosis. In the present study, we obtained 106 bacteria strains by enrichment culture method using PAH compounds as the only carbon source without nitrogen source. We increased the concentration of PAH chemicals to 1000mg/L to isolate endolichenic bacteria strains having higher PAH-degrading activity, and then 12 bacteria strains were obtained. Most of these bacteria were identified as *Burkholderiales* by 16s rDNA sequences, which belong to Beta proteobacteria. We investigated on the environmental factors which can influence the growth of endolichenic bacteria, found that fructose and mannitol are the best carbon sources, for the nitrogen source, asparagines is the most suitable one, 30°C and alkaline condition are the perfect environment for their growth. At the same time, the degradation course of PAH compound by endolichenic bacteria was studied by GC-MS analysis, 96.79% and 99.36% of PAH were degraded within 6 days under 200mg/L and 500mg/L, respectively. The successful culture of endolichenic bacteria is important not only to study lichenism of endolichenic microbial community, but also to provide new biological resources to clear up non degrading compounds such as PAHs in the contaminated environment.

**Keywords:** Desert lichens, Endolichenic bacteria, PHA degradation

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**B-39**

A Proenvironmental Removal and Reuse Process of Nitrogen and Phosphate

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In the agriculture nitrate and phosphate are important for plant nutrition. With this study the recycling of these minerals are stimulated with domestic natural resources. Swine manure and livestock wastewater can be treated with a chemical simple process and at the same time the environmental problems also can be reduced.

The phosphorus and nitrogen components in the artificial manure are precipitated to MgHPO₄ ·H₂O or MgNH₄PO₄ ·6H₂O. Struvite precipitation is known as a removal process of nitrogen and phosphorus with higher efficiency. In this study, firstly, we tried to optimize the whole precipitation process. Secondly we tried to produce an environment-friendly slow release fertilizer, which contains nitrogen, phosphorus and magnesium for plant growth.

**Keywords:** nitrate, phosphate, struvite

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**B-40**

Nitrogen Fixation Abilities Test for Fertilizer

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The utilization of N₂ as a source of cell nitrogen is called nitrogen fixation. The ability to fix N₂ free an organism from dependence on fixed form of nitrogen, such as ammonia or nitrate. Only certain prokaryotes can fix N₂. This experiment was conducted to search high efficiency free living nitrogen fixation bacteria from soil and to investigate effect to plant growth by the bacteria affected plant growth. Soils were collected from Munhyeong and Yeon-cheon, in South Korea. In screening test, mannitol and malic acid were used as the carbon sources. Essential elements were added to fix N₂. Isolation was conduct by dilution plate technique. Each strain was incubated for 5 days. Nitrogen fixation efficiency was measured by acetylene reduction assay (ARA). Total 102 strains were measured by ARA. And 3 bacteria (*Cedexas lapugae GTC 346⁷*, *Citrobacter youngae* CECT5335⁵ and *Enterobacter luhsvigi* EN-119⁵) were showed efficiency. *C. lapugae* GTC 346⁷, *C. youngae* CECT5335⁵ and *E. luhsvigi* EN-119⁵ have nitrogen fixation efficiency of each 100 %, 97.8 % and 83.8 %. This experiment will be conducted to search the optimum condition (O₂ concentration, temperature, pH and carbon source) of nitrogen fixation and to investigate effect to plant growth by the bacteria affected plant growth.

**Keywords:** Nitrogen Fixation, Acetylene Reduction Assay, *Cedexas lapugae* GTC 346⁷
Study of Changes in the Microbial Community Associated with Climate Change in the Laboratory Scale

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Due to global warming and weather since the average temperature has been increasing every year continuously, has had a major impact on the ecosystem. Climate change is possible to affect the frequency of occurrence of more harmful microorganisms and harmful viruses. Severe disease cases due to adverse microorganisms have been reported annually. In this study, two microcosms set up were used to confirm the change in the microbial community due to temperature changes. One microcosm fixed at 20 °C as control, another one was with increasing in the temperature 1°C per 2 month. The microbial community was analyzed through DGGE and pyrosequencing. The result of the DGGE from soil, the temperature difference increases as the banding position in the test, that a change in thickness and brightness was observed. These tendencies, as it has been seen in the water samples, in the control samples showed a 89.7 to 82.9% similarity to the reference, in the test are found to from 87.1 to 77.6% similarity, changes in the microbial community had appeared. Pyrosequencing result, in the case of the test of the soil, in the configuration ratio of test group of Acidobacteria has changed significantly from the initial 16.74% to 1.97%. And in the Thermodesulfovibrio and Bacteroidales, it tended to increase. In this data, we can assume that the microbial community receives the long-term change and that need be studied continuously to understand the effects of natural ecosystems.

Keywords: Climate change, Microbial community

Characterization of the Viable but Nonculturable State in Ralstonia solanacearum

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_Ralstonia solanacearum_ is a plant pathogenic bacterium causing lethal wilt in various plant species. Viable but nonculturable (VBNC) state of this bacterium and other Gram-negative bacteria considered to be a bacterial survival mechanism. In this study, VBNC state was induced in _R. solanacearum_ SL341 strain at low temperature using modified artificial soil microcosm (mASM). There were no distinct differences of total DNA, RNA and protein contents of SL341 strain at two different temperatures. Moreover, the VBNC cells maintained respiration activity in the mASM. Culturability of VBNC cells was recovered by supplementation of catalase. Expression of _omp, rpoS, dps, oxyR_ and the 16s rRNA gene were not different from bacterial cells in mASM at two temperatures by RT-qPCR. To get a more in-depth knowledge about the gene expression of _R. solanacearum_ in the VBNC state, total RNAs of _R. solanacearum_ derived from mASM were linearly amplified and subjected to RNAseq analysis. Transcriptome analysis by RNAseq showed that 254 genes were down-regulated at VBNC state, however, 182 genes were clearly up-regulated. Differentially expressed genes in VBNC state were functionally annotated by COG analysis, showing that genes responsible for the transport, lipid metabolism and coenzyme biosynthesis were highly expressed in VBNC state. Our result suggested that the VBNC cells in mASM induced by low temperature are viable in a physiologically and genetically unique state.

Keywords: Ralstonia solanacearum, Transcriptome, Viable but nonculturable

Rhizosphere Analysis of Field-cultivated and Mountain-cultivated Ginsengs

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Panax ginseng C.A. Meyer is classified as either field-cultivated or mountain-cultivated ginsengs and differences of cultivation environment have effect on lifespan such as lignification. In this study, we investigated the relationship between the microbial communities and environmental differences of ginseng field’s soil and determined which environmental factors might have the most influence on microbial diversity. High-resolution 16S rRNA tag pyrosequencing was used to obtain the bacterial diversity and community structure of soil around ginseng farmland, which soils were from field-cultivated ginseng (FCG) and mountain-cultivated ginseng (MCG). Moreover, we try to find some rhizosphere microorganisms in FCG and MCG’s roots. Sampling from the soil at each site revealed highly diverse bacterial communities containing up to 9982 estimated Operational Taxonomic Units (OTUs). The _Proteobacteria_ and _Acidobacteria_ were the most abundant groups among all samples. _Janthinobacterium agarricidamnosum, Phenyllobacterium immobil, Myxococcum fluorenthorivorans_ were only existed in MCG. As a part of result, different cultivation environments of ginseng contribute to the dynamic diversity of the bacterial community.

Keywords: ginseng, cultivation, metagenomics, microbial community, 16S rRNA

Optimization of the Culture Conditions of Halobacteria and Its Effect in Growing Crops in High Salinity Areas

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Ten halophilic or halotolerant bacteria were isolated from seawater or madlait soils in 5 coastal areas. All of the isolates can grow at 18% salinity but can’t in culture condition without NaCl. Solar salt was used as the NaCl source. Ingredients for culture medium preparation were monosodium glutamate, yeast extract, and solar salt. All of the halophiles or halotolerants produced compatible solutes that are ectoine and betaine. Both organic compounds metabolically synthesized in cytoplasm of the halophiles or halotolerants function to control osmotic pressure between cytoplasm and environment. In this study, 10 halophiles or halotolerants were isolated using the culture medium and analyzed production of the compatible solute as a basic study. After a basic study, cabbages and spinaches were grown for a month with a treatment divided into four groups: salt and water, water, water with bacteria, and bacteria with salt. By comparing the weight and size of the leaves, it was shown that bacteria influenced to increase plant cultivation and reduced the salinity stress in plant. The way compatible solutes work and whether real life application is possible would be further investigated.

Keywords: Halotolerant bacteria, Agriculture, Compatible Solute
Production of 2-Hydroxyalkanoates from Endolichenic Bacteria Using Xenobiotic Compounds as Sole Carbon Source
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Poly lactic acids (PLAs) (2-hydroxyalkanoic acid) are the biodegradable polymers, having diversified applications in scaffolds and pharmaceutical industries. The risk of anthropogenic pollution through xenobiotic compounds is increasing worldwide. Endolichenic bacteria can play an important role to degrade and utilize these compounds, since they are capable to survive in extreme environmental and nutritional status. Although several studies have been conducted on xenobiotic degrading bacteria/xenobiotic degradation, very few have been focused on the functional aspect of endolichenic bacteria. In the present study, bacteria had been isolated from different species of lichen thallus and GC-MS analysis had been done for further studies after staining with Nile blue A. A total of 53 isolated bacterial strains, 35 exhibit fluorescence after staining. In limited nutrient condition, 5 isolates produced 0.133, 0.171, 0.142, 0.741, and 0.153 mg of 2-hydroxydecanoate, whereas 3 of them produced 15.147, 5.888, and 4.999 mg of 2-hydroxyhexadecanoate. Most of the isolates were detected as Pseudomonas sp. by 16s rDNA sequencing. Additionally, we observed that 7 isolates of Pseudomonas sp. can utilize naphthalene effectively as a sole carbon source. Our findings suggest that endolichenic Pseudomonas sp. can effectively degrade toxic compound, naphthalene and produce eco-friendly substituted PLA monomers 2-hydroxydecanoate and 2-hydroxyhexadecanoate.

Keywords: endolichenic bacteria, 2-Hydroxydodecanoate, Pseudomonas sp., Xenobiotic compounds

Effects of Photosynthetic Bacteria on Odor Reduction and Pollutants Degradation of Swine Waste Water
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Photosynthetic bacteria have been isolated and selected for applications in the areas of improved agricultural environment because they are well degraded organic matters. To develop biodegradation agents of live-stock wastes, photosynthetic bacteria were isolated and investigated biodegradation activities. The 26 isolates of photosynthetic bacteria were isolated from soils collected form paddy field, greenhouse, riverbeds, and pond. The 9 isolates were selected by growth test in artificial waste water. The selected isolates were identified as 5 species (Rhodobacter capsulatus, Rhodobacter sphaeroides, Rhodobacter johrii, Rhodopseudomonas faecalis, and Rhodopseudomonas palustris) by 16S rDNA sequence analysis. To determine the odor causing materials and pollutants reducing activities of swine waste, each strains were inoculated in 20% of swine waste and cultured at 30°C for 25 days with continuous shaking. By treated with R. capsulatus PS-2, R. johrii PS-66, and Rhp. faecalis PS-56, ammonia and amine which are kinds of odor causing materials were 90-96% (after 25 days) and 93.8-95.8% (after 18 days) decrease, respectively. Also these 3 strains were able to reduce ammonium nitrogen 54-56%. The BOD reduction obtained with R. capsulatus PS-2, PS-6, Rhp. faecalis PS-56, and R. johrii PS-66 showed 63-69% decrease compared to the untreated control. However, reducing effects of total phosphate, total nitrogen, and nitrate nitrogen were not shown as photosynthetic bacteria treatment.

Keywords: photosynthetic bacteria, biodegradation, swine wastes

B-47
Biocontrol of Sclerotia by Plant Growth Promoting Bacillus thuringiensis C25
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Sclerotinia is a phytopathogenic genus of fungi causing sclerotia. Bacterial strains showing antifungal activities were isolated from Sclerotinia-infected field in Iksan Agriculture Research Station, Korea, which had a potential for suppressing the broad ranges of sclerotia-forming fungi. The selected bacterial strains were examined for in vitro antagonistic effects against soilborne plant pathogenic fungi such as Sclerotinia minor, Sclerotinia sclerotiorum, Sclerotinia rolfsii and Athelia rolfsii. Among isolates, strain C25 identified as Bacillus thuringiensis effectively suppressed the mycelial growth of the S. minor and S. sclerotiorum. The strain C25 exhibited activities for cell wall degrading enzymes such as chitinase and 1,3-β-glucanase. In addition, in vivo studies revealed that strain C25 was effective in suppressing the incidence of sclerotic disease on tomato.

Keywords: Sclerotinia, Biocontrol, Bacillus thuringiensis

Arbuscular Mycorrhizal Fungi Diversity in Post-mining Area and Natural Forest Area in Je-cheon, Korea
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Arbuscular Mycorrhizal Fungi (AMF) are one of the most important symbiont in ecosystem. AMF provide better nutrient absorptivity to host plant, enhance tolerance against plant root pathogen, and also improve resistance against soil contamination by heavy metal. Therefore, AMF are very important in harsh environments such as mine. In this study, we compared the difference of AM fungal diversity in post-mining area and natural forest area in Je-cheon, Changcheong-bu-do, Korea. We sampled 10 abandoned post-mining area soil samples, and 10 natural forest area soil samples with host plants. We extracted AMF spores in field soils, and identified spores using morphological and molecular analysis. As a result, we discovered 7 species from 4 genera. Acaulospora was the most abundant genus in both areas, however, Ambispora leptoticha was the most dominant species in natural forest area while Acaulospora mellea was the most dominant species in post-mining area. These results showed that the AM fungal diversity of mine area soil is different to diversity of natural forest soil.

Keywords: Arbuscular Mycorrhizal Fungi, Diversity, Post-mining Area, Symbiotic
Diversity of Endophytic Fungi Isolated from Leaves of *Camellia japonica* in Jeju, Korea

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Endophytic fungi are non-pathogenic microorganisms, they can have positive effect upon the host plant such as promoting growth, increasing resistance against environmental stress. *Camellia japonica* is distributed over the southern areas in Korea, and an evergreen broad-leaved tree. But studies on the endophytic fungi of *Camellia japonica* are unusually performed. This study was conducted to identify diversity of endophytic fungi which lives symbiotically in leaves of *Camellia japonica* in Jeju, Korea. A total 70 fungal strains were isolated from surface-sterilized leaves of 12 host plants. They identified using morphological characteristics of the fungal hyphae and growth in culture. Genomic DNA was extracted from the isolated fungal strains and the internal transcribed spacer (ITS) region was amplified using the ITS1-OF and ITS4-OF primer pair. The 17 genera and 23 species were identified, genera Nemania was discovered most frequently.

Keywords: Endophytic fungi, Diversity, *Camellia japonica*

Enrichment and Isolation of Microorganisms Degrading Triazole Fungicides from Agricultural Soils

Jae-Hyung Ahn, Gwan-Hyeong Lee, Ji-Young Kim, Yu-Mi Ro, Jaeong Yoo and Incheol Park
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Triazole fungicides, which contain an 1,2,4-triazole ring, are widely used in Korea and some are known to be persistent in soil. They inhibit biosynthesis of sterols, which are important components of fungal cell membranes. In this study, the isolation of microorganisms degrading four triazole fungicides, tebuconazole, tricyclazole, fluquinconazole, and difenoconazole, was tried using agricultural soils collected throughout the country. Soil samples were treated with a technical product of each of the fungicides dissolved in acetone and incubated for two months. 1 g of the incubated soil was transferred to three types of media containing the corresponding fungicides: 1) minimum medium + a technical product of the fungicide 2) minimum medium + commercial product of the fungicide, which contains surfactants and preservatives besides the fungicide, and 3) R2A broth + a technical product of the fungicide. After two months later, 10% of the culture medium was transferred to the same medium and incubated for one month. Among the four fungicides tested, only difenoconazole showed a significant decrease in concentration in all three types of the media in the last incubation. This result reflects the reported half-lives of the fungicides in soil: tebuconazole (597 days), tricyclazole (450 days), fluquinconazole (350 days) and difenoconazole (318 days) ([www.pesticideinfo.org](http://www.pesticideinfo.org)). We isolated four difenoconazole-degrading bacterial strains and identified them.

Keywords: Triazole fungicide, Biodegradation, Soil, Bacteria

The Transmembrane Hybrid Histidine Kinase BmsA Acts Independently of the Gac Regulon But Both Are Required for Surface Spreading and Biofilm Formation in *Pseudomonas alkylphenolica*

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Bacteria have motile or sessile lifestyle depending on environment and cellular stimuli. The sensory systems such as GacS, LadS and RetS have been known to be involved in the lifestyle in *Pseudomonas*, notably in *P. aeruginosa*. *P. alkylphenolica* KL28 can exhibit diverse multicellular behaviors such as aerial structures with p-cresol vapor, circular pellicles on the surface of LB liquid medium and spreading on the semi-solidified LB agar medium. This strain can also show swimming motility in liquid medium. During mutagenesis study it has been found that when a gene (named *bmsA*) encoding hybrid histidine kinase (PSAKL28_21690 containing CHASE3, GAF, histidine kinase and three CheY-like response regulator domains was mutated, spreading motility and biofilm formation were defective but the swimming activity was increased. Deduced amino acid sequence showed that it contains two transmembrane domains at the N-terminal flanking the CHASE3 domain. The gene is co-transcribed with genes encoding proteins with CheR and CheB domains and a histidine kinase. Deletion of these genes did not show apparent changes in surface-related phenotypes. Complementations of *gacA* to *bmsA* mutant and *bmsA* to *gacA* or *gacS* mutant did not restore the wild phenotype. This result showed that the two regulons are independent but both are required for surface-related behaviors.

Keywords: *Pseudomonas*, Biofilm, Motility, Histidine Kinase, Signal transduction

Anaerobic-Anoxic-Oxic (A2/O) Process by Nitrogen and Phosphorus Removal Bacteria with Sediment for Protect Red Tides

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The purpose of this study is preventing Red tides bloom (HAB) through the biological treatment of wastewater generated from fish farm. The Nitrogen and Phosphorus in the seawater is the major nutrient of harmful algal bloom (HABs). This study was carried out to investigate the removal of nitrogen and phosphorus in seawater depending on existence of Eco-friendly carrier as biological coated sediment with nutrient high removal efficiency bacteria in Anaerobic-Anoxic-Oxic (A2/O) reactor. In the Batch test, Nitrogen and Phosphorus removal efficiency have more than 95% on the optimum condition. A2/O reactor is a specific process to Nitrogen and Phosphorus removal. A2/O reactor is composed three tanks of anaerobic, anoxic, andoxic. By repeating the Anaerobic-Aerobic condition, phosphorus released at anaerobic tank. Nitrification and phosphorus uptake occurred atoxic tank. Denitrifications take place inanoxic tank by internal recycle. The models were used to investigate variations of HRT, CNP ratio and F/M ratio on nutrients removal and microbial activity. In the removal COD, T-N (NH3-N, NOx-N), T-P, the best efficiency of the process showed in the condition, 1Q external recycle rate and 1:0.95:1.4 of the volumetric ration in Anaerobic-Anoxic-Oxic process. Ammonia and Phosphorus was removed 99.9%, 70% at optimal condition. In this study, A2/O process is suitable for using biological treatment of seawater and we expect to prevention of harmful algal blooms.

Keywords: Red tides bloom (HAB), biological treatment, A2/O reactor
**B-53**

**Newly Isolated Bacteriophages Targeting Pectobacterium carotovorum subsp. carotovorum to Control Soft-Rot Disease**

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Pectobacterium carotovorum subsp. carotovorum (formerly Erwinia carotovora subsp. carotovora) is a plant pathogen, which causes the soft rot and stem rot diseases in several crops, including Chinese cabbage, potato, and tomato. Here, we collected soil samples from various areas (where Chinese cabbage was being grown) to control for P. carotovorum subsp. carotovorum. Five bacteriophages which can make clear and large plaques were isolated and their antibacterial activities were confirmed. The phage morphologies were observed under the electron microscope, and were found to be tailed and placed in the order Caudovirales. Belonging to Siphoviridae were two phages, one phage was classified to Siphoviridae and the other two phages were classified to Podoviridae. All of the phages showed rapid and strong lytic activity against their host bacteria in liquid medium. The bacteriophages showed high specificity for P. carotovorum subsp. carotovorum, and bacteria belonging to different species of the phylum were resistant to these phages. Phage receptor candidates were analyzed by confirming adsorption ability of each phage to phage resistant Pectobacterium mutant. All phages were sequenced and the genome sizes of each phage were analyzed. Open reading frames and their functions were predicted. All of the phages contain no integrase- or repressor-coding genes related to the lysogenic cycle, and lifestyle prediction using PHACT software suggested that they are virulent bacteriophages.

Keywords: bacteriophage, Pectobacterium, biocontrol

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**B-54**

**Plant Growth Promotion and Bioactive Compounds Production by Endophyte Isolated from Halophyte in Coastal Wetland**

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Suaeda maritima Dumortier was collected from the coastal wetland in west coast. Fifteen endophyte fungi with different colony morphologies were isolated from the roots of S. maritima. The culture filtrate of SM105 strain promoted the growth of the waturc-c rice seedlings. The culture filtrate of the SM105 strain was revealed as containing gibberellins by using HPLC and GC/MS with selected ion monitoring. This strain was identified as novel Penicillium cf. spinulosum that producing new GAs with microscopic observation and further molecular analysis with beta-tubulin gene sequence. These results indicated that P. cf. spinulosum SM105 improves the growth of plants and produces various gibberellins (GAs: GA1, GA3, and GA4 and GA7), and may participate in the growth of plants under diverse environmental conditions. In the biochemical analysis revealed that amino acids were higher in P. cf. spinulosum SM105 treated coastal plants, when compared to their control. The result of present study suggests that P. cf. spinulosum SM105 treatment could be act as biofertilizer to improve the plant growth.

Keywords: bioactive compound, coastal plant, endophyte, plant growth promotion

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**B-55**

**Investigation on Removal Effect of Attached Algae in Spillway of Sedimentation Basin Depending on Materials**

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Attached algae, moss and microorganism are major causes of bad influence on coagulation and precipitation process in water treatment plant. We tried to study removal effect of attached algae depending on various materials in sedimentation basin. The spillways and walls of sedimentation basin used to epoxy-coated cement in Korea. Recently, the material of stainless steel, various matrix by coating on stainless steel has been published for preventing attached algae. We were compared to biomass of algae and bacteria on three materials of sedimentation basin. The biomass of attached algae species in ceramic coated stainless steel was reduced the most than stainless steel and epoxy-coated cement. When water temperature began to rise, filamentous cyanobacteria grew up in stainless steel and epoxy-coated cement by dominant species. Phormidium sp. and Beggiatoa sp. in kinds of filamentous cyanobacteria, were covered the sedimentation basin during June. Attached algae species increased significantly after July, distributed similarly with algae species in Nakdong river. Ceramic coated and stainless steel did not show any significant differences in distribution of bacteria biomass. In side of cleaning time and visual appearance for the efficiency elevation of sedimentation basin, ceramic coated material was the most useful among the other.

Keywords: sedimentation basin, epoxy-coated cement, stainless steel, ceramic coated stainless, Phormidium and Beggiatoa

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**B-56**

**Inhibition of Norovirus Replication by Plant Extracts in Replicon-Bearing Cell**

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Human noroviruses are recognized as a major cause of nonbacterial food-borne illness worldwide. We investigated anti-noroviral effects by plant extracts, which are regarded as safe and inexpensive control measures. Here we investigated the inhibitory effect of 31 plant extracts on human norovirus replication through a Norwalk virus-replicon bearing cell model(HG23). After cytotoxicity test of respective plant extract on HG23 cells, 20µg/ml was chosen as a screening concentration. Plant extract-treated HG23 cells were incubated for 72hr at 37°C and changes in noroviral replication was quantified by qRT-PCR normalized with beta-actin expression levels. Ginger extract significantly decreased nearly 80% of noroviral RNA levels in HG23 cells with 33% of control group. Lemon and jujube extract also reduced viral RNA by 22.3% and 20.9%, respectively. Eucalyptus and horseradish extract showed inhibitory effect on noroviral replication less than 20%. Previous studies to assess anti-noroviral activities of plant extracts were mostly based on results from surrogate systems such as murine norovirus and feline caliciviruses. This is the first report to investigate anti-noroviral effect of various plant extracts by using a human norovirus-replicon bearing cell system and the findings of this study could be applied to develop potent and safe antivirals with no harmful by-product.

Keywords:
Screening of 18 Phytochemicals with Anti-Noroviral Effects in Replicon-Bearing Cells
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Noroviruses are widely known as a major cause of acute gastroenteritis worldwide. We investigated anti-noroviral effects by various phytochemicals. Phytochemicals have received attention as potential norovirus inhibitors because of their low toxicity and no side effect. The efficacies of phytochemicals were evaluated in human Norwalk-virus replicon bearing cells (HG23). The cytotoxicity of 18 phytochemicals was assessed and treated with a concentration of 20µg/ml in HG23 cells. After incubation for 72 hr at 37°C, replicated noroviral RNA levels were quantified by qRT PCR with normalization by beta-actin levels. We found that 10-gingerol reduced a replicon RNA level with 40%, and capsaicin and caffeic acid also inhibited replicon RNA levels with 22% and 17%, respectively. Interestingly, ginsenoside Rg3 increased a replicon RNA level with 72% and other ginsenosides we tested also showed a tendency to increase RNA levels (Rh2, Rg3, F2, Rd, Rb1). This study firstly reported the effect of phytochemicals in human norovirus-replicon bearing cells and further studies would be necessary to confirm the molecular mechanisms of different effective compounds against replication of human noroviruses.

Keywords: noroviruses, mechanisms of different effective compounds against replication of human norovirus inhibitors.

Improved PCR Assay for the Species-Specific Identification and Quantitation of Legionella pneumophila
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Legionellosis outbreak is a major global health care problem. In current, over 90% of legionellosis cases are caused by infection of L. pneumophila. Until now, many studies have been performed to develop sensitive and specific technologies to improve the bacterial pathogen detection and ensure successful control measures. However, current Legionella risk assessments may be compromised by uncertainties in Legionella detection methods, infectious dose, and strain infectivity. These limitations may place public health at significant risk, leading to significant monetary losses in health care. Therefore, in the present study, a primer set was designed based on a LysR-type transcriptional regulator (LTTR) family protein gene of L. pneumophila subsp. pneumophila str. Philadelphia 1 because it was found that this gene is structurally diverse among species through BLAST searches. The specificity of the primer set was evaluated using genomic DNA from 6 strains of L. pneumophila, 5 type strains of other related Legionella species, and other 29 reference pathogenic bacteria. The primer set used in the PCR assay amplified a 264-bp product for only targeted six strains of L. pneumophila. The assay was also able to detect at least 1.39 × 10^3 copies/μl of cloned amplified target DNA using purified DNA, or 7.4 ×10^0 colony-forming unit per reaction when using calibrated cell suspension.

Keywords: Legionella pneumophila, Detection, Real-time PCR
**B-61**

Biodegradation of Octadecane, Eicosane and Docosane Hydrocarbons by Different Strains Isolated from the Oil-Contaminated Soil of South Korea

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Hydrocarbons (alkanes: C_{18}-C_{30}) are hazardous to the plants and are also carcinogenic, mutagenic & potent immune-toxins to human and animal health. Biodegradation of hydrocarbons by natural population of microorganisms allows for the conversion of hazardous substances into less or non-toxic & represent one of the primary mechanisms by which petroleum & diesel products are removed from the environment inexpensively. In this study, 27 strains were isolated using modified transwell plate culture technique. Among which 4 (14.8%) isolates were considered to be probable new species based on 16S rRNA sequencing. Similarly, out of 27 strains isolated, 6 strains (22.2%) belongs to Rhodococcus, 3 (11.1%) equally belongs to Acinetobacter and Pseudomonas, & 2 (7.4%) equally belongs to Chrysobacterium and Enterobacter genus. The hydrocarbon degradation efficiencies of these isolates were tested using mineral salt media (MSM) containing 900 ppm of hydrocarbons (300 ppm each for octadecane, eicosane & docosane) as a sole source of carbon and the degradation rates ranges from 5% by strain D62 to 85% by strain K6 based on gas chromatography analysis. These finding suggested that these isolates may be considered as higher alkanes (hydrocarbons) tolerant and some of the selected strains could be potent higher hydrocarbons degrader.

Keywords: Biodegradation, Isolation, Hydrocarbons, Transwell plate, Oil-degrading bacteria

**B-62**

Comparison in Structures of Microbial Communities between Mangrove Rhizosphere and Intestine of Mangrove Crabs on a Coral Beach

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Mangrove forests on coral-derived sands are an example of tightly-regulated self-sufficient ecosystem. While the predominant flora being mangroves, the predominant fauna in the ecosystem are mangrove crabs. In this study, we estimated the extent of interactions between crab microbe and mangrove rhizosphere by analyzing the bacterial community shared by the two habitats. Intestinal contents of crabs in mangrove forest in Olango Island, Philippines were inoculated into Tryptic Soy Agar medium, with pH level 10 at 37°C. The high pH medium was used to narrow down the spectrum of bacteria for comparison. Isolates were analyzed for genetic diversity using GTGS fingerprinting technique and then identified based on their 16S rDNA gene sequences. Comparative analysis showed that the alkali-tolerant bacterial community in the crab’s gut was not significantly different from the bacteria community found in the mangrove rhizosphere. Bacteria belonging to genera Bacillus predominated in both crab intestine and mangrove rhizosphere. Although B. flexus was found most in crab intestine with 60% dominance, B. horikoshii constituted 44% of rhizosphere community and 29% of intestinal community. The results of this study imply that diatom grazing activity of crabs have a great influence on microbial community structure on coral sand rhizosphere.

Keywords: Microbial Community, Mangrove Rhizosphere, Crab, Coral Beach, Microbiome

**B-63**

Control of Harmful Algal Bloom by Using a Bio-Ceramic Biofilm System

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Harmful algal blooms (HABs) found naturally in surface waters have caused many environmental problems worldwide. One of the most potential techniques is by using algicidal bacteria as a biological control agent for controlling the negative impacts of red tides without any negative effects on other organisms. The main aim of this study was to develop the possible use of immobilized algicidal bacteria as an alternative technique for controlling phytoplankton blooms. In this study, eight bacterial strains designated as the most effective strains were isolated from Misun Bay, Jinhuae Bay, Dol Island, the Guduri Sea, and the Tongyeong Sea in Korea and then were screened furthermore for the characteristics on algicidal activities against Cochlodinium polykrikoides, Chattonella marina, Skeletonema costatum, Heterosigma akashiwo, Heterocapsa triqueta, Prorocentrum minimum, and Scrippsiella trochoidea. Compared to freshly suspended cells, the use of immobilization technique in the bio-ceramics biofilm system could enhance the algicidal activity up to about 90% at the flow rate of 28.6 mL algal culture per min, becoming more effective and faster in killing algal cells than batch culture system. Thus, these results indicate that the system developed in this study might be useful to control harmful red tides in a large scale in ocean.

Keywords: Harmful algal blooms, algicidal bacteria, Cochlodinium polykrikoides, bio-ceramics biofilm system

**B-64**

Prediction of Potential Invasion of Crops Fungal Pathogen Species in Tropical and Subtropical Regions Due to Climate Change

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Invasion of exotic pests due to climate change can cause serious problems on food security because a variety of crop diseases are spread via insect vectors. Monitoring of invasion of exotic pathogen via insect migration requires an inventory of potential pathogens. In this study, we surveyed fungal species that accompany thrrips in tropical and subtropical regions. We collected thrrips in evening primrose, bean and chili pepper plants in Korea, Vietnam, Taiwan and Indonesia. By using pyrosequencing of the inter-transcribed sequence regions of RNAs, phylogenetic contents of fungal microbe in the insects were identified. From the total of 346,586 reads, 56-661 OTUs per sample were found. The OTUs detected in thrrips from Vietnam, Taiwan and Indonesia but absent in thrrips from Korea comprised 74%, 54%, and 69% of the total OTUs, respectively. Dominant fungal species were Ustilago maydis, a maize smut pathogen, Cercospora vignigena, a leaf spot pathogen, and Ustilago sparsa, a wheat smut pathogen, Trichaptum abietinum, a sap rot pathogen, Pseudoxynia rugulosa, a powdery mildew pathogen, Magnaporthe oryzae, a rice blast pathogen, and Stemphylium, a leaf spot pathogen. Although some of these fungal species are already reported to exist in Korea, the exotic strains might carry novel pathogenicity. Considering the trends of climate change, this finding warrants that a close monitoring of insect-commensal pathogens is crucial for prevention crop disease outbreaks by novel pathogens.

Keywords: Fungal Pathogen, Tropical Regions, Climate Change, Thrrips, Network Analysis
Bacterial Diversity in the Tomato Root Environment: a Culture-Dependent Approach Using Various Media and Antibiotics

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Plants are colonized by a variety of microbes both inside and outside of their roots designated as endosphere and rhizosphere, respectively. These microbes are related to plant growth and development. However, the majority of the microbes in the root regions are still unculturable owing to lack of efficient cultivation techniques. In this study, we have carried out culture-dependent methods for tomato plants collected from 2 geographical locations by using a combination of 2 media and 12 antibiotics to recover various, rare and uncultured bacteria. As a result, we isolated 864 bacterial strains, assigning to 241 bacterial species from 5 phyla and 93 genera by 16S rRNA gene sequencing in the tomato root environment. Alphaproteobacteria (28.3%), Gammaproteobacteria (23.7%) and Bacteroidetes (18.2%) were dominant bacterial phyla/class. Among them, 115 strains (13.3%) have been considered as possible novel species. They are significant fractions of microbial community.

We suggest that a combination of present techniques and novel culture-dependent approaches will improve the cultivation of hidden significant fractions of microbial community.

Keywords: bacteria, tomato root, diversity, cultivation

Diversity and Community Structure of Soil Bacteria Across Four Land-Use Types in Korea

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Soil bacteria play an essential role in functioning of soil ecosystem and maintaining various environmental factors. However, little is known about the responses of soil bacterial communities to land use and soil properties at large spatial extents. A total of 856 agricultural soil samples were collected from 4 different land-use types (paddy, greenhouse, upland soil, and orchard soils) across Korea and subjected to pyrosequencing analysis. The predominant phyla of soil bacteria across land-use types (>5% of all sequences) were Proteobacteria (33.7-38.1%), Acidobacteria (7.9-20.4%), Actinobacteria (8.7-15.7%), Chloroflexi (5.0-12.2%), Firmicutes (2.4-9.3%), Bacteroidetes (3.7-7.0%) and Gemmatimonadetes (1.2-3.3%). The greenhouse soils harbored 19,379 and 19,485 putative bacterial species based on Chao1 and Ace richness estimators, respectively, which is the highest among 4 land-use types. Simpson and Shannon indices were higher in upland and orchard soils compared with the other agricultural soils. In addition, paddy soils had very different microbial communities compared with soils of dryer land-use types. Multivariate analysis showed that physical property had little effect on bacterial communities of 4 agricultural soils compared with chemical properties. This study provides the first extensive map of microbial communities in agricultural soils and reveals distinct microbial communities were associated with land-use types.

Keywords: agricultural soil, bacterial community, biogeography, diversity

Anaerobic Crude Oil Degradation in Oil-Polluted Subtidal Sediment

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Coastal area is frequently exposed with oil spills or organic pollutants due to accidents or inadequate practices. On end of January, 2014, an oil tanker collided with bridge containing oil pipelines causing at least 164,000 liter of crude oil to spill into the water near the shores of Yeosu, Korea. Three month after clean-up, still there was remained hydrocarbons. To assess the response of microbial community to anaerobic crude oil degradation, microcosms were established with crude oil impacted tidal sediment under sulfate-reducing condition and methanogenic condition. linear-alkanes (nC8-nC32) were degraded up to 70% over a 180 day period in oil-amended microcosms, in contrast alkane degradation was minimal in microcosms which were inhibited with sodium molybdate. However, Under methanogenic condition, linear alkanes were degraded over 50%. Bacteria from the phyla Proteobacteria (Epsilon-, Delta-classes) and Clostridiales were enriched in oil-degrading microcosms relative to control microcosms. The addition of furmarate in the microcosms, acetA (allylsuccinate synthase), dsrA (dissimilarity sulfite), drrA (dissimilarity sulfite), 16S rRNA gene was increased but mcrA gene was slightly increased compared with other treatment. These data suggest that other groups of organisms in addition to conventional sulphate-reducing microorganisms play a role in the anaerobic degradation of crude oil in some sulphate-containing environment.

Keywords: anaerobic, crude oil, sulfate-reducing bacteria, methanogen

Selective Flushing of Pyrosequencing Reads of 16S rRNA Genes Predominant in Soil by CaO Treatment

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In metagenomic analysis, it is difficult to detect DNAs of rare species due to high-copy DNAs from genomes of predominant species. CaO can destroy cells in general. Therefore, we hypothesized that relative abundance of species in soil may be changed by destroying cells of predominant species in a great number. In the case, we can expect chances of detecting DNA of rare species to increase. We applied CaO on a forest soil and compared relative frequencies of bacterial species based on 16S rRNA genes sequence amplified from metagenomes of CaO-treated and native soil samples by pyrosequencing. Differences in distributions of operational taxonomic units (OTU) by CaO-treatment was analyzed. In comparison of numbers of OTUs and diversity indexes, diversity of bacterial species increased from 0%-CaO(w/w) to 6%-CaO samples. In addition, community structures of samples treated with CaO were similar while that of the native sample showed a low similarity to those structures. In this result, relative abundance of predominant species reduced more than those of rare species by treatment with CaO, which resulted improved sensitivity of detecting genes of rare species. In conclusion, it could be suggested that vegetative-state cells of predominant species are more sensitive to CaO than dormancy cells of rare species.

Keywords: Metagenomic Analysis, Pyrosequencing, CaO Treatment, Soil
In our previous study, we suggested that Lkh1, LAMMER kinase homolog 1, controls the cell size and G1/S cell-cycle progression by phosphorylating Thr110 of Rum1, which acts as an inhibitor of the cyclin-dependent kinase Cdc2. Through the in vivo and in vitro experiments with wild type and T110A mutant form of Rum1, we inferred multiple phosphorylation of Rum1 by Lkh1. Analysis with NetPhosK 1.0 had revealed several putative Lkh1-dependent phosphorylation residues on Rum1, thus we constructed phosho-defective mutants of the putative Lkh1-dependent phosphorylation sites and tested their effects on phenotypic changes. Experiments such as observing cell morphology, in vitro pull down assay and DNA profiling using FACs showed that the amino acid residues, Thr5, Thr16 and Ser212, were not related to Lkh1 phosphorylation site on Rum1 unlike Thr110. Peptide mass fingerprinting using Rum1T110A and Rum1T110E revealed that S129 was another Lkh1-dependent phosphorylation site on Rum1. The effect of single and double mutation on Rum1 function will be discussed.

Keywords: fission yeast, cell cycle, LAMMER kinase, CDK inhibitor, G1/S transition

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**C-2**

**Analysis of a Laccase Promoter Expressed at Low pH in Coprinellus congregates: Induction or Repression?**

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Coprinellus congregates, an inky cap, synthesizes and secretes laccase at acidic pH (4.0-4.5) transiently only in the dikaryotic mycelium. The vector pARGEMG7-1 inserted with green fluorescent protein (GFP) reporter gene regulated by different lengths of the laccase promoter obtained from 5'-sequential deletion constructs was used to determine the dikaryotic-responsive and acid-stress responsive DNA elements. These expression vectors were introduced to C. congregatus monokaryons (α1 and α2) by restriction enzyme-mediated integration, and the transformants were used to generate dikaryons. These transformants were grown in neutral pH liquid media, and then transferred to acidic media to examine the GFP expression by ConfoMicroscope. The heterozygotic transformants which had full-length and shortest-length-promoter both showed GFP expression. We will analyze the other length of promoter, and also construct shorter expression vector to determine the locations of responsive elements. These results will confirm the sites of the DNA elements which respond to the dikaryotic signal and acidic-stress signal in the laccase promoter.

Keywords: Coprinellus congregates, Acid stress, Laccase

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**C-4**

**Establishment of the Protocol for Recombinant PrP Mass Production**


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Recombinant prion protein (PrP) is an extremely useful resource to study misfolding processes and subsequent protein aggregation. Here, we report the cloning of full length and truncated human and murine PrP genes and the mass production protocol of recombinant murine PrP. We cloned PrP genes into pET100/D-TOPO vector for protein expression. For murine recombinant PrPs, 1mM IPTG was optimal to induce gene expression. The optimal induction temperature for truncated murine recombinant PrP was 30°C, while murine full length PrP was expressed best at 37°C. Human truncated PrP was normally expressed at 1mM IPTG and 30°C, but the higher concentration IPTG (5mM) was required for expression of human full length PrP. For mass production of murine recombinant PrP, the transformed cells were cultured by the high cell density fermentation method with 1mM IPTG. The collected inclusion body from bacteri lysis was washed, and solubilized. PrPs were refolded with refolding buffer containing β-mercaptoethanol at 4°C for 24 h. Subsequently, PrPs were purified in order by affinity chromatography using HIPEP FF resin, cation-exchange chromatography using SP sepharose fast flow resin, and reversed-phase chromatography using C8 prep HT resin. The purified proteins via such procedures were in α-helix-rich conformation in measurement by circular dichroism spectrometry. Compared to the conventional method, recombinant PrP of high purity was obtained at least 10-fold more by this procedures.

Keywords: Prion, Recombinant Protein, Purification, His tag, Mass production
Inhibition of the SenX3-RegX3 Two-Component System by polB
Overexpression in Mycobacterium smegmatis
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Recent studies have revealed the convergent signaling pathways between two major signal transduction systems, Ser/Thr protein kinases (STPKs) and two-component systems, in mycobacteria. The SenX3-RegX3 two-component system of mycobacteria is involved in the response of mycobacteria to phosphate-deficient conditions. PknB, a STPK of MTB, negatively affected the transcriptional activity of the RegX3 response regulator. When polB was overexpressed in Mycobacterium smegmatis, expression of polA, which is regulated by RegX3, was approximately 10-fold decreased. Yeast two-hybrid assay showed specific interactions of RegX3 with PolB. Purified PknB strongly phosphorylated RegX3, NarL, KdpE, Trcr, DosR and MtrA response regulators. Site-directed mutagenesis and in vitro phosphorylation assay revealed that Thr191 and Thr217 in RegX3 were phosphorylated by PknB. Overexpression of STPK genes in M. smegmatis revealed that several other STPKs also negatively affected the transcriptional activity of RegX3, suggesting that wide signaling convergence might take place between the SenX3-RegX3 two-component system and STPKs in mycobacterial signal transduction pathways.

Keywords: Gene expression, mycobacteria, Ser-Thr protein kinase, signal transduction, two-component system

Protein Secretion by Asexual Spores of Aspergillus nidulans in the Presence of Water before Isotropic Growth
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Germination of spores of filamentous fungi such as Aspergillus nidulans and Botrytis cinerea was stimulated in the presence of water and nutrients. Without nutrients such as sugar or amino acid, spore germination was not initiated rapidly within 24 hrs. Even isotropic growth of spore was not observed in the absence of sugars for 24hrs, therefore there would be sensory machinery for nutrients in the surface of spores. In this study, proteins were secreted from ungerminated spores before isotropic growth was observed in the presence of water without nutrients. When the spores were incubated at 25°C for 2 hrs, more than 15 protein bands were separated on the 12% SDG gel electrophoresis. The molecular weights of proteins ranged from 30 to 100 kD judging from gel electrophoresis. Protein secretion was maintained for more than 4hrs as examined. In this study, we will discuss the identified proteins and their function in the aspect of spore germination.

Keywords: Germination

Transcriptional Factors Regulating the ahpC Gene Encoding Alkyl Hydroperoxide Reductase in Mycobacterium smegmatis
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Mycobacterium smegmatis has three homologs of ferric-uptake regulator A (FurA). They utilize iron as a cofactor for specific DNA binding. Through in silico analysis, an inverted repeat for FurA binding was found at +16 position relative to the transcription start site +1 of ahpC encoding alkyl hydroperoxide reductase. Comparison of ahpC expression in the wild-type and FurA combinatorial mutant strains and measurement of the promoter activity using ahpC-lacZ transcriptional fusions revealed that three FurA homologs function as negative transcriptional regulators for ahpC expression. The result of zone inhibition assay with a FurA triple mutant supported that FurA is responsible for the repression of oxidative stress-responsive genes including catalase-peroxidase genes (katG1 and katG2) as well as ahpC. Putative FurA-binding sites are located upstream of each furA. In the case of furG2, only right half of the FurA-binding site is present. Analysis of expression patterns of the furA genes in the wild-type and combinatorial FurA mutant strains indicated that expression of furA1 and furA3 are regulated by all FurA, and that of furA2 is controlled by only FurA2. The quaternary structures of FurAs in the presence of 1 mM DTT were determined using gel filtration chromatography. Purified FurA2 had a quaternary structure of a monomer whereas FurA1 and FurA3 were present as mixtures of monomer and homodimer, implying that FurA1 and FurA3 occur as homodimers under cellular conditions.

Keywords: Alkyl hydroperoxide reductase, Ferric-uptake regulator, Gene expression, Mycobacteria, Oxidative stress response

Different Role of N-terminal Domain and Middle-Domain on Chaperone Activity and Hexamer Stability of ClpL
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ClpL (Hsp100 family) of Streptococcus pneumoniae is one of the important chaperones that is involved in survival at harsh environmental stress conditions. In the other Hsp100 members, M-domain is known to function in cochaperone interaction, disaggregation, and controlling the Hsp100 activity and N-terminal domain is responsible for binding to aggregated protein. However, the roles of M-domain and N-terminal domain in pneumococcal Hsp100, particularly in ClpL, remain unknown. In this study, ClpL, M-domain and N-terminal domain deletion mutants were generated to elucidate the role of these domains in chaperone functions. Our result showed that M-domain deletion mutant still retained ATPase, foldase, holdase, disaggregation activity compared to the ClpL, while N-terminal domain mutant did not. Interestingly, M-domain deletion decreased hexamer stability in vitro and in vivo, while N-terminal domain deletion significantly inhibited the ATPase and chaperone activity. In conclusion, M-domain of the ClpL seems to be dispensable for the chaperone activity while N-terminal domain play a key role in regulation of the chaperone activity.

Keywords: ClpL, HSP 100 family, Streptococcus pneumoniae, Chaperone
C-9
Comparison of Wood Decomposition Abilities (Quercus spp.) among Lentinula edodes (Shiitake) Cultivars
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This study was conducted to investigate the effects of wood species on the productivity (fruitbody formation) among different Lentinula edodes cultivars and to give basic information of suitable tree species for each cultivar. As the first step, we conducted wood decay test to understand the decomposition abilities of Lentinula edodes cultivars on the Quercus spp. Eight cultivars of Lentinula edodes representing different fruiting temperature types (high-temperature, mid-temperature, and low-temperature) and 6 tree species Quercus spp. Q. Mongolica, Q. aliena, Q. variabilis, Q. serrata and Q. acutissima were used for this study. The results are summarized as follows:1. Overall, it showed the highest decomposition rates in Q. serrata2. After 30 days, of cultivation, the decomposition rate was the highest in Q. serrata, followed by Q. variabilis and Q. aliena. In terms of cultivars, Poongnyunko showed the highest decomposition rate with 3. After 60 days, of incubation, the decomposition rate was the highest from SANJO701HO followed by Baekhwahyang and 357.4. There was no significant difference among the fruiting temperature types.

Keywords: Decomposition, Cultivars, Lentinula edodes, Quercus spp., Wood

C-10
Genomic Mutations Shortened the Lag Phase and Accelerated the Growth Rate of Escherichia coli BL21 (DE3) in Succinate Minimal Medium
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As programmed in the genome, bacterial cells can regulate genes and grow optimally under various environmental conditions. It has been known widely that E. coli laboratory strains can grow in various defined culture media such as M9 minimal medium via potent anabolic pathways encoded by the genome. In M9 minimal medium containing succinate as the sole carbon source, E. coli BL21(DE3) grew very slowly unlike E. coli BW25113, and the length of lag phase of BL21(DE3) strain is much longer than that of BW25113. After one round of flask culture, we isolated large colonies in succinate minimal broth. Next, genome sequencing characterized that such adaptations result from single genomic mutations in the regulatory region of a gene encoding a dicarboxylic acid transporter. Seed dilution experiments showed those adaptive mutations occurred during the flask culture containing succinate minimal medium. Taken together, microbial cellular systems can be reprogrammed by adaptation via genomic mutations to change metabolic or regulatory network for optimal cell growth, when appropriate methods coping with certain environmental conditions are not available in the genome. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning[NRF-2015R1A2A2010054042]]

Keywords: BL21(DE3), Succinate minimal medium, adaptive mutations, lag phase

C-11
Anti-Bacterial Abilities of Essential Oil on Pathogenic Bacteria
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Mokwon university

The purpose of this experiment is to find out the best suitable Essential Oil which contains anti-bacterial abilities on pathogenic bacteria that you can encounter common in everyday life such as Escherichia coli, Staphylococcus aureus and Streptococcus mutans. To investigate anti-bacterial ability of essential oils on each bacterium, it was measured the size of clear zone. The results are like following: 1. Cinnamom, Basil and Spearmint had anti-bacterial ability on Escherichia coli by dosedependent manner. Basil had the highest anti-bacterial ability against Escherichia coli. 2. Basil, Rosemary, Tea tree and Lemongrass had anti-bacterial ability on Staphylococcus aureus. Lemongrass had the highest anti-bacterial ability against Staphylococcus aureus. 3. Spearmint, Tea tree, Cinnamon leaf and Eucalyptus had anti-bacterial ability on Streptococcus mutans. In conclusion, Basil will be able to prevent diarhea, Lemongrass will help to prevent from acne and Tea tree will take part in protecting our teeth.

Keywords: Essential oil, Pathogenic bacteria

C-12
Morphological Characteristics of Sarcodon aspratus Mycelial Colonies Grown on Different Culture Media
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Sarcodon aspratus is one of the most commercially important edible mushrooms with a value of artificial cultivation in Korea. However it is not easy to cultivate because the mushroom forms ectomycorrhizas with oak trees such as Quercus mongolica. The first step for artificial cultivation of S. aspratus is to secure the pure cultures available as an inoculant. The morphological characteristics of pure cultures on culture media may help to identify a specific strain or to distinguish them from other species. With these perspectives, we investigated the morphological characteristics of mycelial colonies in Korean S. aspratus strains grown on different culture media. All strains of S. aspratus formed the mycelial colonies with 2–3 different colors within a culture medium depending on the strains and culture media. Gray was a dominant colony color in all strains on potato dextrose agar (PD). All strains also formed the wrinkled colonies on PD. The colony shape was a circular form on all media tested. Velvety colony was observed in all strains on PD, while cottony colony was observed in all strains on Modified Melin-Norkrans’s agar (MMN). The pigmentation of culture media by mycelial colonies was observed in two strains among all four strains. S. aspratus strain derived from spore showed a brown pigmentation when cultured on PD or sabouraud dextrose agar (SD), S. aspratus strain isolated from a fresh tissue of fruit body showed a brown pigmentation on PD, SD and MMN.

Keywords: Morphological characteristics, Mycelial colony, Sarcodon aspratus
**C-13**

**Comparison of Morphological Characteristics of Amanita spp. Mycelial Colonies Grown on Two Different Solid Media**

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The cultural characteristics of *Amanita* spp. (total of 11 species) collected from Korean forests were analyzed based on colonial morphology. We compared the morphological characteristics of mycelial colonies grown on two different culture media. The dominant color of mycelial colonies in all species was white or yellow on both potato dextrose agar (PD) and Modified Melin-Norkrans’s agar (MMN). Most of species formed a circular colony on both PD and MMN, whereas two species (*A. rubescens* and *A. pantherina*) formed a filamentous colony on both PD and MMN. *A. eiji* formed an irregular colony on MMN. PD was often pigmented with a brown or yellowish color by some species of *Amanita* sp. There are no MMN media pigmented by *Amanita* spp. The mycelial colonies with uneven surface on both PD and MMN were observed in two species (*A. therense* and *A. melliceps*). However, the other species showed different surface morphologies on two different media. Except for two species, many species formed the cottony colonies on both PD and MMN. The mycelial colonies with velvety texture were observed in *A. volvatus* on PD and *A. hemibaphus* on MMN, respectively. Two species (*A. eiji* and *A. verna*) released a transparent exudates on the surface of their mycelial colonies when cultured on PD. Brown exudates were released from *A. pantherina* grown on PD. None of strains grown on MMN produced any exudates.

Keywords: *Amanita* spp., Morphological characteristics, Mycelial colony

**C-14**

**Effect of Low Temperature for Mycelial Growth of Ectomycorrhizal Mushrooms**

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In terrestrial ecosystems, ectomycorrhizal (ECM) fungi play an important role in plant growth under cold and barren environmental conditions. To decide minimum temperature for mycelial growth, we examined in mycelial growth of thirteen ECM mushrooms (*Amanita rubescens*, *A. pantherina*, *Hygrophorus russula*, *Leneocarpus leucobates*, *Macroboletus procerus*, *Bisporophorus sp.*, *Suillus pictus*, *S. viscidus*, *Tylopilus chromatopogon*) on potato dextrose agar (PDA) according to temperature conditions (4, 6, 8, 10, 25°C) for one month. All strains did not grow at 4 and 6°C. But they started mycelial growth at 8°C and showed less mycelial growth and density than 10°C and 25°C. Lignocellulolytic enzyme and cellulase activity measured on plate containing 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)(ABTS) and carboxymethylcellulose. Two enzyme activity of ECM mushrooms started to appear at 8°C. As a result, mycelial growth and enzyme activity of ECM mushrooms showed at 8°C and the temperature was critical temperature for mycelial growth of ECM mushrooms. In conclusion, we suggest that ECM mushrooms start to grow at more than 8°C under the ground soil.

Keywords: Ectomycorrhizal fungi, Minimum temperature, Mycelial growth, ABTS, carboxymethylcellulose

**C-15**

**Biochemical Characterization of a Novel SGNH Hydrolase (Nm21) from Neisseria meningitidis 053442**

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A novel SGNH-hydrolase (Nm21) from *Neisseria meningitidis* 053442 was identified, purified, and characterized by biochemical and biophysical methods. Multiple sequence alignment of Nm21 with other SGNH family member proteins confirmed a putative catalytic triad (Ser29-Asp169-His172), and conserved sequence motif of Ser(S)²-Gly (G)²-Asn (N)²-His(H)². Biochemical properties of Nm21 were investigated with fluorescence analysis, dynamic light scattering (DLS), molecular modeling, electron microscopy, mutant studies, and time of flight (TOF) mass spectrometry. Furthermore, cross-linked enzyme aggregates (CLEAs) of Nm21 were shown to exhibit good durability after repeated usages. Collectively, these molecular characteristics of Nm21 highlight its industrial potential for further applications in the near future.

Keywords: Nm21, SGNH hydrolase, CLEAs

**C-16**

**Hyphal Growth Condition and Cellulase Activity of Agaricus spp.**

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Agaricus species have been widely used as edible and medicinal mushrooms. The mushroom exhibits various therapeutic as well as beneficial properties. Especially, *A. subrufescens* has become a hot issue in various scientific studies. We examined the four species of *Agaricus* spp. (*A. arvensis, A. silvaticus, A. subrufescens, A. subrutilescens*) to understand their cultural characteristics. We have focused on the optimization of the hyphal growth and the cellulase activity. All species except for *A. subrufescens* grew well on the potato dextrose agar (PDA) at 25°C. The minimum and maximum temperatures for mycelial growth were 10°C and 30°C, respectively. The optimal pH of all species was observed on the Potato Dextrose Broth (PDB) medium incubated at pH16. *A. subrufescens* grew at various pH values (pH5, 6, 7, 8). Carboxymethylcellulose (CMC) was used as the substrate of cellulase activity. All species showed the cellulase activity and *A. arvensis* showed the higher enzyme activity than other species.

Keywords: Agaricus spp., A. subrufescens, Cellulase
C-17
Optimal Condition of Mycelial Growth and Laccase Activity of Coprinopsis spp.
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Coprinopsis species are commonly known as the ink cap and belong to the family Psathyrellaceae that has black spore print. These species are easy to find common in urban and forest areas in Korea. Four species of genus Coprinopsis collected from Korean forests during field research from 2010 to 2014. We have investigated the optimal conditions for mycelial growth and laccase activity in the four species of genus Coprinopsis (C. atramentaria, C. cinerea, C. friesi, and C. insignis). After 21 days of incubation, C. cinerea and C. friesi showed the highest growth on potato dextrose agar (PDA), malt extract agar (MEA) and sabouraud dextrose agar (SDA), whereas C. atramentaria and C. insignis were well grown on the PDA. The average temperature for mycelial growth of Coprinopsis spp. was 25°C. All species showed the laccase activities on the agar plate containing 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS).

Keywords: Coprinopsis spp., Coprinopsis cinerea, Laccase activity.

C-18
Enhancement of the Level of FK506 Production by Manipulating Regulatory Genes
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FK506 is a clinically important drug and possesses 23-member polyketide macrolide. The sequences of the entire FK506 biosynthetic gene cluster from Streptomyces sp. strain KCTC 11604BP was reported. Three putative regulatory genes, tcs2, tcs7, and fkbN, are identified by sequence analysis. These genes encoded an AntC family transcriptional regulator, LysR-type transcriptional regulator, and LAL (large ATP-binding regulators of LuxR) family regulator, respectively. This study examined the functions of tcs2, tcs7, and fkbN in the regulation of FK506 biosynthesis in Streptomyces sp. KCTC 11604BP through the overexpression, in-frame deletion, complementation, and transcriptional analysis of FK506 biosynthetic genes by semiquantitative reverse transcription-PCR (RT-PCR) in the wild-type and mutant strain. Overexpression and in-frame deletion of tcs2 did not affect the production of FK506, indicating that tcs2 is not involved in FK506 biosynthesis. In contrast, deletion of tcs7 resulted in a 1.9-fold increase in the level of FK506 production compared to the wild-type strain. Overexpression of fkbN in the wild-type and tcs7 deletion strain gave rise to a 2.1-fold and a 4.0-fold (21 mg liter-1) increase in FK506 production, respectively. These results suggest that fkbN and tcs7 play key roles as positive and negative regulators of FK506 biosynthetic pathway. Moreover, higher-producing industrial strains might allow to enhance more developed strains through this strategy.

Keywords: Polyketide, Streptomyces, genetic engineering, regulatory gene, FK506.

C-19
Analysis of Non-Ribosomal Peptide Synthetase Gene Cluster for Tolasaon Biosynthesis in Mushroon Pathogen Pseudomonas tolosi KACC10082
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Non-ribosomal peptide synthetase gene cluster for tolasaon biosynthesis was sequenced. Total 74kb gene cluster contained eight genes such as Polyketide synthase(PKS), macA, macB, diaminobutyrate-2-oxoglutarate amino transferase, succinate-semialdehyde dehydrogenase, alcohol dehydrogenase, aldehyde-activating protein, and ribonucleotide-diphosphate reductase subunit. Among these PKS gene was composed of 18 adenylation domain, 18 condensation domain, 18 thiolation domain, and one thioesterase domain. Whole genome sequence of Pseudomonas tolosi PMS117, Pseudomonas tolosi 6264, and Pseudomonas tolosi NCPPB 2192 was reported to NCBI. PKS gene in this study showed 89% DNA homology with Pseudomonas tolosi PMS117, whereas the other two, Pseudomonas tolosi 6264 and Pseudomonas tolosi NCPPB 2192 show homology below 50%. The two genes, macA and macB, were also reported in NRPS gene cluster in Pseudomonas putida strain PCL1445 producing putisoluvin. Therefore, macA and macB were suggested to play a role in exporting the tolasaon. Diaminobutyrate-2-oxoglutarate amino transferase was located neighbor to mac, however, the role of the enzyme was not investigated the relationship to the production of tolasaon. In contrast to that, the genes for succinate-semialdehyde dehydrogenase, alcohol dehydrogenase, aldehyde-activating protein, and ribonucleotide-diphosphate reductase subunit clustered at 9kb location from PKS TE domain were seemed not to be related to production of tolasaon.

Keywords: NRPS

C-20
A Novel Mechanism of Reduction of Dimethyl Sulfide in Thermococcus onnurineus NA1
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To date, a variety of microbes are known to grow by respiration with dimethyl sulfide (DMSO) as an electron acceptor. Several bacteria, such as Escherichia coli and Rhodopseudomonas capsulida, have been characterized on DMSO respiratory systems consisted of electron carriers and a terminal DMSO reductase. In this study, we presented that cysteine-cysteine redox shuttle serves as an electron mediator for DMSO reduction. Our experimental results show that thioredoxin reductase-protein disulfide oxidoreductase redox couple has a potential suitable for catalyzing the reduction of cystine to cysteine, permitting recycle of cysteine. Based on this mechanism of growth stimulation by DMSO via a redox-regulating system and an extracellular electron mediator, DMSO reduction is proposed to function simply by disposing of excess reducing power rather than conserving energy.

Keywords: DMSO, Thermococcus onnurineus NA1, Cysteine-Cysteine redox shuttle.
C-21 Cellulolytic Enzyme Activity in Basidiomycetes after Low Temperature Storage
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Fungal preservation is very important task that maintains its vitality to use in future. However, preserved fungal strains have an effect on mycelial vitality according to preservation periods. Cellulase is useful enzyme for industrial process and produced by chiefly by fungi and bacteria. Cellulase activity of preserving strains has an important values for various area. We investigated in cellulolytic enzyme activity of fungal strains preserved as an agar slant at low temperature for more than one year. Cellulase activity by fungi was measured by using carboxymethylcellulose (CMC) agar plate and spectrophotometry essay at 540 nm. The enzymatic index (EI) of tested strains showed Gloeostereum incarnatum (1.51), Gymnosporangium (1.87), Stilbus granulatus (2.04), Tricholoma sp. (3.21), Xeromyces subbotentosum (4.06) on CMC agar plate. Strains that showed EI higher than 1.50 were considered to be potential producers of cellulases. In submerged culture medium, cellulase activity of G. aggaricus (0.05 UI/ml), Tricholoma sp. (0.05 UI/ml) showed low. That of G. incarnatum (0.17 UI/ml), S. granulatus (0.11 UI/ml), X. subbotentosum (0.17 UI/ml), Xerula pudens (0.12 UI/ml) was better than the others. Quantitative test on CMC plate demonstrated a directed relationship with a qualitative test. And two methods should be used complementary to use screening assay for the detection of cellulase activity.

Keywords: Preservation, Cellulase, Enzymatic Index

C-22 Fluorometric Assay of α-factor Affinity for GPCR Receptor of S. cerevisiae
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The α-factor pheromone receptor of S. cerevisiae, Ste2p, belongs to the large family of GPCRs associated with signaling pathway present in diverse living organism from higher animals to microbes. α-Factor binding onto a type yeast pheromone receptor (Ste2p) causes the signal transfer into the cell causes physiological and morphological change. To assess α-factor affinity onto the receptor, we designed fluorophoric detector (Edan-a-factor and AMC-a-factor) and measured affinity of non-chromogenic analogs onto the receptor by competitive analysis using fluorophoric detector. Fluorometric assay was more sensitive than colorimetric assay. Eight analogs of non-chromogenic α-factor of S. cerevisiae with isoflectic replacement at position 5, 6, and 9, assessed their receptor binding affinities. The analogs substituted at position 6 with positive charged residues, [X,D-Ala]α-factor, exhibited the increased activity with 0.4 to 2.4-fold in affinity compared to native α-factor. Among 18 analogs, [Dap], D-Ala]α-factor and [Orn], D-Ala]α-factor showed highest affinities with 2.4-fold higher potency than native α-factor.

Keywords: α-factor, GPCR, S. cerevisiae, receptor affinity, fluorescence

C-23 Detection of Protein Interaction with 22kDa-Peptidyl Prolyl cis/trans Isomerase and 70kDa-Heat Shock Protein DnaK of Vibrio anguillarum
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The present study was conducted to detect the interaction between FKBP22 (22kDa FK506 binding protein) and DnaK in Vibrio anguillarum and perhaps, might be the first report on FKBP in Vibrio anguillarum and in vitro (bacterial two hybrid system), in vitro (GST pull down assay) and in silico (computational analysis) methods. The Va FKBP22 consists of N and C terminal domains, similar with the Mip (Macrophage infectivity protein) from Legionella pneumophila predicted by secondary structure forecast. GST pull down assay verified that the full length and C-terminal domain of Va FKBP22 can interact with DnaK. Moreover, it was found that FK506(FKBP inhibitor) and ATP do not influence the interaction. FKBP (one of the members of PPIase family; FK506 binding protein) and DnaK (one of the members of heat shock protein 70, HSP70, from prokaryote) have chaperone activity and have role in protein folding. So we carefully expect cooperating effect about chaperone activity by forming folding helper complex.

Keywords: protein interaction, peptidyl prolyl isomerase, heat shock protein 70(DnaK), Bacterial two hybrid system, GST pull down assay

C-24 Evaluation of the Nutritive Value from Korean Pollens Treated with Fungi
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Pollens have been known to possess various biological properties. But stiff pollen wall hindered dissolution of polysaccharides and lowered extraction efficiency. We measured the antioxidant activities as DPPH radical scavenging and the total polyphenol content of the lyophilized pollen was higher than the refined pollen and the pulverized pollen inoculated with Armmariella mellea and was lowest in the pollen inoculated with Lentinus edodes. Total polyphenol content of the lyophilized pollen was higher than the refined pollen and the pulverized pollen in acorn pollen germinated with Armmariella mellea. The antioxidant activity measured by the DPPH(2.2 diphenyl-1-picyrhydrazyl) free radical scavenging method exhibited that the lyophilized acorn pollen germinated with Armmariella mellea had the highest and that germinated with Lentinus edodes lowest in antioxidant activities. Pollen cell walls were torn and the cytoplasm was exposed after lyophilizing, while the pulverized cell walls were disrupted or cut. Many germinated cells were formed around pore of acorn pollen inoculated with Lentinus edodes, while those were formed at the end of hyphae derived from acorn pollen inoculated with Armmariella mellea.

Keywords: Pollen, Total polyphenol, DPPH radical scavenging
Characterization of Human Skin Bacterial Strains to Develop Vegetable Antibacterial Substances

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Sixty bacterial strains were isolated from human skin of scalp, cheek, and nose. The chemical and physiological characteristics were examined as the basic research to develop the vegetable antibacterial substances. Twelve chemical, physiological and morphological tests were accomplished. All strains from scalp showed positive reaction of urea test and the others reacted negatively. The most strains responded positively in Gram stain except two strains. The other tests showed various responses according to bacterial strains of isolated part of skin.

Keywords: human skin, characterization

Molecular and Functional Characterization of Hansenula polymorpha SAT1 Encoding Serine O-Acetyltransferase Involved in de novo Cysteine Biosynthetic Pathway

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Glutathione (GSH) and its precursor L-cysteine are the essential sulfur-containing compounds, which are important materials regarding their applications in the industries of pharmaceutics, cosmetics, and food as well as their vital functions in cellular metabolism. Here we report the molecular and functional characterization of HpSAT1, encoding a homologue of serine O-acetyltransferase, involved in the OAS pathway of the thermotolerant methylotrophic yeast Hansenula polymorpha. HpSat1p consists of 499 amino acid residues and shows a high sequence identity to serine O-acetyl transferases of other yeast and filamentous fungal species, ranging from 51.3 % to 59 %. While it displays very low identity in vitro activity assay showed that the activity of HpSat1p was approximately 100 times lower than that of the E. coli cysE protein. Together with its low transcript level, the weak catalytic activity of the HpSAT1 protein indicates that de novo cysteine biosynthesis via the OAS pathway, which is the only pathway to synthesize sulfur amino acids from inorganic sulfur in this yeast, might be the rate-limiting step in providing sulfur compounds in H. polymorpha.

Therefore, we propose that enhancing the flow to cysteine synthesis through overexpression of HpSAT1 or E. coli cysE would be good strategies to increase the production of L-cysteine and GSH in H. polymorpha.

Keywords: serine O-acetyl transferase, Hansenula polymorpha, cysteine, glutathione, sulfur pathway

Sugar Signal Transduction by N-acetylglucosamine-specific Enzyme II in Vibrio vulnificus

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The bacterial phosphoenolpyruvate: sugar phosphotransferase system (PTS) regulates a variety of physiological actions in addition to sugar uptake. Various regulatory roles have been known for the PTS components, especially in Escherichia coli. Although, the PTS is conserved in the opportunistic human pathogen, Vibrio vulnificus, as compared to E. coli, much less is known. Chitin, the oligomer form of N-acetylglucosamine, is one of the most abundant nutrient sources in the ocean. V. vulnificus has an N-acetylglucosamine specific PTS component namely EIICBGNp in order to utilize N-acetylglucosamine more efficiently. In this study, we identified a homolog of the global repressor Mcr in E. coli as a binding partner of the cytosolic part of EIICBGNp in V. vulnificus, and that this interaction is strongly dependent on the phosphorylation state of EIICBGNp. We revealed that only the dephosphorylated form of EIICBGNp interacts with Mcr. The interaction between dephosphorylated form of EIICB, the glucose transport enzyme, and Mcr is well known in E. coli and that this interaction induces transcription of Mcr-regulated genes in the presence of glucose. However, the regulatory mechanism of the Mcr regulon in the presence of N-acetylglucosamine is yet to be known in V. vulnificus. This novel regulatory mechanism in V. vulnificus that differs from E. coli may shed light on the unique bacterial physiology of this organism.

Keywords: Vibrio vulnificus, Sugar transport system, Protein-protein interaction, N-acetylglucosamine, Global repressor Mcr

Life Span Extension of Caenorhabditis elegans by Oxyresveratrol and Its Mechanism

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Oxyresveratrol (OXY), an aglycone of mulberroside A and an isomer of hydroxylated resveratrol, purified from the ethanol extract of Ramulus mori. Resveratrol has been studied on the lifespan extension of C. elegans. We investigated the effect of OXY on the lifespan extension of C. elegans and elucidated the mechanism underlying OXY-mediated lifespan extension in C. elegans by using loss-of-function mutants. Resveratrol is known to extend the lifespan of C. elegans by SIR-2.1 dependent manner. The results showed that OXY significantly (p < 0.05) extended the lifespan of C. elegans compared with the negative control. OXY significantly (p < 0.05) increased the mRNA and protein expression levels of SIR-2.1 and AAK-2 in a dose dependent manner. The lifespan was extended in loss-of-function mutant of DAF-16 by the treatment of OXY, which suggested the lifespan extension of C. elegans was not by activating DAF-16, while OXY did not extend the lifespan in the loss-of-function mutants of sir-2.1 and aak-2. Therefore, OXY extends the lifespan of C. elegans by activating SIR-2.1 which is related to the lifespan extension by calorie restriction and AMP-activated protein kinase (AMPK) pathway that is activated by dietary restriction.

Keywords: Oxyresveratrol, Caenorhabditis elegans, Lifespan extension, SIR-2.1
Molecular Determination of the Interaction Surface of the RNA Pyrophosphohydrolase RppH and the Diaminopimelate Epimerase DapF

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Similar to decapping of eukaryotic mRNAs, the degradation of a subset of mRNAs in *Escherichia coli* is initiated by the RppH-catalyzed conversion of 5’-terminal triphosphate to monophosphate, which triggers rapid endonucleolytic cleavage by endonucleolytic cleavage by RNase E in *E. coli* and 5’ exonuclease activity of RNase J1 in *Bacillus subtilis*. Despite the physiological importance of RppH-mediated mRNA decay, the regulation of RppH activity has little studied. A recent study identified a novel regulator of RppH-mediated mRNA degradation by showing that the diaminopimelate (DAP) epimerase DapF tightly interacted with RppH and increased its RNA pyrophosphohydrolase activity. DapF catalyzes the stereo-inversion of L,L-DAP to D,L-DAP, the penultimate step in the lysine biosynthetic pathway. To further characterize the interaction between RppH and DapF, we conducted a genetic screen to search for each variant exhibiting decreased interaction with its partner protein. Only one mutation of RppH showing a significant decrease in interaction with DapF was identified. We also isolated several DapF mutants exhibiting decreased interaction with RppH. From these data, we predict the interaction surfaces of two proteins. This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201529201).

Keywords: pyrophosphohydrolase, protein-protein interaction, diaminopimelate epimerase

Characterization of the Possible Intermediates Involved in the Post-PKS Modification in FK506 Parallel Biosynthetic Pathways

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FK506 is an ß-keto amide bonding-containing macrolide natural product that exhibits potent immunosuppressive activity and moderate antifungal activity. It also possesses numerous promising therapeutic potentials, which include neuroprotective and neuroregenerative activities. The biosynthesis of FK506 involves a hybrid polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS) system to construct the 23-membered macrolide. Although the entire biosynthetic gene cluster of FK506 and biosynthetic pathway to allylmalonyl-CoA have been reported until quite recently, the detailed post-PKS modification route has remained unsolved. Here we demonstrate that there are two independent biosynthetic routes to the final formation of FK506. First, FkbD catalyzes the hydroxylation at the C9-position of 9-deoxo-31-O-demethylFK506 to form 9-hydroxy-31-O-demethylFK506, which undergoes further oxidation to yield 31-O-demethylFK506 by FkbD. Then, FkbM performs 31-O-demethylation to afford FK506. Second, 9-deoxo-31-O-demethylFK506 might be converted to 9-deoxoFK506 by the action of FkbD, which then undergoes hydroxylation to form 9-hydroxyFK506, followed by oxidation of the hydroxyl group by FkbD to finally give FK506. It also proves that FkbD and FkbM can provide a potential tool for the combinatorial biosynthesis of novel macrolide derivatives as substrate-flexible post-PKS modification enzymes.

Keywords: FK506, intermediates, PKS, derivatives

Distinct Characteristics of the Substrate Binding Mode in Thermophilic L-Arabinose Isomerase

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L-Arabinose isomerase (AI) catalyzes sugar conversions like interconversion of L-arabinose to L-ribulose. Thermophilic and mesophilic AIs showed the distinct metal dependency for their catalysis and thermostability at elevated temperatures. However, it still remains unclear how thermophilic AIs showed different substrate preferences and metal requirements at molecular levels. Here, we solved the crystals of the apo, hol, and L-arabinol bound hexameric Al from *G. kaustophilus* with decent resolutions of 2.40 Å, 2.30 Å, and 2.25 Å, respectively. The structure of L-arabinol bound Al revealed that the substrate binding pocket is comprised of the C-terminal region of one subunit and the N-terminal region of neighboring subunit and the role of H-bonding network in the active site. Notably, Mn2+ and L-arabinol bound Al underwent significant displacements of several residues near the substrate binding site comparing with apo-Al. These structural data propose a metal-mediated substrate binding model for the isomerization reaction at elevated temperatures. Also, GKAI tightly forms salt bridges on interface of both two trimer and two subunits of one timer by non-conserved Arg residue comparing with other AIs. Taken together, thermophilic Al has adopted distinct active site features that promote the broadness of substrate specificity as well as thermostability.

Keywords:
D-1  
Microbial Phenol Production in Metabolically Engineered E. coli Using Synthetic sRNA Technology
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Microbial phenol production has been challenged by low metabolic flux through the biosynthetic pathway of aromatic compounds and microbial toxicity of phenol. To overcome these hurdles, we metabolically engineered 18 Escherichia coli strains to enforce the metabolic flux toward the immediate precursor, L-tyrosine, using the synthetic sRNA technology for target gene knockdown and developed a biphasic fermentation strategy to minimize the effects of phenol on cell growth. E. coli strains overexpressing Pantoea aeruginosa tyrosine phenol-lyase with tyrR and coreA downregulated using sRNA efficiently produced phenol from glucose. Among the strains tested, an engineered BL21(DE3) strain showed a highest phenol titer of 419 mg/L in flask culture and 1.69 g/L in fed-batch fermentation. The titer was increased up to 3.79 g/L in biphasic fed-batch fermentation. This work was supported by the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031693) of the Ministry of Science, ICT & Future Planning through the National Research Foundation of Korea.

Keywords: Phenol, Escherichia coli, sRNA, Metabolic Engineering

D-2  
Production of 1,3-Diaminopropane from Microbial Cell Factory Development by Metabolic Engineering
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Cellular production of chemicals is essential for sustainable chemical industry. We metabolically engineered Escherichia coli for production of 1,3-diaminopropane (1,3-DAP), a monomer for polyamide. Preparation of heterologous C2 and C3 pathways for 1,3-DAP production by in silico flux analysis revealed that the C3 pathway employing Acinetobacter baumannii and acl gene encoding 2-ketoglutarate 4-aminotransferase (KGD) and ald gene encoding 2-ketoglutarate 4-aminotransferase (KGD) and ald gene encoding 2-ketoglutarate 4-aminotransferase (KGD) and ald gene encoding 2-ketoglutarate 4-aminotransferase (KGD) and ald gene encoding 2-ketoglutarate 4-aminotransferase (KGD) and ald gene encoding 2-ketoglutarate 4-aminotransferase (KGD), was chosen for 1,3-DAP production. Also, knocking out pgdA was found to increase 1,3-DAP production by applying 128 synthetic small RNAs. Overexpression of the ppc and aspC genes in the pgdA deleted strain resulted in even higher production of 1,3-DAP. Fed-batch fermentation of the final engineered E. coli strain allowed production of 13 g/L of 1,3-DAP in a glucose minimal medium. This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT & Future Planning (NRF2012-0001751, NRF2012-0001761), through the National Research Foundation of Korea (NRF-2012-0001751, NRF2012-0001761).

Keywords: 1,3-DAP, 1,3-diaminopropane, metabolic engineering, Escherichia coli

D-3  
Development of L-Arginine Producing Microbial Cell Factory through Metabolic Engineering Based on Corynebacterium glutamicum
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Microbial cell factory for production of L-arginine was developed based on Corynebacterium glutamicum. Random mutagenesis was performed on C. glutamicum to increase tolerance against L-arginine. Arginine operon repressor proteins were inactivated. The PPP flux was strengthened to increase the pool of NADPH by downregulating the pgi gene and overexpressing the opaA, pgd, tal, dta, and zos genes. Next, the Ngl1221 gene encoding L-glutamate exporter was inactivated to channel L-glutamate to L-arginine formation. Also, the expression levels of the argB, argD, and car genes were optimized for converting L-ornithine to L-arginine effectively. Finally, the argGH operon was overexpressed. Fed-batch fermentation of the final strain was performed in a 1,500 L bioreactor resulting 81 g/L of L-arginine production. The approaches described here will be useful in developing strains of Corynebacteria regarding the production of arginine and its derivatives.

This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT & Future Planning (NRF2012-0001751, NRF2012-0001761) through the National Research Foundation of Korea (NRF-2012-0001751, NRF2012-0001761).

Keywords: Corynebacterium glutamicum, metabolic engineering, L-arginine

D-4  
Deoxyviolacein Mass Production with High Cell Density Culture and Aggregation of Deoxyviolacein in the Culture Using Escherichia coli
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Deoxyviolacein is one of violacein derivatives which is produced by various natural violacein producing strain such as: Vibrio, Cytophaga spp., Pseudoviolaceum, and Collimonas sp. Violacein is well known material which has antimicrobial, anti-cancer, antiprotozoan and antiviral effect. Most of mechanism how violacein and its derivative is harmful to several gram-positive bacteria, cancer cells and protozoans like C.elegans is unknown but several reports of this derivative suggests an effective and noble mechanism against this organism. Therefore the mass production of violacein and its derivative is important. This study is focused on the simple mass production of deoxyviolacein with high density cell culture of E. coli and the aggregation of deoxyviolacein in cell culture. Deoxyviolacein is one of the easiest forms of violacein derivative which is produced by violacein3hABC. Unstable expression of violA gene in all violacein mass production is a major problem. For this reason, several violacein producing groups move to deoxyviolacein production. In this study we make W3110 dhio BH2 strain which contains deoxyviolacein production and bacterial hemoglobin plasmid. This strain produce 0.63g/L of violacein for 24hours in broth. Moreover overproduced deoxyviolacein is aggregated down to the bottom of the fermenter bottom. Total deoxyviolacein amount produced including deoxyviolacein aggregates is 1.12g/L in 24hours.

Keywords: Deoxyviolacein, Virulacein, HDCC
**D-5**

**Extraction of Biosurfactant from Streptomyces blastomyceticus**

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Surfactant decreases interfacial or surface tension of liquid and are used in various fields such as detergents, perfumes, plasticizers and lubricants. In this experiment, we have extracted biosurfactant from yeast extract malt extract medium of Streptomyces blastomyceticus strain 12-6 and analyzed its characteristics. We verified surfactant activity of culture medium by drop collapse test, emulsification assay and oil displacement assay. 6N HCl was added to adjust the pH of culture medium around 2 and precipitation occurred over night at 4°C. Supernatant was obtained by centrifugation at 4°C. The supernatant was extracted by ethyl ether and acetate was tested for the optimal developing solvent. Again, the precipitate was separated and extracted for surfactant by hexane. The precipitate obtained from this concentrate was further extracted by ethyl ether and concentrated by rotary vacuum evaporation. The surfactant was stained by phosphomolybdate. Its surfactant activity was verified by micro plate assay, oil displacement assay and emulsification assay. The surfactant was tested for purity by thin layer chromatography. Various combinations of hexane, ethyl ether and acetate was tested for the optimal developing solvent. The surfactant was stained by phosphomolybdate.

Keywords: Streptomyces, biosurfactant, screening

**D-6**

**Comparison of Methyl Orsellinate and Sparassol Concentrations via Submerged Culture of Sparassis latifolia**

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Methyl orsellinate (methyl-2,4-dihydroxy-6-methyl-benzoate) and sparassol (methyl-2-hydroxy-4-methoxy-6-methylbenzoate) which are known as antifungal compounds of Sparassis latifolia were isolated from submerged culture medium (potato dextrose broth, PDB) of mycelia. And these chemical structures were identified by nuclear magnetic resonance (NMR) and liquid chromatography-Mass Spectrometry (LC-MS). We were compared with concentrations of methyl orsellinate and sparassol in culture conditions (temperatures and pHs). In the case of methyl orsellinate, strain KFRI 645 was produced the highest concentration in pH 6.0 at 23°C and strain KFRI 747 was also made high level in pH 6.0 at 30°C. And in the case of sparassol strain KFRI 645 produced the highest concentration in pH 4.0 at 23°C and sparassol content of strain KFRI 747 was highest in pH 5.0 at 30°C. Thus, we made sure that production of methyl orsellinate and sparassol was depend on the culture conditions of the fungus.

Keywords: Methyl orsellinate, Sparassol, Sparassis latifolia

**D-7**

**Expression Analysis of Genes Related in Two Pathway of Sesquiterpene Biosynthesis in Wood Rot Fungus, Polyporus brumalis**

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Terpenoids form the largest group of called secondary metabolites and are widely explored as perfumes, food additives, and pharmaceutical agents. In our previous study, sesquiterpene biosynthesis has been researched in wood rotting fungus, Polyporus brumalis. However, despite these efforts, the synthetic pathways responsible for terpene metabolites in these fungi remain unclear. The basic terpene building blocks are generated in cells by one of two distinct biosynthetic pathway, mevalonate (MVA) and methylerythritol phosphate (MEP) pathway. To verify the enzymes in terpene metabolism of P. brumalis, analysis of transcriptome was conducted using NGS tool. As the results, genes in both pathways have been co-expressed in P. brumalis mycelium. Co-regulation of genes in two pathways was unanticipated, as this has been identified in plants. Therefore, the relation of two pathways through analysis of gene expressions related in two pathways and metabolite under various culture conditions in P. brumalis.

Keywords: Polyporus brumalis, terpene metabolism, wood rot fungi, gene expression

**D-8**

**Proteomic Analysis of Differences in Korean Traditional Wheat-Based Nuruk**

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Nuruk, a traditional starter culture, is used extensively for brewing the cereal-based Korean alcoholic beverages Makgeolli. Various microorganisms of Nuruk are involved in the saccharification, fermentation, and ripening processes to produce Makgeolli. In particular, the fungal act as major source of amylolytic and proteolytic enzymes for saccharification process during fermentation. Therefore, this study focused on proteomic analysis of extracellular protein for fermentation in traditional wheat-based Nuraks fermented at two representative traditional temperature conditions for 30 days. Nurak A was fermented at a uniform temperature of 36°C for 30 days and Nurak B was fermented at a high initial temperature of 45°C for 10 days followed by 35°C for 20 days. To provide the particular patterns of secreted proteins from fungal community according to fermentation conditions of Nuraks, we carried out a comparative proteomic analysis using two-dimensional polyacrylamide electrophoresis (2D-PAGE). We analyzed 25 protein spots, which were differentially expressed in accordance with the fermentation condition, from 12 different gels. Identified proteins were mainly amylolytic enzymes as amylase.

Keywords: Wheat, Nurak, Proteomics, 2D-PAGE, Amylase
Study on the Large Scale Fermentation of Recombinant E. coli for the Production of N-SPERSE
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N-SPERSE (10-Hydroxy stearic acid) is natural product prepared by bioconversion method from oleic acid, having a moisturizing and surfactant effect can be used as cosmetic raw materials. For the mass production of N-SPERSE, bacterial expression vector was constructed using oleate hydratase from Stenotrophomonas maltophilia. In this study, we compared the two bacterial host (E. coli MG1655(DE3) and E. coli BL21(DE3)) in large-scale fermentation. E. coli MG1655(DE3) showed cell lysis and lag time for 12 hours in initial culture time. We confirmed using plaque assay that the cell lysis caused by phage contamination. Accordingly, we used the E. coli BL21(DE3) having a resistance to T1 phage. In E. coli BL21(DE3), decrease of cell growth rate and cell lysis was not observed in large-scale fermentation. These results suggest that using the pluge resistant E. coli overcome the phage contamination in large-scale fermentation.

Keywords: N-SPERSE(10-HSA), E. coli, Oleate hydratase, Large scale fermentation, Plage

454 Pyrosequencing Reveals Mycofloral Community Dynamics in Traditional Wheat-Based Nuruk Based on Varying Moisture Content
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Nuruk, is extensively used in the brewing of Korea’s popular alcoholic beverages thereby stressing the needs for quality enhancement. The nuruk mycobiome greatly influences both fermentation as well as palatability enhancement. In continuation to our study of nuruk mycobiome characterization, we further evaluated mycobiota dynamics in a wheat based nuruk C, consisting of barley, green gram and wheat in the ratio 3:3:3:3 under varying moisture content of 20%, 26% and 30% for a period of 30 days. This revealed a total of 151, 151 and 148 OTUs respectively, from a total of 35,065 ITS sequences. The earlier stage of nuruk fermentation was dominated by Aspergillus whereas later stage from 10 to 30 days by Zymomonas. Taxonomic assignment indicated that nuruk C mycobiota was dominated by the genera Aspergillus during day 3 and 6, whereas Lichtheimia predominated, during day 10, 20 and 30 of nuruk fermentation. There was a decreasing trend in Aspergillus population with increase in moisture content in day 10, 20 and 30 samples. Most of the mycobiota communities present abundantly in the 0 day samples either perished or diminished with the progress in fermentation. Species-level identification indicated that Aspergillus candidus showing a decreasing trend whereas, Saccharomyces sp. indicated an increasing trend in population with increase in moisture content throughout the fermentation process. These findings are a step forward in our goal of nuruk quality enhancement.

Keywords: nuruk, mycobiota, Aspergillus, fermentation

Characteristics of Ethanol Fermentation with Low Temperature Adaptation Yeast for Yakju Brewed
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The objectives of this study were to isolate and characterize the low temperature adaptation yeast and to obtain the industrially useful yeasts for brewing of Korean traditional yakju. In this study, 482 wild yeasts were isolated from a Korean fermented foods. Among them, the 5 yeasts strains were examined for their ethanol, glucose and salt tolerance. In addition, the pH, acidity, amino acid, ethanol content, organic acid, free sugar and volatile flavor components in the mash of yakju prepared with the yeasts isolated. The five isolated yeast identified by comparison of nucleotide sequences for PCR-amplified D1/D2 region of 26S rDNA large subunit using BLAST. Overall, this results indicated that using isolated wild yeast strain in the fermentation process affects the chemical characteristics of the Yakju brewed.

Keywords: Low temperature, Yakju, Fermentation, Saccharomyces cerevisiae

Reconstruction of Genetic Circuit for 1-Deoxynojirimycin (DNJ) Biosynthesis in Escherichia coli
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1-Deoxynojirimycin (DNJ) is a D-glucose analogue, in which a nitrogen atom substitutes for the ring oxygen. It is relevant to the pharmaceutical industry for the treatment of various diseases, especially the treatment of non-insulin-dependent (type II) diabetes. A previous study has demonstrated that recombinant E. coli and Bacillus subtilis strains, harboring the DNJ biosynthesis gene cluster of B. subtilis MORL, were able to produce DNJ as indicated by the α-glucosidase inhibition activity. However, the level of DNJ production by recombinant E. coli strain was a lot lower than that of B. subtilis MORL strain. In this study, we constructed the vector, pSYK175, containing three codon optimized monoeisotronic DNJ biosynthetic genes for the biosynthesis of 1-DNJ in E. coli. In addition, we developed a novel screening method to visually distinguish between the low- and high- DNJ producing cells, based on the suppression of the tyrosinase by DNJ resulting in reduced melanin production. Our DNJ biosynthetic circuit and screening tool will be useful for identification of engineering targets and optimization of DNJ biosynthesis in recombinant strains. Supported by the Intelligent Synthetic Biology Global Frontier Program.

Keywords: 1-deoxynojirimycin (DNJ), B. subtilis MORI, anti-tyrosinase activity, screening
Effect of Metal Ions on Production of Fusaricidin A and B of *Paenibacillus kribbensis* CU01 Isolated from Yellow Loess

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A novel bacterial strain was isolated from the yellow loess in Haenam, Jeollanam-do province, South Korea. On the basis of 16S rDNA sequence and biochemical tests, the isolate was named as *Paenibacillus kribbensis* CU01. Cells were cultured in a modified M9 medium. Culture broth showed strong antifungal activity against several phytopathogenic fungi such as *Sclerotinia sclerotiorum* KACC40457, *Colletotrichum gloeosporioides* KACC40003, *Phytophthora capsici* KACC40157, *Fusarium oxysporum* KACC40031, and *Botrytis cinerea* KACC40574. Culture broth was centrifuged and the supernatant was extracted by 100% butanol. The extracted fraction was evaporated and then dissolved with 100% methanol. The solution was applied to HPLC and the eluted fractions having antifungal activity were analyzed by LC/MS. Major m/z values of 883 and 897 were observed from mass spectrum. Based on the results of MALDI-TOF/TOF MS, the antifungal substances was found to be LI-F type antibiotics (fusaricidin A and B). The effect of six different metal divalent cations (Fe²⁺, Mn²⁺, Ni²⁺, Mo²⁺, Co²⁺, Zn²⁺) on the production of fusaricidin A and B by *Paenibacillus kribbensis* CU01 was tested. The production of fusaricidin A and B significantly increased by the addition of Fe²⁺ and Mn²⁺. On the other hand, addition of metal cations did not change the ratio of fusaricidin A and B.

Keywords: *Paenibacillus kribbensis*, Fusaricidin, LI-F type antibiotics, antifungal activity

Hericidin A Production in Submerged Culture of *Streptomyces scopuliridis* M40 Strain

Sanghyun Ha¹, Keon Jin Lee¹, Sang-Hi Lee¹, Hyun Jung Gwak¹, Jong-Hee Lee¹, Tae-Woon Kim¹, Hak-Jong Choi¹, Ja Young Jang¹, Jung-Sub Choi², and Hae Woong Park¹,

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Hericidin A, which show herbicidal activity, was produced in submerged culture of *Streptomyces scopuliridis* M40. At a rotational speed of 300 rpm, serious oxygen depletion was present from 24 h to 168 h, resulting in low herbicidin A yield of 385 mg/L. The length of *Streptomyces* cells was longest, showing 190 ± 68 um. There was a positive correlation between oxygen uptake rates (OUR) and herbicidin A yield. As the rotational speed increased from 300 to 600 rpm, dissolved oxygen tension (DOT) increased proportionally. OUR increased with increasing rotational speed by 500 rpm where the highest herbicidin A yield of 1,025 mg/L was obtained. However, herbicidin A yield dramatically decreased at 600 rpm although DOT was maintained over 70%, showing the length was less than 60 um. The highest OUR was obtained with the mean hypha length of approximately 100 um which induced the highest specific substrate consumption rate. When the rotational speed was over 600 rpm, *Streptomyces* cells were severely damaged by the high mechanical shear forces, resulting in poor herbicidin A productivity of 1.5 mg/L/h.

Keywords: *Streptomyces scopuliridis*, herbicidin A, submerged culture, oxygen uptake rate, hypha length

Multi Drug Resistant *Staphylococcus aureus* Growth Inhibition by Violacein from Natural Isolated Strain N28

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Nowadays, human beings have many diseases like cancer and infections by microorganism. Multi drug resistant organism (MDRO) is one of the upcoming threats to health issues, however, there is no many methods to solve this problem without noble antibiotics development or rediscovery of traditional antibiotics. In this study, we introduce the versatile agent: violacein which has various effects such as anticancer, antibiotics, antifungal and antiprotozoans. *Pseudokugelluma* sp. N28 strain, violacein producer, is found from soil and has 98% 16s rRNA similarity to type strain *Pseudokugelluma violaceigra* YLM3127. The violacein produced by *Pseudokugelluma* sp. N28 has antibacterial activity against *Staphylococcus aureus* ATCC25923 and other multi drug resistant *S.aureus* isolated from hospital patient. Antibacterial effect of purified violacein was tested on *Staphylococcus*. Using ethanol extraction, 25μM of purified violacein can kill 95% of *S.aureus* after 24h. As a result, minimal inhibitory concentrations of violacein were 5 mg/L in Mueller Hinton broth and 0.6 mg/L in M9 modified media. Hence, antibacterial effect of violacein against multi drug resistant *Staphylococcus aureus* has shown possible development of treatment for the multi drug resistant gram positive bacterial infection.

Keywords: MDRO, *S.aureus*, *Pseudokugelluma*, Violacein

Improvement of the Antioxidant Activity of Safflower Seed (*Carthamus tinctorius* L.) by Fermentation with β-glucuronidase Producing Lactic Acid Bacteria

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Department of Life Science, Kyonggi University

Safflower seeds (*Carthamus tinctorius* L.) are used as traditional medicine for the treatment of osteoporosis and rheumatoid arthritis in Korea. In the present study, the influence of two types of lactic acid bacteria (*Lactobacillus acidophilus*, *Leuconostoc mesenteroides*) on antioxidant activities of fermented safflower seeds was determined and compared with those of their unfermented safflower seeds. Safflower seeds fermented with *L. acidophilus* showed the highest antioxidant activity and the greatest increase in the contents of acacetin. The percentage of DPPH and ABTS in safflower seeds fermented with *L. acidophilus* increase from 59.48% and 48.16%, respectively, to 71.43% and 63.76 after 28 days. Results of the HPLC analysis indicated the change of phenolic compounds during the fermentation of safflower seeds with *L. acidophilus*. Acacetin which is a flavonoid compound in safflower seeds has anti-inflammatory, anti-cancer activity and was increased from 0.27 mg/g to 1.23 mg/g which is a flavonoid compound in safflower seeds has anti-inflammatory, anti-cancer activity and were increased from 0.27 mg/g to 1.23 mg/g by *L. acidophilus* but composition of acacetin was not changed by *L. acidophilus* as well in the fermentation by the fermentation bacteria to improve antioxidant activity of safflower seeds and potentially as a functional starter to fermentation safflower seeds.

Keywords: Safflower seeds, Fermentation, Lactic acid bacteria, Phenolic compounds, Acacetin
Characterization and Identification of MJM7007 as Novel Producer of Gentamicin B
Longbin Qi1, Ji-su Han1, Yingyu Jin2,3, and Joo-won Suh4
1Department of Biomodulation, Myongji University, 2Korea Lichen Research Institute, 3Korean Lichen Natural Resources, 4Emerging global importance of Korea’s alcoholic beverages emphasizes the need for the quality enhancement of nuruk, used extensively in their brewing. Bacterial microflora known to be ubiquitously present, is also a part of the nuruk ecosystem and are known to influence the fermentation activity by influencing fermentation favourable factors. In continuation to our study of nuruk microbiome characterization, we further evaluated bacterial dynamics in a wheat based nuruk C, consisting of barley, green gram and wheat in the ratio 3:3:0:52:3.3 under varying moisture content of 20%, 26% and 30% for a period of 30 days. A total of 91424 16S reads were analyzed, which were assigned to a total of 231, 320 and 298 OTUs under 20%, 26% and 30% moisture content respectively. Diversity parameters such as Chao1 clearly indicated nuruk C with 26% moisture to be higher in bacterial richness than that of 20% and 30% with the exception of day 30 sample. Taxonomic assignments indicated the phylum Cytophagia to be predominant, followed by Pseudoalteromonas throughout the fermentation period. But, from day 6 onwards, the Cytophagia population had a decreasing trend, whereas the Pseudoalteromonas population had an increasing trend in 20%, 26% and 30% samples. Among Pseudoalteromonas, Alpha-Pseudomonas were predominant, whereas Firmicutes included mostly Bacilli. The findings of this study is a step forward towards bacterial community structure in traditional nuruk starter production.

Keywords: nuruk, microflora, Proteobacteria, Firmicutes

A New Potential UV Filter, 5-Hydroxymellein Isolated from Endolichenic Fungus
Lu Zha1,2 and Joo-won Suh1,4
1Korean Lichen Research Institute, Sunchon National University, 2Korean Lichen Natural Resources, 3Emerging global importance of Korea’s alcoholic beverages emphasizes the need for the quality enhancement of nuruk, used extensively in their brewing. Bacterial microflora known to be ubiquitously present, is also a part of the nuruk ecosystem and are known to influence the fermentation activity by influencing fermentation favourable factors. In continuation to our study of nuruk microbiome characterization, we further evaluated bacterial dynamics in a wheat based nuruk C, consisting of barley, green gram and wheat in the ratio 3:3:0:52:3.3 under varying moisture content of 20%, 26% and 30% for a period of 30 days. A total of 91424 16S reads were analyzed, which were assigned to a total of 231, 320 and 298 OTUs under 20%, 26% and 30% moisture content respectively. Diversity parameters such as Chao1 clearly indicated nuruk C with 26% moisture to be higher in bacterial richness than that of 20% and 30% with the exception of day 30 sample. Taxonomic assignments indicated the phylum Cytophagia to be predominant, followed by Pseudoalteromonas throughout the fermentation period. But, from day 6 onwards, the Cytophagia population had a decreasing trend, whereas the Pseudoalteromonas population had an increasing trend in 20%, 26% and 30% samples. Among Pseudoalteromonas, Alpha-Pseudomonas were predominant, whereas Firmicutes included mostly Bacilli. The findings of this study is a step forward towards bacterial community structure in traditional nuruk starter production.

Keywords: nuruk, microflora, Proteobacteria, Firmicutes

Bacterial Diversity in Traditional Wheat-Based Nuruk Based on Varying Moisture Content
Jyotirajnan Bal1, Suk-Hyun Yum1, Myeong Jin Jo1, Soo-Hwan Yeo2, Jung-Mi Kim1, and Dae-Hyuk Kim
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In nature, the microbial degradation of tryptophan produces different products depending on bacterial species. For example, tryptophan is degraded to anthranilate in Pseudomonas aeruginosa, but indole in Escherichia coli, respectively. In previous study, we found anthranilate and indole has an opposite effect on P. aeruginosa biofilm formation. While indole enhances the biofilm formation of P. aeruginosa, anthranilate was able to disintegrate the mushroom structure of biofilm. In P. aeruginosa, the transcription of antiABC operon encoding anthranilate dioxygenase complex that functions to degrade anthranilate, was activated by the indole treatment and as consequence, the anthranilate level in P. aeruginosa culture supernatant decreased. We investigated whether the presence of indole could enhance the activity of AntiR, the transcriptional activator of antiABC. Interestingly, although indole alone failed to activate AntiR, co-addition of indole with anthranilate boosted the activation of AntiR by anthranilate. Here we suggest a clue about how indole can reduce the anthranilate level and modulate AntiR activity. In addition, anthranilate also deteriorated the biofilm formation of other bacteria, suggesting that it can be used as an agent to inhibit the bacterial biofilm formation.

Keywords: Pseudomonas aeruginosa, Anthranilate, Biofilm formation, Indole

Mutual Antagonism between Anthranilate and Indole in Biofilm Formation of Pseudomonas aeruginosa
Xi-Hui Li, Soo-Kyeong Kim, Jangmin Oh, and Joon-Hee Lee
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In nature, the microbial degradation of tryptophan produces different products depending on bacterial species. For example, tryptophan is degraded to anthranilate in Pseudomonas aeruginosa, but indole in Escherichia coli, respectively. In previous study, we found anthranilate and indole has an opposite effect on P. aeruginosa biofilm formation. While indole enhances the biofilm formation of P. aeruginosa, anthranilate was able to disintegrate the mushroom structure of biofilm. In P. aeruginosa, the transcription of antiABC operon encoding anthranilate dioxygenase complex that functions to degrade anthranilate, was activated by the indole treatment and as consequence, the anthranilate level in P. aeruginosa culture supernatant decreased. We investigated whether the presence of indole could enhance the activity of AntiR, the transcriptional activator of antiABC. Interestingly, although indole alone failed to activate AntiR, co-addition of indole with anthranilate boosted the activation of AntiR by anthranilate. Here we suggest a clue about how indole can reduce the anthranilate level and modulate AntiR activity. In addition, anthranilate also deteriorated the biofilm formation of other bacteria, suggesting that it can be used as an agent to inhibit the bacterial biofilm formation.

Keywords: Pseudomonas aeruginosa, Anthranilate, Biofilm formation, Indole
Chemical Constituents of the Fruiting Body of Glaziella splendens

Ji-Yul Kim, Byung Soon Hwang, Dae-Won Kim, E-Eum Woo, Yoon-Ju Lee, Van Minh Nguyen, In-Kyoung Lee, and Bong-Sik Yun

Mushrooms are valued as a nutritionally functional foods and also as an important source of beneficial medicinal components. They produce various primary and secondary metabolites which have interesting biological activities and unusual chemical structures. Glaziella splendens, belonging to the family Xylariaceae, is characterized by hollow, gelatinous stromata that accumulate liquid. As part of our ongoing investigation on chemical constituents of Korean indigenous mushrooms, G. splendens was collected and investigated. G. splendens was extracted twice with chloroform : methanol (1:1) at room temperature, and the extract was concentrated to eliminate chloroform and methanol, and then partitioned consecutively with hexane, ethyl acetate, and butanol. The ethyl acetate-soluble layer was concentrated and separated by silica gel column chromatography, MPLC, and Sephadex LH-20 column chromatography, followed by preparative reversed-phase HPLC to provide four compounds. The structures of these compounds were determined by spectroscopic methods, mainly NMR and mass analyses.

Keywords: Mushroom, Glaziella splendens, Secondary metabolites
D-25
Diversity and Multifunctional Properties of Actinomycetes
Jae-min Lim, Youngjun Ju, Yujung Oh, Dong-Jin Park, and Chang-Jin Kim*
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Actinobacteria, especially Streptomyces sp., are recognized as the producers of many bioactive metabolites that are medicine, such as antibacterials, antifungals, antivirals, antithrombotics, immunomodifiers, anti-tumor drugs and enzyme inhibitors; and in agriculture, including insecticides, herbicides, fungicides and growth promoting substances for plants and animals. Actinobacteria-derived antibiotics that are important in medicine include aminoglycosides, anthracyclines, chloramphenicol, macrolide, tetracyclines etc. In this study, actinomycetes as the useful resource were isolated from multiple soil and were analysed 16S rRNA sequences, enzyme (protease, amylase, lipase, CMCase) and antimicrobial activities, culture conditions depend on temperature, pH, NaCl and LC/MS profiles. Ultimately, we gather and supply not only actinomycetes secondary metabolites, but also their informations of valuable characteristics and new functions, so that the researches can speed up the development of bio-R&D, strengthen the competitive power of bio-industry.

Keywords: actinomycetes, secondary metabolite

D-26
Medium Optimization of a Potential Herbicide from Streptomyces scopuliridis N29
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†Industrial Bio-material Research Center, Korea Research Institute of Bioscience and Biotechnology, ‡Eco-friendly and New Materials Research Group, Korea Research Institute of Chemical Technology

With environmental issues increasing from synthetic chemical herbicides, microbe-originated herbicides could be a fascinating alternative in current farming. In this study, we isolated a Streptomyces strain that could produce herbicidally active metabolites against a grass weed Digitaria sanguinalis. It was identified as a member of the Streptomyces scopuliridis cluster. In order to develop the medium composition, effects of ingredients including carbon sources, nitrogen sources, metal ions and phosphate were examined. Among various carbon and nitrogen sources used, glucose, potato starch and soybean meal were the most suitable for a potential herbicide production. The productivity was increased as the increasement of carbon sources and decreasement of nitrogen sources. The maximum productivity was reached approximately 1,300 mg/L in the optimized medium, which consist soybean meal 1.5%, glucose 3%, potato starch 1%, ZnSO4 1mM, Sodium alginate 0.1%. We would like to acknowledge the financial support from the R&D Convergence Program of MSIP (Ministry of Science, ICT and Future Planning) and NST (National Research Council of Science & Technology) of Republic of Korea (Grant 000-00-000).

Keywords: Streptomyces, Herbicide, secondary metabolites, Medium optimization

D-27
Adaptive Laboratory Evolution of Starch-utilizing Yeast Saccharomyces fibuligera for an Improved Stress Tolerance
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2Fermented Food Science Division, Department of Agro-Food Resource, NASS, RDA

Saccharomyces fibuligera is amylolytic yeast that exhibits a raw starch-degrading activity. Its ability to directly digest raw starch is a technologically interesting trait and property. However, low tolerance to heat and ethanol limit the potential of S. fibuligera for use in a single step conversion of starch-rich substrates into value-added products. In this study, adaptive evolution was performed to improve tolerance of S. fibuligera to temperature, lactic acid and ethanol by prolonged exposure to stress conditions. Evolved S. fibuligera strains exhibited significantly enhanced tolerance to grow in stress conditions such as high concentration of ethanol (10%) and lactic acid (2.5%) and increased growth temperature (39°C).

Keywords: Saccharomyces fibuligera, Adaptive laboratory evolution, Stress condition

D-28
Quality Comparison of Detoxified Rhus Verniciflua Vinegars Produced by Various Acetobacter Bacteria
Ji-Seon Kim, A-Ra Jo, Ji-Young Mun, Soo-Hwan Yeo, Dong-Jun Seo, SeongYeol Baek*  
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To investigate the effect of Acetobacter on qualities of detoxified Rhus verniciflua Vinegars (DRV), DRV were produced through acetic acid fermentation using three Acetobacter strains such as A. pasteurianus KACC16934 (A.p), A. malorum V5-7 (A.m), Gluconacetobacter entanii RDAF-S (G.e). There were little difference in pH (2.87–2.90) and titratable acidities (5.33–5.68%) of three DRV. The content of acetic acid in DRV(A.p, A.m, G.e) were 99.51mg/ml, 96.43mg/ml and 101.06mg/ml. Except acetic acid in each DRV, the content of oxalic acid, malic acid, succinic acid, fumaric acid in DRV (A.p), malic acid, succinic acid, fumaric acid in DRV(A.m) and malic acid in DRV (G.e) were in the range of 0.1~0.7mg/ml. The content of glutamic acid, alanine, valine, leucine, tyrosine, lysine, amoseric and arginine were high in all DRV. A sensory evaluation of DRV indicated that A. malorum V5-7 (A.m) vinegar strain was better than the other samples in aspect color and G. entanii RDAF-S (G.e) vinegar strain was better than the other samples in aspect flavor, tasted and overall preference.

Keywords: Fermentation, Vinegar, Acetobacter Bacteria, Rhus verniciflua, stockes, Organic
Screening and Selection of *Bacillus* Strains as Potential Starter Cultures for the Fermentation of Low-salted Doenjang

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For the screening of potential starter cultures for fermentation of low-salted doenjang, Korean traditional doenjang samples were collected in Gyeonggi and Gangwon provinces, Republic of Korea and 640 *Bacillus* strains were additionally collected from Kangwon National University and RDA. We first screened bacterial strains based on protease activity on TSA supplemented with 7% NaCl and 1% skim milk, as a result total 263 strains were screened. Then antipathogenic activity was tested and prominent 11 strains showing high protease and antipathogenic activities were selected. Functional, phenotypic, and food safety-related properties of 11 strains were additionally tested and these strains showed various enzyme activities including fibrinolytic activity, amylase, and cellulase. Among 11 strains, B3-2, B7-1, and B16-8 showing most inhibitory effect against major food-borne pathogens were finally selected as the best potential doenjang starter cultures. Phylogenetic analysis based on 16S rRNA gene sequences showed that two strains were closely related to *B. methylotrophicus*, while one strain was closely related to *B. stearothermophilus*. In particular, the growth test of three strains shows that these strains can use as a starter under fermentation environment of commercial low-salted doenjang containing 7–10% NaCl concentration. In conclusion, three strains are considered good candidates of starter culture for fermentation of low-salted doenjang with high quality.

Keywords: Bacillus, doenjang, starter, screening, protease activity
E-1
Complete Genome Sequence of Mycobacterium tuberculosis K from a Korean High School Outbreak, Belonging to the Beijing Family

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*Mycobacterium tuberculosis K, a member of the Beijing family, was first identified in 1999 as the most prevalent genotype in South Korea among clinical isolates of M. tuberculosis from high school outbreaks. M. tuberculosis K is an aerobic, non-motile, Gram-positive, and non-spore-forming rod-shaped bacillus. A transmission electron microscopy (TEM) analysis displayed an abundance of lipid bodies in the cytosol. The genome of the M. tuberculosis K strain was sequenced using two independent sequencing methods (Sanger and Illumina). Here, we present the genomic features of the 4,385,518-bp-long complete genome sequence of M. tuberculosis K (one chromosome, no plasmid, and 65.59% G+C content) and its annotation, which consists of 4,194 genes (3,447 genes with predicted functions), 48 RNA genes (3 rRNA and 45 tRNA) and 261 genes with peptide signals. This research was supported by the Basic Science Research Program through the Ministry of Science, ICT, and Future Planning (NRF-2013R1A2A1A0100932).

Keywords: Mycobacterium tuberculosis, Korean Beijing strain, Outbreak, TB clinical strain

E-2
Metagenome-based Microbial Community Structure of the Arctic Soil from MidtreLovénbreen Glacier Foreland, Svalbard

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Climate change influences glacial ecosystems by affecting the advance and retreat of glaciers. The exposed glacier foreland provides great opportunities to study the structure change of microbial communities through soil age of glacier retreat. This study focused on the influence of glacial retreat on the bacterial community structure from the MidtreLovénbreen glacier foreland in Svalbard (79°N). Six soil samples of different ages were collected and their microbial community structures were analyzed by metagenome sequencing analysis using the Ion Torrent Personal Genome Machine (PGM) platform based on semiconductor technology. Millions of reads were respectively utilized for the analysis of microbial structure based on the metagenome sequences of the arctic soil samples. Proteobacteria (39.0-50.3%) and Bacilli (16.7-25.0%) were showed to be predominant bacterial groups in arctic soil samples. Euryarchaeota (78.1-86.0%) and Crenarchaeota (9.3-12.7%) were major group of Archaea. In case of Eukaryote, Ascomycota (17.9-45.9%) and Streptomycota (18.0-23.4%) were majorly showed. The current study finally suggests that the Ion Torrent PGM platform could be suitably applied to analyze the whole microbial community structures, giving the basis of assessing the relative importance of predominant groups on the each microbial community in the glacier foreland of MidtreLovénbreen with a high resolution.

Keywords: Arctic, MidtreLovénbreen, Microbial community, Metagenome, Ion Torrent PGM

E-3
Completion of the Circular Mitochondrial Genome of Pepper Anthracnose Caused by Colletotrichum acutatum

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Colletotrichum acutatum is a fungal plant pathogen that causes pre- and postharvest anthracnose on a wide range of plants worldwide. The genome sequences of several Colletotrichum species including C. gloeosporioides, C. graminicola, C. higginsianum and C. orbiculare have been uncovered, providing a valuable resource for large scale functional genomic approaches. Here, we report the analysis of the complete mitochondrial DNA sequence of a C. acutatum isolate KC05 obtained from an infected pepper collected in Kangwon province of South Korea. The mitogenome of C. acutatum KC05 was sequenced using platforms of the PaclBio RS II system and MiSeq system. This study revealed that the mitogenome of C. acutatum is a closed circular molecule of 30,892 bp in length, with a G + C content of 34.7%, which include 15 protein-coding genes, 22 tRNA genes and 2 rRNA genes. All the protein-coding genes, accounting for 46.6% of the C. acutatum mitogenome, start with the standard ATG codon and end with the TAA termination codon except for nad6 gene using the TAG termination codon. The mitogenome information of C. acutatum can provide molecular basis for further studies on molecular systematics and evolutionary dynamics.

Keywords: Mitogenome, Colletotrichum acutatum, anthracnose, pepper, ascomycota

E-4
Useful Gene Identification of Bacillus methylotrophicus CBMB205 Isolated from Rhizosphere Soil of Rice (Oryza sativa L.)

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Bacillus methylotrophicus, designated strain CBMB205, was isolated from the rhizosphere soil of field-grown rice. The genomic DNA was isolated and sequenced using PaclBio platform with 259 coverage. Total 77,620 reads were produced, and the reads were assembled by PaclBio SMRT Analysis 2.3.0. The genome of B. methylotrophicus CBMB205 had 3,929,745 base pairs circular chromosome with 46.50% GC contents and consists of 2,846 genes. It contained 27 rRNA and 86 tRNA sequences, and 10% and 8% of the total genes involved in metabolism including amino acid transport and transcription, respectively. By MUMMER analysis, CBMB205 genomic sequence was similar with B. amyloliquefaciens. In addition, we detected genes involved in plant growth promotion. CBMB205 has 53 genes related with siderophore production, 42 genes related with heavy metal resistance, and 3 genes related with sulfur oxidation activity. This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries(IPET) through Agricultural Microbiome R&D Program, funded by Ministry of Agriculture, Food and Rural Affairs(MAFRA)(No. 914004-04).

Keywords: Bacillus methylotrophicus, genome, rhizosphere, siderophore
Isolated from Rhizosphere Soil of Sesamum indicum L.

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Methylobacterium phyllophoraeae, designated strain CBMB27, was isolated from leaf tissues of rice. CBMB27 utilize the methanol emitted from plants as a plant symbiont and promote plant growth. The genomic DNA was isolated and sequenced using PacBio platform with Illumina MiSeq platform with 556.18 coverage. PacBio platform produced total of 83,123 reads and Illumina MiSeq platform produced total of 10,649,669 reads. And these were assembled by PacBio SMRT Analysis 2.3.0 and CLCbio CLC Genomics Workbench.

In addition, three plasmids were detected and their lengths were 144,167 bp (designated CBMB27-p1), 52,290 bp (CBMB27-p2) and 36,169 bp (CBMB27-p3), respectively. Each GC contents were 63.13% in CBMB27-p1, 63.25% in CBMB27-p2, and 63.20% in CBMB27-p3. The CBMB27 genome consists of 5,796 CDS containing 12 rRNA and 57 tRNA sequences. In CBMB27, we identified several genes modulating plant growth such as 1-aminoacyltransfer-1-carboxylic acid deaminase activity, siderophore production, heavy metal tolerance and reduction of phytotoxicity. This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agricultural Microbiome R&D Program, funded by Ministry of Agriculture, Food and Fisheries.

Keywords: Microevolution of Methylobacterium phyllophoraeae, genome, plant symbiont

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A simple Screening Method Using Lignocellulose Biodegradation for Effective Breeding in Agaricus bisporus

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The white button mushroom, Agaricus bisporus is commercially the fifth most edible mushroom, accounting for the production of 6,678 tons in Korea, 2013. The genus Agaricus has been known for its potential to degrade lignocellulosic materials. In chemical analysis during the cultivation of A. bisporus, the cellulose and hemicellulose and lignin fraction were changed preferentially for each vegetative growth and sexual reproduction. We screened strains with effective biodegradation under celluose, xylose and laminoyetic enzyme to shorten mycelial running period of A. bisporus. The enzyme biodegradation were conducted as follows; mycelia of collected strains were cultivated in 0.5% CMC-MMP (malt-mops-peptone, 0.5 Xylan-MMP, 0.5% lignin-MMP) media for 14 days. And cultured mycelia were stained with 0.2% Congo-red. The mycelial growth was examined to group on different enzyme biodegradation in MMP media. Twenty-four strains were decided 6 groups. These strains also were cultivated in compost media to identify correlation of mycelial growth between MMP and compost media. Selected strains were confirmed expression of genes related to lignocellulose biodegradation. It was suggested this method is simple screening of strains of rapid growth to improve breeding efficiency of A. bisporus.

Keywords: Screening method, Agaricus bisporus, Lignocellulose, Biodegradation

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Genome Analysis of Psychrophilic Arthrobacter sp. PAMC 25486 Isolated from the Arctic Region Reveals Evolutionary Implications of the Genus Arthrobacter

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The genome diversity of closely related species has shown the micro-evolution processes that occur during environmental adaption. Arthrobacter species are ubiquitous aerobic bacteria isolated from various environmental niches such as food, soil and Antarctica. Recently, we newly isolated the psychrophilic strain PAMC 25486 from Spitsbergen Island in Artic region (78°55'41.7"N, 11°56'11.9"E) and sequenced its genome completely. It comprises a 4,593,579-bp long circular chromosome with 4,366 protein coding genes and 71 RNA genes. To investigate the evolutionary changes, we systematically characterized the genetic variation of strain PAMC 25486. The phylogenetic analysis at the genomic level and MLSA analysis showed that the species of Arthrobacter were divided into four large groups and strain PAMC 25486 was deeply branched from other strains. Gene repertoire relatedness (GRR) based on ortholog gene clustering revealed that the Arthrobacter strains have shared more than 60% of gene families within each group, but Arthrobacter core-genome contains only 515 families corresponding to 5% of the size of pan-genome families. Although PAMC 25486 belonged to none of the four groups, it shared gene families (approximately 44%) with each of the four groups, suggesting that PAMC 25486 is a highly divergent strain. These genetic variations among Arthrobacter strains can provide insight into evolution changes in their traits in response to environmental adaptation.

Keywords: Arthrobacter sp. PAMC 25486, Psychrophile, Artic region, Genetic evolution, Genome sequencing
**E-9**

**Comparative Genome Analysis of Three Genera Belonging to the Family Streptomycetaceae**

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The family Streptomycetaceae currently encompasses three genera, *Streptomyces*, *Kitasatospora* and *Streptacidiphilus*. The family is characterized by a complex life cycle involving morphological differentiation, a rich repertoire of secondary metabolites, large and linearized chromosomes, and high G+C contents. Members of the genera *Kitasatospora*, *Streptacidiphilus* and *Streptomyces* share a number of key characteristics, but each forms a stable monophyletic branch in the 16S rDNA tree. Members of these genera are widely distributed in various environmental conditions, and their importance as producers of useful metabolites such as enzymes and bioactive compounds is well recognized.

In this study, the genomes of three genera *Kitasatospora*, *Streptacidiphilus* and *Streptomyces* were subjected to comparative analysis, and genes involved in metabolic and physiological activities were searched. The size of the genome ranged from 7.81Mb to 10.43Mb, the DNA G+C content from 71% to 74%, and the number of predicted protein-coding genes were between 6,920 and 9,239. The three species were subjected to comparative analysis, and genes involved in metabolic and physiological activities were searched. The size of the genome ranged from 7.81Mb to 10.43Mb.

**Keywords:** genome, *Streptomycetaceae*, *Streptomyces*, *Streptacidiphilus*, *Kitasatospora*

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**E-10**

**Complete Genome Analysis of Dyella thiooxydans ATSB10 Isolated from Rhizosphere Soil of Sunflower (Helianthus annuus L.)**

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*Dyella thiooxydans* designates strain ATSB10 was isolated from rhizosphere soil of sunflower. Cells were gram-negative, aerobic, motile, and rod-shaped. We investigated the genetic properties of ATSB10 using next generation sequencing technology. The genomic DNA was isolated and sequenced using PacBio platform and Illumina MiSeq platform with 10/70:06 coverage. PacBio platform produced total of 81,855 reads and Illumina MiSeq platform produced 13,081,160 reads. And these were successfully assembled by PacBio SMRT Analysis 2.3.0 and CLCbio CLC Genomics Workbench 7.5.1. The genome of *D. thiooxydans* ATSB10 had 4,227,172 base pairs circular chromosome with 67.98% GC contents and consists of 3,862 CDSs. It contained 6 rRNA of 16S, 23S and 5.8S rRNA sequences, 8% and 7% of total genes involved in cell wall/membrane/envelope biogenesis and amino acid transport, respectively.

These results were similar to the previously reported genes *Dyella* genome sequences. Strain ATSB10 had thiosulfate-oxidizing function, so it is thought that the strain related to plant growth promotion. In further studies, genes involved in plant growth promotion of ATSB10 should be identified. This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries(IPET) through Agricultural Microbiome R&D Program, funded by Ministry of Agriculture, Food and Rural Affairs(MAFRA)(No. 914004-04)

**Keywords:** *Dyella thiooxydans*, genotype, rhizosphere

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**E-11**

**Biochemical Characterization of Laccase Isozymes in Pleurotus ostreatus**

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In this study, transcriptome analysis of twelve laccase genes in *Pleurotus ostreatus* revealed that their expression was differentially regulated at different developmental stages. Lacc5 and Lacc12 were specifically expressed in fruiting bodies and primordia, respectively, whereas Lacc6 was expressed at all developmental stages. Lacc1 and Lacc3 were specific to the mycelial stage in solid medium. In order to investigate their biochemical characteristics, these laccases were heterologously expressed in *Pichia pastoris* using the pPIC3HOL1-2 expression vector. Expression of the laccases was facilitated by intermittent addition of methanol as an inducer and sole carbon source, in order to reduce the toxic effects associated with high methanol concentration. The highest expression was observed when the recombinant yeast cells were grown for 5 days at 15°C with intermittent addition of 1% methanol at a 12 h interval. Investigation of enzyme kinetics using 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diaminomine salt (ABTS) as a substrate revealed that the primordium-specific laccase Lacc12 was 5.4 fold less active than Lacc6 at low substrate concentration with respect to ABTS oxidation activity. The optimal pH and temperature of Lacc12 were 0.5 pH units and 5°C higher than those of Lacc6. Lacc12 showed maximal activity at pH 3.5 and 50°C, which may reflect the physiological conditions at the primordiation stage.

**Keywords:** Heterologous expression, Laccase, Mushroom, Primordia

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**E-12**

**Genetic Heterogeneity of Varicella-Zoster Virus**

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Primary infection of Varicella-zoster virus (VZV) results in chickenpox (varicella) and reactivation from latency often leads to shingles (zoster). Live attenuated vaccines have been developed based on Japanese Oka and Korean MAV/06 strains. There have been reports suggesting that the Oka-derived VZV vaccines contain quasispecies with mixtures of attenuated vaccine-types and wild-types, which could be supported by genome sequence data. Thus, we attempted to understand the nature of VZV genetic heterogeneity by determining the relative frequencies of 4 nucleotides at each position of VZV whole genome. Three vaccine strains (Suduvax, Varilrix, Varivax) and 3 clinical strains from Korean patients with 3 different passage histories in vitro cell culture were subjected to next-generation sequencing. The numbers of genetically heterogeneous sites were found to be greater in vaccine strains than in clinical strains. Percentages of wild-type sequences and vaccine-type sequences were obtained for each of the 24 vaccine-specific sites from NGS data. The 18 sites in vaccine strains were found to be genetically heterogeneous where the percentage of minor base was relatively high, up to even more than 40%. Interestingly the numbers of genetically heterogeneous sites in high passed clinical strains were greater than those in low passed clinical strains. Thus, attenuation and in vitro passaging appear to increase the genetic heterogeneity of VZV, resulting in quasispecies.

**Keywords:** Varicella-zoster virus, quasispecies, genetic heterogeneity, vaccine-specific sites
Characterization of Copper-Inducible Fungal Laccase Promoter in P. pastoris expression system

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The promoter region of copper-inducible laccase gene, LCC1, from Pycnoporus coccineus was explored in the heterologous expression of foreign protein in P. pastoris. The promoter region (P_{PLCC1}) was isolated and used to replace the methanol-inducible AOXI promoter (P_{AOXI}) of pPICHOLI-2, an episomal expression vector for P. pastoris, to generate a new copper-inducible expression vector. The promoter activity of P_{PLCC1} was compared with those of P_{AOXI} and P_{EXP1}, a copper-inducible promoter of a commercial vector pPIC3K-C, using a laccase gene as a reporter gene in P. pastoris G5115. Reporter laccase activity of the culture broth reached 182 units/L and 43 units/L for P_{PLCC1} and P_{EXP1}, respectively, after induction with 0.2 mM CuSO4 at OD600=1 and culture for 120 h at 15°C in complex medium containing 1% glucose. For P_{AOXI} activity, yeast cells harboring P_{AOXI}-laccase plasmid were cultured for 120 h at 15°C in complex medium with intermittent feeding with 1% methanol every 12 h to avoid methanol toxicity. Laccase activity of culture broth was 124 units/L. Conclusively, P_{PLCC1} is a new copper-inducible promoter that shows superior performance in terms of efficiency of laccase production compared to commercial vectors. P_{PLCC1} is additionally superior to P_{AOXI} since it does not require laborious feeding with a carbon source.

Rapid Gene Knockout Method Using Single Helper Plasmid Expressing Red and Cre Recombinases

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Development of rapid gene manipulation tools is essential for efficient metabolic engineering approaches. In this study, integration helper plasmid was developed for rapid gene manipulation. The integration helper plasmid pCW611 expresses two recombinases (Red and Cre) by using two independent (IPTG and Arabinose) inducible systems. Required time and effort can be significantly reduced compared to conventional method by using integration helper plasmid because the iterative transformation of the helper plasmid and curing steps are not required. We could delete one target gene in 3 days by using pCW611. To verify the usefulness of this gene manipulation system, the deletion experiments were performed for knocking out four target genes individually (adaB, sfoA, fadABCD, and uckA) and two genes simultaneously for two cases (adaB-espI and sfoA-aspD). Also, furamic acid producing E. coli strain was developed by deleting four target genes (fumB, iclR, fumE, and fumC) in 10 days as a proof-of-concept study. Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MISP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AA001-2012M1A2A2026556)

Keywords: Integration Helper Plasmid, Metabolic Engineering, Escherichia coli

Water Extracts from Spent Mushroom Substrate of Hericium erinaceus induce Expression of Defense Genes in Tomato Plants

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Previously, Ralstonia solanacearum, which causes bacterial wilt of tomato plants was suppressed by treatment of water extract from spent mushroom substrate (WESMS) of . It was assumed that anti-bacterial compound in SMS is a major disease suppression factor. In addition, this study was carried out to find possibility that SMS extract can induce defense response. The ethylene (ET), Jasmonic acid (JA) and salicylic acid (SA) defense signal transduction pathways have been known in defence resistance mechanisms. WESMS was treated in tomato plants and extracted total RNA from tomato leaves with different hours. Expression quality of the pathways related genes was determined by quantitative real-time PCR. WESMS induced expressions of defense genes encoding α,1,3-glucanase (GluA) and pathogenesis-related protea (PR-1a) associated with systemic acquired resistance (SAR) over 20-50 folds. These results suggest that WESMS triggers significant SA pathway defense gene expression

Keywords: Spent mushroom substrate, Water extract, Defense gene, qPCR, Expression

The MpkB MAPK is Not Essential for Sterigmatocystin Production in Aspergillus nidulans

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Aspergillus nidulans mitogen-activated protein kinase (MAPK) encoded by mpkB was known to coordinate sexual development as well as secondary metabolism. Also, it had been reported that the mpkB gene would regulate sterigmatocystin (ST) gene expression and produce mycotoxin at low levels. However, in the results of our TLC investigation, we found that mpkB constitutively inactivated mutant also showed no significant effect on the ST production. However, ST production of mpkB and mkkB mutants was remarkably delayed in the veA+ background were not different with compared to wild type. Furthermore, MpkB constitutively activated mutant and MpkB constitutively inactivated mutant also showed no significant effect on the ST production. However, ST production of mkkB and mkkB mutants was remarkably delayed in the veA+ background. The biosynthesis genes required for ST production (afrB, stcE and stcJ) was constitutively expressed in each mutant of the MAPK module. These data indicate that mpkB does not affect the expression of genes involved in ST production. Our results suggest that the signal of MpkB MAPK and the ST production pathway were independent.

Keywords: MAPK, mppK, mkkB, Sterigmatocystin, Aspergillus nidulans
Functional Analysis of Transcription Factors Containing CCAAT-DNA Binding Domain

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The homothallic ascomycete fungus Fusarium graminearum is an important plant pathogen of major cereal crops. The CCAAT sequence is present in roughly 30% of eukaryotic promoters. In filamentous fungi, the CCAAT sequence has been known to modulate the expression of several critical genes involved in various developmental stages. In this study, we functionally characterized two transcription factors FCT1 and FCT3 containing the CCAAT DNA-binding domain. Both deletion mutants have similar defects in mycelia growth, sexual reproduction, and virulence. Double deletion of FCT1 and FCT3 resulted in indistinguishable phenotypes compared to single deletion mutants. Moreover, Fct1 and Fct3 physically interact with each other and localized to nuclei, suggesting that Fct1 and Fct3 form protein complex for transcriptional regulation. This is the first study dealing with transcription factors containing CCAAT DNA-binding domain in F. graminearum and will present weighty perception to subsequent studies.

Keywords: Fusarium graminearum, Plant pathogen, Transcription factor, CCAAT-DNA binding domain

The whcD Gene of Corynebacterium glutamicum Is Important for Cell Growth and Affects Responses to Hydrogen Peroxide-mediated Oxidative Stress

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In this study, we analyzed the whcD gene from Corynebacterium glutamicum, which encodes a homologue of WhiB, a Streptomyces coelicolor protein required for the sporulation of aerial hyphae. The ∆whcD strain not only showed retarded growth rate but also showed decreased cell yield on complex media, and lost the ability to grow on minimal media. In addition, the WhcD cells were found to be elongated and aggregated during growth as observed by SEM. The expression of the whcD gene was constitutive during growth, suggesting a housekeeping role. Disruption of the whcD gene resulted in a marked increase in katA mRNA level, and the ∆whcD strain showed a hydrogen peroxide resistant phenotype. But the ∆whcD strain showed increased sensitivity to oxidant diamide and menadione. Subsequently, determination of the proteins under the regulation of whcD using two-dimensional electrophoresis identified several proteins that were regulated in the ∆whcD strain. While expression of the protein fatty acid synthase (fasB) and two component response regulator (frsA) increased, expression of pyruvate dehydrogenase E1 subunit (aceE) decreased in the ∆whcD strain. Our results suggest that the whcD gene plays an important role in cell growth and response to oxidative stress. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A4401004556).]

Keywords: Corynebacterium glutamicum, oxidative stress, WhiB

sRNA Regulates the Anaerobic Induction of FrsA Expression

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Fermentation respiration switch (FrsA) is an enzyme catalyzing pyruvate decarboxylation. Since the cellular level of FrsA was minimal in Vibrio vulnificus cells grown under aerobic conditions, the regulatory mechanism for the anaerobic induction of FrsA was investigated in this study. Transcript quantitation showed no significant difference of frsA mRNA levels in cells grown in the presence or absence of oxygen. This result suggests that regulation at the post-transcription level is important in FrsA expression, leading us to examine the presence of a putative regulatory sRNA. A candidate FrsA-regulating sRNA (Frr), including the sequences complementary to the 5′-UTR of frsA mRNA, was identified in V. vulnificus genome. A northern blot revealed the presence of 350 nucleotide-long sRNA. Its regulatory role was investigated by comparing the cellular contents of FrsA in wild type and its isogenic fr deletion mutant. In the absence of Frr, the negative effect of oxygen on the FrsA level was abolished. This oxygen-dependent repression of FrsA expression by Frr was achieved through the repression of Frr expression by Frr under anaerobic conditions. Thus, this study demonstrates the cellular content of FrsA is minimized during aerobic growth via repression by Frr. This repression, however, is relieved during anaerobic growth via repression of the frr transcription by Frr, resulting in higher FrsA activity.

Keywords: FrsA, small RNA, post-transcription, translational repression

Comparative Genome Analysis of Five Lichen-Forming Fungi and Two Symbiotic Algae

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Lichens are symbiotic organisms, composed of a fungal partner (the mycobiont) and at least one eukaryotic algal or cyanobacterial species (the photobiont). As demonstrated by the world-wide distribution of lichens in various kinds of habitats from the tropics to the polar regions, lichen symbiosis seems to be a highly successful adaptation to a diverse range of environmental conditions. In order to understand the symbiosis, a total of five lichen-forming fungal isolates and two algal isolates were selected to analyze. For the five sequenced lichen-fungal genomes, average size and the number of predicted genes were 36.05 Mb and 97468, respectively, and two sequenced algal genomes, average size and the number of predicted genes were 55.14 Mb and 8,995, respectively. By integrating information of genome, gene family annotation and bioinformatics tools, a web-based genomics portal will be developed for comparative and evolutionary genomics in lichen. This finding will enhance our understanding of the adaptive evolution of the lichen-forming fungi with the algae to their ecological niches.

Keywords: Comparative genomics, Lichen-forming fungi, Symbiotic algae, Whole-genome sequencing
Hypsizygus marmoreus (or Hypsizygus marmoreus) that is also known as brown beech, is an edible mushroom. It is native to East Asia, one species of the genus Hypsizygus, with the white beech mushroom, white clamshell mushroom, buna-shimeji etc. It is also industrialised due to its inflammatory, anti-bacterial and anti-viral activities are reported. RDA and Korea University consortium launched the genome sequencing project in 2014. The material is diploid strain Haemi, collected in Korea. The two haploid strains 51987-8 and Haemi-18 generated from the diploid. 71X Illumina sequencing data lead the genome feature with 30.3 Mb and 5,899 protein coding genes. About 70% of the annotated genes have no roles in metabolic process. Approximately 22% of genes involved biological process were predicted to play roles in metabolic process.

Keywords: genome, Hypsizygus marmoreus, Hypsizygus marmoreus, brown beech, mushroom

E-22

RDS1 Involved in Yeast-To-Mycelium Transition in Lichen-Forming Fungus Umbilicaria muehlenbergii

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Lichen is a symbiotic organism between a fungus (as mycobiont) and an alga (as photobiont). It is generally known that axenically cultured lichen-forming fungi grow extremely slowly as hyphal form. Unlike other lichen-forming fungi, dimorphic lichen-forming fungus, Umbilicaria muehlenbergii, grows easily and quickly as yeast-form cells in liquid nutrient media. This observation allows establishing the first successful transformation system for lichen-forming fungi. Here we report a gene, RDS1 (Regulation of fungal Dimorphic Switch 1), which controls the dimorphic switching between yeast form and hyphal form of U. muehlenbergii. In the previous study, we generated random insertion transfectants using yeast-form cells of U. muehlenbergii using Agrobacterium tumefaciens-mediated transformation (ATMT). More than 1,000 transformants have been created. Among them, a hyphal form growing mutant, UmT-270, was observed to identify T-DNA insertion region of the mutant UmT-270, thermal asymmetric interlaced polymerase chain reaction was employed and reveal that the T-DNA was inserted in upstream region of a putative C2H2-type zinc finger transcription factor RDS1. Light microscopic and scanning electron microscopic observation clearly showed that the mutant UmT-270 was apparently hyphal form and suggested that the responsible gene, RDS1, might be involved in dimorphic switching of U. muehlenbergii.

Keywords: ATMT, Dimorphic fungi, Lichen-forming fungus, Transcription factor, Umbilicaria muehlenbergii

E-23

Draft Genome of the Streptomyces rubrolavendulae MJM4426, a Strain Producing Staurosporine

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Streptomyces rubrolavendulae MJM4426, a producer of the staurosporine, has isolated from soil in the Korea. Here we summarize the features of the strain MJM4426 and described its permanent draft genome sequence. The genome contains one linear chromosome consists of 6,543,262 base pairs, with an average G+C content of 74.8%, 5,425 coding sequence, 18 rRNA genes, and 96 tRNA genes. A large number of genes were associated with the COG functional categories of 8.02% transcription, 3.80% energy production and conversion, 5.38% carbohydrate transport and metabolism, and 5.77% amino acid transport and metabolism.

Keywords: Streptomyces rubrolavendulae, MJM4426, Genome sequence, Staurosporine gene cluster

E-24

Analysis of Complete Genome Sequence and Virulence Factors of Bacillus cereus FORC_010, Food-borne Pathogen Isolated from Fresh Kimchi in Korea

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B. cereus FORC_010 was isolated from fresh kimchi in South Korea, and its genome was sequenced by hybrid of two Next-Generation Sequencing (NGS) platforms, Illumina Miseq and PacBio RS II. Genome analysis was performed by various bioinformatics tools: RBS finder, Rapid Annotation using Subsystem Technology (RAST) server and Virulence factors (VFs) database. B. cereus FORC_010 has single chromosome and one plasmid. The chromosome is 5,326,039-bp in length and contains 5,147 ORFs with G+C content of 35.28%. The plasmid pFORC10-F is 12,252-bp in length and contains 17 ORFs with G+C content of 34.11%. 5% of ORFs encoded hypothetical proteins, while 3,886 ORFs and 8 ORFs were predicted to encode functional proteins, respectively. Analysis of VFs revealed all of three major enterotoxins: cytoxin K, hemolysin enteroxin (HLE) and nonhemolytic enteroxin (NHE). These findings suggest B. cereus FORC_010 can be regarded as a potential factor related to enteric illnesses in human. This research was supported by a grant (14162MFDS972) from the Ministry of Food and Drug Safety, Korea in 2015.

Keywords: Bacillus cereus, Food-borne pathogen, Complete genome sequence, Next-Generation Sequencing
Whole Genome Sequencing of *Faecalibaculum rodentium* ALO17, Isolated from C57BL/6J Laboratory Mouse Feces

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Intestinal microorganisms affect host physiology, including aging. In this study, we report on the complete genome of *Faecalibaculum rodentium* ALO17, a bacterium that was isolated from the feces of a 9-month-old female C57BL/6J mouse. This strain will be utilized in future in vivo studies detailing the relationships between the gut microbiome and aging. The whole genome sequence of *F. rodentium* ALO17 was obtained using single-molecule, real-time (SMRT) technique on a PacBio instrument. The 16S rRNA gene of ALO17 was 86.9% similar to that of *Allobaculum stercoricanis* DSM 13633T, and the average overall nucleotide identity between strains ALO17 and DSM 13633T was 66.8%. After confirming the phylogenetic relationship between *F. rodentium* ALO17 and *A. stercoricanis* DSM 13633T, their whole genome sequences were compared, revealing that *F. rodentium* ALO17 contains more fermentation-related genes than *A. stercoricanis* DSM 13633T. Furthermore, *F. rodentium* ALO17 produces higher levels of lactic acid than *A. stercoricanis* DSM 13633T as determined by high-performance liquid chromatography. The availability of the *F. rodentium* ALO17 whole genome sequence will enhance studies concerning the gut microbiota and aging. This work was supported by the Research Program for Agricultural Science & Technology Development (PJ010168012015).

Keywords: whole genome sequencing, comparative genomics

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Development of Open Source Genome Annotation Pipeline and Interactive Visualization Server

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For understanding genomes in a functional context, the accurate annotation of genome sequences is essential. Although many open-source or commercial software platforms are available for prokaryotic genome annotation, it is still not an easy task for general researchers to process and visualize genome sequencing data in currently available annotation platforms. Here we introduce fully automated prokaryotic genome annotation pipeline server based on the open-source softwares and R package. The pipeline takes a set of contig or scaffold sequences from user’s submission and automatically proceeds the annotation by predefined steps in the pipeline server. Users can download final annotation results in the submission ready-format for public database deposition. To improve the accuracy of gene prediction and functional annotation, we integrated multiple programs and developed selection algorithms at each step in the pipeline. The platform also provides the web-based genome browsing for interactive analysis of genome data. The benchmark test was proceeded to validate the precision of the pipeline. The genome assembly data in the NCBI genome database was used and the annotation result of pipeline showed comparable or better quality for submission. Our prokaryotic genome annotation service is expected to perform as easily-accessible resource for making annotation of bacterial and archaeal genomes to many researchers who are less familiar with bioinformatic tools.

Keywords: Genome annotation, Genome browser, Bioinformatic resource, NGS data, Genomic data analysis

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Developing Fungal Genome Annotation Pipeline: From Gene Prediction to Functional Annotation

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In order to understand the diversity and ecological function of fungal species, a variety of fungal genome sequencing projects (e.g., 1000 fungal genome project) are ongoing. Accurate gene prediction is one of the most critical parts to understand diverse ecological functions of fungi on earth. Many public or commercial annotation pipelines are available but no fungal genome specific annotation pipeline is known yet. Here, we developed a fungal genome annotation pipeline (FGAP) combining open-source gene prediction and annotation programs. The developed pipeline take various types of genomic sequences as an input file (genome assembly, ESTs or ORFs, etc.) and give the annotation results in the GFF3 format. We benchmarked a yeast genome and retrieved 4,480 genes models with a scoring function based on BLASTp. The FGAP annotate the gene models with the Pfam, and KEGG, and GO database and compared the results to the original annotation. The FGAP is a single-stream procedure to improve accuracy of gene prediction.

Keywords: Fungi, Gene annotation, Gene prediction pipeline, Fungal genome

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A Study on Biological Diversity with Bioinformatics

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Biological diversity, also known as biodiversity, is an important criterion for measuring the value of an ecosystem. As biodiversity is closely related to human welfare and quality of life, many efforts to restore and maintain the biodiversity of species have been made by government agencies and non-governmental organizations, thereby drawing a substantial amount of international attention. In the fields of biological research, biodiversity is widely measured using traditional statistical indices such as the Shannon-Wiener index, species richness, evenness, and relative dominance of species. However, some biologists and ecologists have difficulty using these indices because they require advanced mathematical knowledge and computational techniques. Therefore, we developed VBioindex, a user-friendly program, which is capable of measuring the Shannon-Wiener index, species richness, evenness, and relative dominance. VBioindex serves as an easy to use interface and visually represents the results in the form of a simple chart and in addition, VBioindex offers functions for long-term investigations of data sets using time-series analyses.

Keywords: Biodiversity, Bioinformatics, Ecology
**E-29**

**Analysis of Mutational Events of Varicella-Zoster Virus Passaged in vitro Cell Culture**

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Varicella-zoster virus (VZV) is a causative agent for chickenpox in primary infection and shingles after reactivation from latency. Some attenuation mutations were also found in highly passaged clinical strains such as Ellen and 32p72. In this study, we attempted to understand mutations in highly passaged VZV strains. Clinical strains YC01, YC02 and YC03 isolated from Korean patients were passaged in vitro cell culture more than 100 times. Different passages of YC01 (p13, p61, p110), YC02 (p14, p61, p110) and YC03 (p6, p61, p110) were subjected to next-generation sequencing and their full genome sequences were compared with each other. Mutations were detected at 300, 256 and 126 sites in the strains YC01, YC02 and YC03, respectively. Most of the mutations were A→G or T→C substitutions. Approximately 70 – 80% of mutations were found in open reading frames, and ORF 62 exhibited the most number of mutations. Fifteen mutations were common to three strains, including a read-through mutation at position 560 and 11 substitutions in ORF62. Change in the codon usage at mutation sites between low and high-passaged strains were investigated. Codon adaptation index (CAI) values decreased while %GC values increased at mutation sites between low and high-passaged strains. Analysis of mutational events of Varicella-Zoster Virus passaged in vitro cell culture further studies of in vitro passaging with different strains and under attenuating conditions will help to understand the mechanism of attenuating mutations in VZV.

Keywords: Varicella-zoster virus, high-passaged strain, Codon adaptation index

**E-30**

**Complete Genome Sequence of Multidrug-Resistant Staphylococcus haemolyticus S167 Strain Which Forms icu-Independent Biofilm**

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Biofilm is considered determinants of pathogenicity in staphylococcal infections. Biofilm forming ability of newly isolated strains from leaf vegetables were tested with crystal violet assay. One of isolated strain which produces thick biofilm matrix was identified with vegetables were tested with crystal violet assay. One of isolated strain from leaf vegetables was identified with 32s rRNA sequencing. The strain was identified with Staphylococcus haemolyticus and named Staphylococcus haemolyticus S167. To investigate various mechanisms of biofilm associated genes, complete genome sequencing was performed. The genome of Staphylococcus haemolyticus S167 consists of one chromosome with 2,549,338 bp (GC content of 32.83%) and one circular plasmid with 10,808 bp. The entire genome contains 2,456 protein-encoding sequences (CDS), 19 rRNA genes and 59 tRNA genes. Interestingly, there was no icu operon, which have been reported to play an important role in biofilm formation in Staphylococcus species. Several icu-independent mechanisms for biofilm formation and those related genes such as bap, fnbAB, arRS and arf have been reported. Two component system genes arRS and major autolysin gene arf was found in sequence analysis of Staphylococcus haemolyticus S167. This information could be the basis of icu-independent mechanism of biofilm formation studies.

Keywords: complete genome sequence, biofilm, Staphylococcus haemolyticus

**E-31**

**Production and Role of Nitric Oxide During Development in Neurospora crassa**

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Nitric oxide is a signal molecule that plays important role in the immune, nervous and cardiovascular systems of mammals while in plants it is involved in regulating germination, development, and disease resistance. Studies on nitric oxide are very rare in fungi compared to mammals and plants. In this study, we used Neurospora crassa (a model filamentous fungus) to investigate the production and role of nitric oxide during asexual development. Intracellular nitric oxide detected by using DAF-FM diacetate (20 μM) was increased during hyphal growth in liquid VM after 8 h and its level was reached to the highest after 16 -24 h. After fungal mycelial mat was incubated on VM agar media for 16 hrs (conidiation), higher level of nitric oxide was detected in conidiophores. When fungus was treated with cPTIO, a nitric oxide scavenger, intracellular nitric oxide was scavenged and hyphal growth in liquid VM and conidiophore development were slightly slowed down. cPTIO treatment during conidiation (16 h) also caused a reduction in expression of conidiation related genes, particularly con-6, con-10, and con-13. Exogenous nitric oxide (treat with SNP or NO producing nanoparticle) seems to enhance hyphal growth and conidiation in wild type. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP), No. 2010-0027963 and No. NRF-2013R1A1A01011245.

Keywords: nitric oxide, conidiation, fungal development, Neurospora crassa, hyphal growth

**E-32**

**Development of Highly Sensitive Cadmium and Lead Microbial Biosensors Using Synthetic CadC-T7 Genetic Circuits**

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Biological components with relevant functions can be excrated from enormous genomic and metagenomic sequence databases, and then reassembled to generate synthetic circuits for biosensors. However, we still lack specific knowledge and/or screening tools that meet the demand for mining useful biological modules from similar or redundant genes in the databases. Here, we demonstrated how to obtain sensory and regulatory components with distinct specificities from reiterated paralogous CadC genes in the genome of Bacillus oceanocystemains 2691 that was isolated from ocean sediment. Quantitative RT-PCR showed that each CadC regulator differentially responded to heavy metal ions at nano-scale concentrations. This study therefore suggests that our synthetic microbial cells may be potentially applicable for the development of highly specific heavy metal biosensors [This work was supported by the National Research Foundation of KOREA (NRF), Center for Women In Science, Engineering and Technology (WISET), and by Next-Generation BioGreen 21 Program (SSAC, PJ01111802), RDA, Korea].

Keywords: Cadmium, Lead, Biosensor, Synthetic CadC-T7, Genetic circuits
Functional Analysis of Putative Peroxidases in *Fusarium graminearum*

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Fungal pathogens encounter toxic environments generated by the host defense mechanisms during infection. The oxidative burst, a rapid production of reactive oxygen species (ROS), is one of the host’s earliest responses to pathogen infection. Several fungal pathogens produce ROS-scavenging enzymes to protect themselves against the plant-derived oxidative stress. Peroxidases are group of antioxidant enzymes including catalase, catalase-peroxidase, peroxiredoxin, NAD(P)H oxidase, and so on. In this study, we performed a functional analysis of putative peroxidase genes in *Fusarium graminearum*, a major plant pathogen which causes *Fusarium* head blight (FHB) on cereal crops. We identified 32 putative peroxidase genes and generated deletion mutants for all 32 genes. These mutants were screened in various developmental stages including sexual and asexual development, virulence, toxin production, and the oxidative stress response. The results showed that all these mutants had no defects in virulence, and only four deletion mutants showed increased sensitivity to extracellular H$_2$O$_2$. Among these four deletion mutants, the deletion mutant of catalase-peroxidase gene, named *FCA7*, produced more trichothecenes compared to the wild-type strain. Our ongoing study will provide the insight into the understanding of peroxidases’ roles in *F. graminearum*.

Keywords: *Fusarium graminearum*, Peroxidase, Oxidative stress, Plant pathogen
Disease, Yonsei University College of Medicine, Seoul

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Formation of PrP Aggregates and their Morphological Analysis

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Tuberculosis(TB) is not only horrible disease that can cause infection everywhere in the body, but also infectious chronic diseases. According to a 2014 WHO report, for the year 2013 new TB patients have been reported in 200 countries and about 9 million people, 4.5 million, which was equivalent to about twice the incidence of TB patients in 2010. In TB laboratories, the core of TB diagnosis can be directly observed in clinical isolates of Mycobacterium tuberculosis(M. tb). The acid-fast stain of smears from respiratory specimen is an important role in the early diagnosis of mycobacterial infections because of simple, rapid method. In this study, we evaluated the stainability and results between manual staining and Smart Auto Stainer through Ziehl-Neelsen method, the most commonly used method. Acknowledgment. This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education Science and Technology (No. NRF-2013R1A1A0209687)

Keywords: Mycobacterium tuberculosis, Acid-Fast Stain, Manual Staining, Auto Stainer

F-2

Formation of PrP Aggregates and their Morphological Analysis

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Prion disease is an obvious example of neurodegenerative disease that often correlated with accumulation of PrP aggregation throughout the disease progression. Using bacterially expressed recombinant PrP and its peptide fragments, various aggregating conditions with each of their aggregating properties were examined. Series of variegated conditions were used to generate aggregated PrP. Results from these analyses demonstrated that ThT binding aggregates of both MoPrP(89-230) and PrP(106-126) were formed in the buffer containing 0.4 M guanidine hydrochloride and PBS. Other aggregating conditions with different concentrations of salt and pH showed distinctive efficacies in generating aggregates of PrP(106-126). The buffer containing 10 mM Na2HPO4, 10 mM NaH2PO4, 300 mM NaCl, and 50 mM glycine generated the highest level of aggregated products. We next visualized the PrP aggregates using atomic force microscopy (AFM) to elucidate their morphological patterns. AFM images revealed that the monomers aggregate into larger structures. However, the morphology of each aggregate generated under various conditions were different.

Keywords: Prion protein, Aggregate, Protein aggregate method, Morphology, Th-T binding assay

F-3

Development of Infectious Clones of a Wild-type Korean Rabies Virus and Evaluation of their Pathogenic Potential

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Most reverse genetic (RG) systems for rabies viruses (RVs) have been constructed on the genome background of laboratory-adapted strains. In this study, we developed an RG system using a street KGH strain to investigate the pathogenic potential of different strains. We developed a RG system with the KGH strain for the first time: The pKGH infectious clones were constructed using the CMV/T7 promoter, and hammerhead ribozyme (HamRz) and hepatitis delta virus ribozyme (HdvRz) were introduced to allow self-cleavage of the synthesized RNA. We successfully recovered the rescued virus by constructing chimeric RVs in which we replaced a part of the construct with the partial gene from the fixed RC-HL strain. The rescued viruses formed clearer and countable plaques in an immunostaining plaque assay, with a distinct plaque morphology. Furthermore, compared with the parental viruses, the pKGHRcimsA4 strain containing the KGH strain G protein exhibited a decreased efficiency of cell-to-cell spreading in BHK-21 cells and significantly reduced (100-1000 fold) replication kinetics. However, pKGHRcimsA4 strain-infected mice revealed 100% morbidity at 11 days post-infection, whereas other chimeric RV strains showed no mortality. Our RG system is a useful tool for studying differences in the cell-to-cell spreading efficiency and replication with respect to the different internalization patterns of street and fixed laboratory-adapted viruses.

Keywords: Rabies virus, KGH strain, RC-HL strain, reverse genetic system, street virus

F-4

Identification of Interacting Partners for Viperin in Human Cytomegalovirus Infection

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Viperin is an interferon-inducible protein that is directly induced in cells by human cytomegalovirus (HCMV) infection. Viperin’s function depends on its expression time point and cellular localization during HCMV infection. When viperin is expressed before HCMV infection, it localizes to endoplasmic reticulum and inhibits viral replication. However, HCMV-induced viperin localizes to mitochondria by interacting with a HCMV protein vMIA at early stage of infection and manipulates the cellular metabolism for efficient viral replication. Viperin co-localizes to the viral assembly compartment (AC) at late stage of infection, although its function is unknown. Viperin interaction with HCMV viral proteins is the key factor to determine viral replication status. Nevertheless, HCMV viral proteins which interact with viperin have not yet been identified except for vMIA. Here, we screened interacting partners for viperin using yeast two hybrid assay. We selected a HCMV tegument protein pp28 that is accumulated at the AC at late stage of infection and essential for viral replication. We confirmed that viperin interacts with pp28 in transient transfection or viral infection. We also observed that viperin and pp28 co-localize to the AC at late stage of infection. The results suggested that viperin has a function in cytoplasmic assembly of HCMV in the AC through the interaction with pp28. Further studies are underway to identify the significance of their interaction in HCMV infection.

Keywords: Viperin, HCMV, Tegument protein pp28, Interacting partners
is the first description of ESBL-producing STEC O103 strain isolated in Shiga toxin-producing Escherichia coli (STEC). Shiga toxin-producing Escherichia coli are a major pathogen that causes diseases in humans ranging from diarrhea to hemolytic uremic syndrome. STEC O157:H7 is still the predominant serotype isolated, whereas infection with non-O157 STEC has been recently reported in outbreak and sporadic cases. In June 2015, we investigated a multistate outbreak of STEC infections affecting attendees and staff at a school camp. STEC O103:H2 strains were isolated from 23 patients and from drinking water. All isolates displayed an identical XbaI pulsed-field gel electrophoresis pattern, except one strain (201501610) having one additional band at ~85 kb. Antimicrobial susceptibility test showed that outburst isolates were susceptible to all antimicrobial agents tested, whereas the isolate 201501610 was resistant to third generation cephalosporins. The isolate harbored the blaCTX-M, and blaoa genes which were encoded by a self-transferable Inc1 plasmid. DNA sequence analysis of the genetic environment of the blaCTX-M gene showed the truncated orf477 downstream, whereas no insertion sequences frequently associated with the blaCTX-M genes were detected upstream. This report is the first description of ESBL-producing STEC O103 strain isolated in humans and indicates that antibiotic resistant plasmid can be successfully transferred to such as pathogenic STEC. Therefore, national surveillance for antibiotic-resistant STEC should be urgently established.

Actinomycin D Inhibits the Virulences of Staphylococcus aureus
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Staphylococcus aureus is a versatile human pathogen that produces diverse virulence factors and its biofilm cells are difficult to eradicate due to their inherent ability to tolerant antibiotics. This study was undertaken to identify new biofilm inhibitors against S. aureus. The antibiofilm activities of the spent media of 252 diverse endophytic microorganisms were investigated using two methicillin-susceptible S. aureus strains and one methicillin-resistant S. aureus strain. We attempted to identify antibiofilm compounds in active spent media and assessed their activity against S. aureus cells to become less hydrophobic - supporting its anti-biofilm effect. In addition, surface coatings containing actinomycin D prevented S. aureus biofilm formation on solid glass surfaces. Together, the FDA-approved actinomycin D warrants further attention as a potential antivirulence agent against S. aureus infections.
Keywords: actinomycin D, biofilm, hemolysis, hydrophobicity, Staphylococcus aureus

Cinnamaldehyde and Eugenol Inhibit Biofilm Formation and Toxin Production against Pseudomonas aeruginosa
Yong-Guy Kim, Jin-Hyung Lee and Jintae Lee*
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Infection by antibiotic-resistant bacteria like Pseudomonas aeruginosa is a worldwide problem, and pathogenic biofilms contribute to reduced susceptibility to antibiotics. In this study, 83 essential oils were initially screened for biofilm inhibition against P. aeruginosa. Cinnamon bark oil and its main component cinnamaldehyde at 0.05% (v/v) markedly inhibited P. aeruginosa biofilm formation. Furthermore, cinnamon bark oil and eugenol decreased the production of pyocyanin and 2-heptyl-3-hydroxy-4(1H)-quinolone, hemolytic activity, swimming motility, and quorum sensing activity of P. aeruginosa. Also, transcriptional analysis showed that cinnamon bark oil down-regulated toxin-related genes pseC, pseE, and pchA in P. aeruginosa. In addition, biodegradable poly(lactic-co-glycolic acid) film incorporating biofilm inhibitors was fabricated and shown to provide efficient biofilm control on solid surfaces. This is the first report that cinnamon bark oil and its components, cinnamaldehyde and eugenol, reduce the production of pyocyanin and PQS, hemolytic activity, swimming motility, and quorum sensing activity of P. aeruginosa.
Keywords: biofilm formation, cinnamon bark oil, eugenol, hemolysis, toxin production
Diffusible Signal Factor

During infection, host and microbial pathogen do dynamic crosstalks and mutually affect gene expressions. Pathogens robustly change gene expression profiles to adapt to host environments. *Vibrio vulgaris* is a predatory bacterium causing very rapidly progressing septicemia. To address how the predator adapts to host environment to cause disease, we studied global in vivo *V. vulnificus* gene expression through transcriptome, proteome, metabolome and flux analyses using an intraperiodontal semipermeable infection model. In comparison with in vitro culture in a rich medium, *V. vulnificus* underwent a genome-wide metabolic reprogramming after infection. Infecting *V. vulnificus* appeared to run microaerobic metabolism with shutting off the TCA cycle and having acetate and formate effluxed. Carbon and nitrogen metabolic pathways were reorganized to maximize siderophore production through upregulation of chorismate production. Energy was primarily produced through glycolysis and functional activation of membrane ATP synthase complex. Gluconeogenesis was inhibited. Fatty acid metabolism did not appear to contribute significantly to ATP production. Nucleotides were synthesized primarily through the salvage pathway. And we proved our hypothesis on metabolic reprogramming by applying molecular genetic experiments. In summary, we found a genome-wide ‘metabolism reprogramming’ after infection by adopting systems biological approach to multi-omics datasets.

Keywords: *Vibrio vulnificus*, Metabolism reprogramming, Systems biology, Transcriptome, Proteome

Gingipains-Mediated Enhancement by Porphyromonas gingivalis of Tannerella forsythia Phagocytosis

Young-Jung Jung1,2, Hye-Kyoung Han1,2, Eun-Ju Ryu1,2, Seok-Joo Lee1,2, and Byong-Kyu Choi1,2,3

Porphyromonas gingivalis is a keystone pathogen that causes dysbiosis in periodontitis pathogenesis. Arg gingipains and Lys gingipain are its major virulence factors. In this study, the role of gingipains in modulation by *P. gingivalis* of *T. forsythia* phagocytosis was investigated. Phagocytosis of *T. forsythia* by macrophages was significantly enhanced by *P. gingivalis* coinfection in an MOI- and gingipain-dependent manner. Mutation of gingipains in coexisting *P. gingivalis* resulted in attenuated enhancement of *T. forsythia* phagocytosis compared to wild-type *P. gingivalis*. Inhibition of conjugation between the two bacteria, which was dependent on gingipains, reduced *T. forsythia* phagocytosis in the mixed infection. Although most internalized *T. forsythia* was cleared in the infected macrophages at 48 hours postinfection, more intracellular *T. forsythia* remained viable in coinfected with gingipain-expressing *P. gingivalis* than in coinfection with the gingipain-null mutant or in monoinfection with *T. forsythia*. These results suggest that gingipains are essential for the augmentation by *P. gingivalis* of *T. forsythia* phagocytosis and that *P. gingivalis* may facilitate the clearance of coexisting *T. forsythia*. Further studies are needed to determine whether *P. gingivalis* contributes to dissemination of coexisting *T. forsythia*.

Keywords: *Porphyromonas gingivalis*, *Tannerella forsythia*, gingipains, Phagocytosis, Macrophages, Periodontitis.
**F-13**
Production of Pseudoviruses for Ebola Virus and Marburg Virus Using Lentiviral Vector System

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Ebola virus (EBOV) and Marburg virus (MARV) belong to family Filoviridae. They can cause severe hemorrhagic fever in humans and nonhuman primates with a high mortality rate. Because EBOV and MARV are required high lab safety, clinical laboratories have a problem with diagnosis of EBOV and MARV. Otherwise, Pseudoviruses can be handled in a BSL-2 laboratory. In this study, We produced pseudoviruses including the glycoprotein (GP) of EBOV and of MARV. For production of pseudoviruses, we performed transfection with plasmids which were expressed GP of EBOV or GP of MARV, lentivirus structural proteins (gag/pol, Rev) and firefly luciferase into the 293FT cells. At about 48hrs post-transfection, we harvested the supernatant from the transfected cells. To confirm rescued pseudoviruses, we infected 293FT cells with these viruses. Then, we performed dual-luciferase assay to target the luciferase gene. Results of dual-luciferase assay in pseudovirus, we confirmed 2 types of rescued pseudoviruses including EBOV GP and MARV GP in 293FT cells. The pseudoviruses produced in this study may help to establish the neutralization assay for ebola and marburg at biosafety level 2 laboratories.

Keywords: Pseudovirus, Ebola virus, Marburg virus, Glycoprotein, Luciferase assay

**F-14**
Generation of Ebola Virus-like Particles in Drosophila Expression System

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Ebola virus disease became a major global public health concern since the huge outbreak began in West Africa in 2014. The estimated mortality is about 40% and the epidemic has affected 10 countries in 3 continents, mainly Guinea, Liberia, and Sierra Leone. There is currently no vaccine or antiviral therapy for ebola. In this study, we report the generation of ebola virus-like particles in Drosophila expression system as a vaccine candidate.

Zaire Ebola virus (EBOV) Guinean strain was used in this study. The coding sequences of GP, VP40, VP, and VP24 proteins of EBOV were cloned into the Drosophila expression vector (pMT/BIp/V5/His). Drosophila cell line S2 was transfected with these 4 plasmids. GP, VP40, VP, and VP24 proteins of EBOV expressed in Drosophila cells were determined by Western blotting using monoclonal antibody against ebola virus. Recombinant VLPs of EBOV consisting of GP, VP40, VP, and VP24 proteins were successfully expressed by using Drosophila expression system.

The Drosophila expression system is a more convenient and safer approach to the production of vaccine candidates for EBOV. Further in vivo studies of the VLP of EBOV are necessary to determine the immune responses of the ebola virus as a vaccine candidate.

Keywords: Ebolavirus, VLP, Drosophila Expression System

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**F-15**
The Function of Lipocalin 2 Production in Macrophages against Mtb Infection

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Iron is essential for survival of intracellular bacteria. Iron homeostasis in macrophage is closely associated with Mycobacterium tuberculosis (Mtb). Lipocalin2 (LCN2) which is a 25kDa glycoprotein and an acute secretory protein is involved in innate immune response by sequestering iron leading to limitation of bacterial growth. In this study, the role of LCN2 during mycobacterial infection was investigated using LCN2 knockout mice and their wild-type mice. LCN2 was produced from immune cells such as dendritic cells or macrophages during mycobacterial infection. Intracellular survival of Mtb was increased in LCN2-deficient macrophages compared to WT cells. To examine which factors of mycobacteria are involved in LCN2 induction, we infected macrophages with live H37Rv and heat-killed H37Rv. The level of LCN2 was increased in live H37Rv infected macrophages, whereas heat-killed H37Rv slightly induced production of LCN2. It is reported that ER stress is associated with LCN2 expression in lung cancer cells because GADD153 binds to LCN2 promoter. We observed Mtb-induced GADD153 production was associated with LCN2 protein expression in macrophages. It was confirmed that the inhibition of IRE1a and PERK with specific inhibitors prevented LCN2 induction in H37Rv infected macrophages. Therefore, we suggest that Mtb-induced ER stress regulates LCN2 protein production, sequentially resulted in the elimination of intracellular survival of Mtb via apoptotic cell death in immune cells.

Keywords: Lipocalin 2, Endoplasmic reticulum stress, Mycobacterium tuberculosis, Macrophage

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**F-16**
Lysophosphatidylcholine Controls Mycobacterium tuberculosis Infection by Promoting the Process of Phagosomal Maturation through PI3K-p38 Pathway in Mouse Macrophage

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Tuberculosis (TB) is caused by the infectious agent known as Mycobacterium tuberculosis (Mtb). Mtb have various survival strategies, including the blockage of phagosome maturation and inhibition of antigen presentation. Lysophosphatidylcholine (LPC) is a major phospholipid component of oxidized low-density lipoprotein involved in various cellular responses, such as the activation of second messengers and bactericidal activity in neutrophils. In this study, to investigate the bactericidal activity of LPC against Mtb, RAW264.7 cells were infected with low infectious dose of Mtb with treatment of LPC. In the cells infected with avirulent Mtb strain, H37Rv, LPC significantly decreased the Mtb growth and increased the production of ROS and NO. LPC also triggered the signaling pathway on the phosphorylation of PI3K and p38 rapidly, however, the inhibition of ROS production did not lead to LPC-induced phosphorylation in H37Ra-infected cells. The ratio of colocalized Mtb with specific markers proved phagosomal maturation, EE/A or LAMP-1, was increased in LPC-treated cells, while was reduced when cells were pre-treated with inhibitor of PI3K or p38. LPC promoted the processing of procathepsin D to an active form and the acidification of H37Ra-infected cells. The location of colocalized Mtb with specific markers proved phagosomal maturation, EE/A or LAMP-1, was increased in LPC-treated cells, while was reduced when cells were pre-treated with inhibitor of PI3K or p38. LPC promoted the processing of procathepsin D to an active form and the acidification of H37Ra-containing phagosomes. These results suggested that LPC could control Mtb growth by means of promoting the process of phagosomal maturation via PI3K-p38 MAPK pathway in macrophages.

Keywords: Mycobacterium tuberculosis, Lysophosphatidylcholine, Phagosomal maturation, Bactericidal activity, Macrophage
Detection of Hantavirus RNA from Anti-HTNV IgG Sero-negative Rodents in a Highly Endemic Area, Republic of Korea

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Hantavirus (HTNV), Bunyaviridae, is a negative sense tripartite RNA virus and harbored by \textit{Apodemus agrarius} as a natural reservoir. Infection with HTNV causes Hemorrhagic Fever with Renal Syndrome (HFRS) in humans, an endemic infectious disease in the Republic of Korea (ROK). Over decades, our studies have demonstrated the phylogenetic analysis and epidemiology of HTNV from anti-HTNV IgG sero-positive rodents in endemic areas. However, the identification of HTNV RNA is little investigated from anti-HTNV IgG sero-negative rodent. Here, indirect immunofluorescence antibody test was performed from \textit{A. agrarius} collected in endemic regions demonstrating 186 (77.5\%) of 240 rodents was negative for HTNV-specific IgG antibody. To detect HTNV RNA, total RNA in lung tissues was analyzed by RT-PCR using HTNV-specific primers. HTNV RNA was identified from \textit{A. agrarius} in 186 samples based on a HTNV M segment partial sequence. The phylogenetic analysis showed HTNV RNA from the rodents forms geographic specific clusters. In addition, we examined the Ct-value of HTNV RNA in various tissues of the rodents by RT-qPCR. Anti-HTNV IgG sero-negative rodents with low Ct-value may indicate the early phase of the rodent with HTNV infection because of the presence of viral genome but the lack of antibody. This study provides a possibility to detect HTNV RNA genome from anti-HTNV IgG sero-negative rodents and an insight for virus-host interaction and coexistence in the natural host.

Keywords: Hantavirus, Hantaan virus, Serological study, Phylogenetic analysis, Epidemiology

Xanthine Oxidase Inhibitory Activity of Extracts Prepared from Aloe vera and Aloe Arborescense \textit{in vitro}

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Xanthine oxidase (XO) which is the main enzyme in the purine metabolic pathway is the major cause of gout. Currently, XO inhibitors are mainly used as a treatment for gout. But, in the case of some items, serious side effects were observed. In this study, we evaluated selective xanthine oxidase inhibitory effects of Aloe vera (A. V.) and Aloe arborescense (A. A.) extracts which are natural products with less side effects. The cell viability was determined by MTT assay on Raw 264.7 cells. A. V. and A. A. extracts dissolved in water, ethanol and methanol demonstrated more than 70\% cell viability compared to untreated control group cells. XO assay was performed to identify XO inhibitory activity of extracts. A. V extracts dissolved in water showed XO inhibition of 12\%, 8\% at 5, 1mg/ml. In the same solvent, A.A extracts inhibited XO by 18\%, 22\% at 1, 0.5mg/ml respectively. All of methanolic and ethanolic extracts of A.V and A.A inhibited XO more than 30\%. In conclusion, this study showed that Aloe of the two species have therapeutic potential for treatment of gout.

Keywords: Aloe vera, Aloe arborescense, anti-gout, xanthine oxidase, Aloe extract
**F-21**

The Effect of Hypoxia-Mimic Agent on Innate Defense against S. Typhimurium Infection in Mouse Macrophages

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Salmonella enterica serovar Typhimurium (S. Typhimurium) is a facultative intracellular pathogen that causes typhoid-like systemic infection in mice. Hypoxia is an abnormally low level of oxygen and an essential feature of chronic inflammatory disease in tissue. The cellular effects of hypoxia are mediated by the hypoxia-inducible transcription factor-1 (HIF-1). Although HIF-1 plays a critical role in tumor proliferation and angiogenesis, it is less well understood that HIF-1 has a role in innate immune response against bacterial infection. In this study, to investigate the effect of cobalt chloride (CoCl2), hypoxia-mimicking agent, on S. Typhimurium infection, macrophages were treated with CoCl2 alone or infected with S. Typhimurium. Treatment of CoCl2 did not affect the growth of S. Typhimurium, however, the host cell viability decreased after 72h treatment of CoCl2. The transcriptional and translational expression level of HIF-1α was increased in a dose-dependent manner in CoCl2-treated cells. Furthermore, the treatment of CoCl2 accelerated the phosphorylation of Id8, MEK, Erk, and Jnk. It also triggered autophagy in accordance to increase the conversion ratio of LC3-I to LC3-II. The production of NO was remarkably decreased in CoCl2-treated cells during S. Typhimurium infection. These results suggested that hypoxia-mimetic agent could modulate innate immune response and it would be provide a potential therapeutic target to modulate S. Typhimurium-killing activity.

Keywords: *Salmonella* Typhimurium, Hypoxia-mimicking agent, Cobalt chloride, HIF-1α, Macrophage

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**F-22**

Differences in Biofilm Formation and Expression of Biofilm-Associated Genes between Epidemic and Minor Clones of Carbapenem-Resistant *Acinetobacter baumannii*

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*Acinetobacter baumannii* is an important nosocomial pathogen. Attachment and adherence to medical equipment and environmental surfaces appears to be important for survival of *A. baumannii* in hospital environment. This study aimed to investigate the differences in biofilm formation and expression of biofilm-associated genes between epidemic and minor clones of carbapenem-resistant *A. baumannii* (CRAB) from a Korean hospital. We selected the CRAB isolates belonging to prevalent pulsotypes 5 and 18 as an epidemic clone and CRAB isolates belonging to pulsotypes 12 and 20 as a minor clone. There was a significant difference in biofilm formation between epidemic and minor CRAB clones. Expression of biofilm-associated genes such as cqsC, cqsD, and cqsE under sessile conditions was significantly different between epidemic and minor clones. However, expression of pgaA, pgaC, and aba genes under planktonic conditions was not different between epidemic and minor clones. Interestingly, minor clones expressed more biofilm-associated genes, including aba, pgaA, pgaC, cqsC, cqsD, and cqsE, than epidemic clone under planktonic conditions. Epidemic CRAB clone harbors more antibiotic resistance genes than those of minor clone. This study shows that there are significant differences in carriage of antibiotic resistance genes, biofilm formation, and expression of cqsCDE genes between epidemic and minor clones of CRAB, which may partly explain the epidemicity of *A. baumannii*.

**F-23**

Outer Membrane Protein A Plays a Role in Antimicrobial Resistance of *Acinetobacter nosocomialis*

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*Acinetobacter nosocomialis* is an important nosocomial pathogen and is highly resistant to many antimicrobial agents by both intrinsic and acquired resistance mechanisms. We investigated the role of outer membrane protein A (OmpA) in antimicrobial susceptibility of *A. nosocomialis*. A ΔompA mutant of the *A. nosocomialis* ATCC 17903 strain was constructed using markerless gene deletion. The ompA gene was complemented by transformation of plasmids carrying the ompA gene in the ΔompA mutant. The minimal inhibitory concentration (MIC) of 10 antimicrobial agents was determined by E-test. The wild-type strain was more susceptible than the ΔompA mutant to imipenem and tetracycline, whereas the ΔompA mutant was more susceptible than the wild-type strain to aztreonam, colistin, and gentamicin. The ompA-complemented strain restored the MICs of aztreonam, imipenem, and tetracycline, but not of colistin and gentamicin. The efflux pump inhibitor phenyl-arginine-β-naphthylamide (PAβN) decreased the MICs of naldixic acid and tetracycline in the ΔompA mutant strain. These results suggest that OmpA plays a role in the antimicrobial susceptibility of *A. nosocomialis*.

**F-24**

PrP Regulates RhoA-mediated Neurite Outgrowth

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The cellular prion protein (PrP), a highly conserved GPI-anchored membrane protein, mainly known for its physiological role in pathogenesis of prion disease, is involved in the initial phase of neurite outgrowth and signal transduction. Ras homolog gene family, member A (RhoA) is a small GTPase protein, known to play an essential role in regulating the neuronal development, neuronal differentiation, neuronal survival and death in the central nervous system. Although recent studies showed dysregulation of RhoA in a variety of neurodegenerative diseases, the role of RhoA in prion pathogenesis is still unclear. Here, we investigated the regulatory mechanism of the PrP and RhoA in neurotogenesis using in vitro and in vivo systems. We found that the overexpression of PrP significantly reduced RhoA inactivation and RhoA phosphorylation in hippocampal neuronal cells and in the brains of transgenic mice. Using siRNA-mediated depletion of endogenous PrP and overexpression of PrP pathogenic mutants, we confirmed the PrP-mediated RhoA inactivation, which reduced RhoA phosphorylation and increased phosphorylated RhoA downstream effectors. We also observed that PrP co-localized with RhoA and that the overexpression of PrP significantly enhanced RhoA-mediated neurite outgrowth in NGF-treated PC12 cells. However, the pathogenic or truncated mutants of PrP decreased the neurite outgrowth compared to PrP. In addition, the inhibition of the downstream effector of RhoA, the Rho kinase (ROCK) substantially facilitated neurite outgrowth in PC12 cells similar to that induced by PrP. Interestingly, we found that the induction of RhoA inactivation is through the interaction of PrP with RhoA and that PrP enhanced the interaction between RhoA and RhoA effector proteins. These findings demonstrate that PrP contributes to the RhoA-mediated neurite outgrowth through the interaction with RhoA and its inactivation and that the pathogenic mutations of PrP impair the RhoA signaling, which in turn develops prion-related neurodegeneration.

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Keywords: Prion protein, RhoA, Neurite outgrowth, PC12 cell
F-25

Neuronal Cell Lines Established from Bank Vole Transgenic Mice
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Prions are infectious agents that cause a devastating neurodegenerative disease in both humans and animals. Unlike other rodents, bank vole (Myodes glareolus) is susceptible to prions from diverse range of species, including humans. Several lines of evidence suggest that neuron have important roles in the pathogenesis of prion disease. Here, we established neuronal cell lines (BVtg) from embryos of transgenic mice that were introduced with bank vole’s prion gene (prnp) by using a plasmid encoding for the large T antigen of SV40. In general bank vole transgenic mice are well known to be susceptible to a wide range of prion strains. The established BVtg cell lines were neuronal cells. After infection of the BVtg neuronal cell lines to 22L scrapie strain, we observed replication and accumulation of disease-associated forms of the prion protein even in 15 passage of BVtg cell lines. Furthermore, BVtg cell lines were susceptible to other scrapie strains. Thus, BVtg neuronal cell lines (BVtg) from embryos of transgenic mice that were infected with various murine prion strains provide a useful tool for the study of the pathogenic mechanisms of prion disease.

Keywords: Prion disease, bank vole, scrapie, prnp, neuronal cell line

F-26

Induced Activation of the Innate Immune Response to Pathogens by Low-dose Radiation in Drosophila melanogaster
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There are numerous reports that Low Dose Radiation (LDR) induces the beneficial effects in various biological fields such as longevity, anti-oxidant defense, DNA repair, enhanced circadian rhythm, etc. Drosophila studies on infection are extensively progressing for systemic immunity although the intestine response of fly to bacterial infection is little known. Enterococcus faecalis (Gram-positive) are known to be able to kill D. melanogaster and induce toll pathway. And many researchers have studied on the relationship between radiation and infection with D. melanogaster. In the study, E. faecalis was infected by feeding in Drosophila by LDR. Interestingly, E. faecalis-fed infected and irradiated flies at low dose showed enhanced development and motor activity against the infection of gram positive bacteria. Also, circadian rhythm in low dose irradiated flies was activated compared to non-irradiated in ROS level when compared to the others. Taken together, LDR influences on the behavior at the molecular level and ROS scavenging. Furthermore, it is carefully expected that innate immune response response on the infection will be intensively induced by LDR.

Keywords: Infection, Drosophila melanogaster, Enterococcus faecalis, ROS, Circadian rhythm

F-27

Five sRNAs, Qr1–5, are Involved in Fine Regulation of Virulence Genes Depending on Quorum Sensing and Iron in Vibrio vulnificus
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Expression of virulence factors of the human pathogen Vibrio vulnificus is closely related to quorum sensing. By primer extension, we confirmed that five Qrs (quorum sensing regulatory sRNAs) are expressed in V. vulnificus, and the transcriptional start site of these sRNAs are also confirmed. We certified central role of five Qrs in the quorum sensing circuit. Five qrr genes are activated by phosphorylated LuxO at different degrees: qrr2 is strongly activated, while qrr1 and qrr4 moderately, and qrr1 and qrr2 are marginally activated. Qrr sRNAs function redundantly to inhibit SmcR expression at low cell density, while the full repression requires all of the five sRNAs. Function of Qrr sRNAs subsequently convey to quorum sensing regulators, including virulence factors VvpE and VvhA. Besides, we found that iron inhibits the expression of qrrs in three distinct ways: Iron-Fur complex directly binds to qrr promoter regions, inhibiting the LuxO activation by competing with LuxO for cis-acting elements of qrrs; qrr genes transcription are also repressed by iron independently of Fur; LuxO expression are also repressed by iron through Fur independent manner, leading to a repression of qrr. Our study suggests that iron and quorum sensing are closely related to each other, and cognate regulatory circuits are linked together to obtain coordinated the expression of virulence factors depending on environmental conditions.

Keywords: Vibrio vulnificus, Qrr sRNAs, Iron, Quorum sensing

F-28

Lactobacilli Isolates from Healthy Vaginal Fluids Inhibited Bacterial Vaginosis- and Vulvovaginal Candidiasis-associated Pathogens
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The prevalence of vaginal pathogenic bacteria as well as the depletion of lactobacilli is associated with numerous adverse health outcomes including bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC). Among BV candidate bacteria, Gardnerella vaginalis is a well-known vaginal pathogen and Snethia spp. are recently emerging as opportunistic pathogens associated with a variety of clinical conditions such as preeclampsia, preterm labor, spontaneous abortion, and HPV infection. In this study, we investigated the growth-inhibitory activities of 53 lactobacilli isolates from 9 healthy subjects on Gardnerella vaginalis, Snethia sanguinegens (BV pathogens), and Candida albicans (VVC pathogens). The tested lactobacilli isolates consist of fifteen Lactobacillus crispatus, thirteen L. jensenii, six L. gasseri, and sixteen L. jensenii strains. After cultivation of lactobacilli strains in MRS broth, the bacterial cell-free supernatant (CFS) were obtained after filtration using 0.22μm syringe filter. The anti-microbial activities of selected lactobacilli isolates in MRS broth, the bacterial cell-free supernatant (CFS) were obtained after filtration using 0.22μm syringe filter. The anti-microbial effect of lactobacilli was evaluated based on the inhibited growth rate of target pathogens after co-culture with respective CFS by measuring optical density at 600nm. Ten lactobacilli isolates showed the greatest inhibition on growth of all tested pathogens. Further research elucidating effective compounds in the CFSs and confirming in vivo inhibitory effects of selected lactobacilli isolates on BV and VVC would be required.

Keywords: Lactobacilli, Gardnerella vaginalis, Snethia, Candida albicans, Bacterial vaginosis
Effect of Incomplete Lipopolysaccharide on Virulence and Immunogenicity in Salmonella enterica Serovar Enteritidis

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Lipopolysaccharide (LPS) in Salmonella enterica is very important for infection of host cells. Therefore, we were focusing on reducing inflammation of vaccine in this study. LPS is a component of the outer membrane of Salmonella and is a major virulence factor. We constructed LPS mutants to attenuate Salmonella virulence through regulation of LPS biosynthesis. RfaH, a transcriptional activator, regulates biosynthesis of LPS O-antigen and core polysaccharide. The rfaH mutant gene encodes acyltransferase that adds an acyl chain to lipid A. The low endotoxin of the rfaH mutant reduces the host inflammatory reaction. In this study, we evaluated the characteristics of SE LPS mutants and performed attenuation test of the mutants’ virulence Protective immunity and efficacy of the mutants were evaluated in mice. Vaccination with the ArfaH and AnmbH mutants induced significantly higher immunity in mice compared to PBS, but ArfaHAnmbH-infected mice surprisingly presented low immunity. We also found that the ArfaH mutant induced Th1-biased immune response and that the AnmbH mutant stimulated a Th1/Th2-mediated mixed immune response. As expected, the ArfaH and AnmbH mutants provided significant levels of efficacy in mice against challenge with the virulent SE, however, the ArfaH AnmbH mutant presented low efficacy. We suggest that the ArfaH and AnmbH mutants of SE are safe and can be used for preventive vaccine studies. Keywords: Salmonella Enteritidis, Lipopolysaccharide, rfaH, Protection, virulence

Comparative Anti-mycobacterial Activity and Cytotoxicity of Deoxypergularinine Derivative 5 and 1st Line Anti-tuberculosis Drugs in vitro

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Tuberculosis is a worldwide concern due to the emergence of increasing drug resistance against conventional anti-tuberculosis drugs and increasing infection rate. In our present study, we found the anti-mycobacterial activity of deoxypergularinine (DPG) with high cytotoxicity. Therefore, we synthesized several derivatives of DPG and found DPG derivative 5 (DPG5) with the lowest cytotoxicity. We found the minimum inhibitory concentration (MIC) ranges from 3.125 to 6.25 µg/mL in both avirulent M. tuberculosis H37Ra and virulent M. tuberculosis H77Rv strains. Our MTT data showed that DPG5 did not have any cytotoxicity on human alveolar A549, human fibroblast L929 and mouse macrophage Raw264.7 cells up to 50 µg/mL concentration. We also evaluated anti-tuberculosis activity and cytotoxicity of 1st line anti-tuberculosis drugs namely Isoniazid (INH), Rifampicin (RIF), Ethambutol (EMB). The MICs of INH, RIF and EMB against M. tuberculosis H77Rv were found 0.039, 0.0195, and 1.25 µg/mL, respectively but they showed no cytotoxicity at 1000 times higher anti-mycobacterial MIC dosages in any cells. A profound generation of reactive oxygen species (ROS) by DPG5 in raw264.7 cells was observed that was significantly diminished by ROS inhibitor, N-acetyl-L-cysteine (NAC). In summary, DPG5 has potential anti-tuberculosis activity with low cytotoxicity and the activity might be due to the activation of macrophage through ROS generation in some extent.

Keywords: anti-mycobacterial activity, deoxypergularinine, cytotoxicity, MIC, reactive oxygen species
High Throughput Detection of β-Lactamase Genes Conferring Antibiotic Resistance
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Fast detection of β-lactamase (bla) genes allows improved surveillance studies and infection control measures, which can minimize the spread of antibiotic resistance. Although several molecular diagnostic methods have been developed to detect limited bla gene types, these methods have significant limitations, such as their failure to detect almost all clinically available bla genes. We developed a fast and accurate molecular method to overcome these limitations using 62 primer pairs, which were designed through elaborate optimization processes. To verify the ability of this large-scale bla detection method (TMAPalaFINDER), assays were performed on previously-reported bacterial control isolates/strains. To confirm the applicability of the TMAPalaFINDER, the assays were performed on unreported clinical isolates. With perfect specificity and sensitivity in 198 control isolates/strains and 403 clinical isolates, the TMAPalaFINDER detected almost all clinically available bla genes. The ability of TMAPalaFINDER to detect bla genes on a large scale enables prompt application to the detection of almost all bla genes present in bacterial pathogens. The widespread use of the TMAPalaFINDER in the future will provide an important aid for monitoring the emergence and dissemination of bla genes and minimizing the spread of resistant bacteria.

Keywords: β-lactamase (bla) gene, large-scale detection, molecular diagnosis

Glucose Metabolism Affects Trimethylamine N-oxide Stimulated Cholera Toxin Production
Young Tack Oh and Sang Sun Yoon
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Vibrio cholerae, a causative agent of pandemic cholera, infects human intestine, which is considered to be anaerobic environment. Production of cholera toxin (CT), a major virulence factor of V. cholerae, is highly induced during anaerobic respiration with trimethylamine N-oxide (TMAO) as an alternative electron acceptor. However, the molecular mechanism of TMAO-stimulated CT production is not fully understood. Herein, we revealed that CT production during anaerobic TMAO respiration was affected by glucose fermentation, another major mode of energy metabolism. When N16691, V. cholerae strain was grown together with glucose, the CT production was markedly reduced. Furthermore, an N16691Δcrp mutant, devoid of cyclic AMP receptor protein (CRP), became defective in CT production during growth by anaerobic TMAO respiration, further suggesting a role of glucose metabolism in regulating TMAO-mediated CT production. TMAO reductase activity was noticeably decreased when grown together with glucose or by the mutation of crp gene. A CRP binding region was identified in the promoter region of torD gene encoding a structural subunit of TMAO reductase and our gel shift assay further confirmed the binding of purified CRP to the torD promoter sequence. Together, our results suggest that bacterial capability to respire using TMAO is controlled by CRP. This is a demonstration that CT production, an important virulence feature, is critically controlled by bacterial metabolism.

Keywords: Vibrio cholerae, cholera toxin, Trimethylamine N-oxide, glucose metabolism, cyclic AMP receptor protein

Nucleotide Composition and Genomic Diversity Pattern in Influenza A Virus
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The genetic variation of influenza A virus is utilized to describe antigenic drift and shift. However, it is difficult to show efficiently viral genomic variations among subtypes by time period. Classification of influenza subtypes is performed using the sequences of HA and NA. Here, through the nucleotide composition analyses of 31,507 viral genome segments, we observed that AT and GC ratios of genome segments of influenza A viruses were able to quantify and visualize genomic diversity among subtypes by time period. Using this method, we found that human, swine, and equine influenza viruses were able to be classified by AT and GC ratio of PB2, not HA and NA which genes are related to determine host range of influenza A viruses. In contrast, this method was not able to separate clearly host specification between avian and other animals, suggesting the avian's role in transmission of influenza A virus. Furthermore, in the human influenza virus, four high pathogenic subtypes (H1N1, H2N2, H1N2, and H5N1) were classified by HA, NA, and PB1 genes. This results supported that the reassortment theory of human influenza virus. In the avian influenza virus, the GC ratio of HA in high pathogen (H5 and H7) viruses was higher than low pathogen (H9) viruses. Therefore, our approach will be a valuable method to classify and determine host specification and pathogenicity as well as to understand genomic diversity of influenza A virus.

Keywords: Influenza A, Nucleotide composition, Genomic diversity, Host specification, Pathogenicity

First Isolation of Severe Fever with Thrombocytopenia Syndrome Virus from Haemaphysalis longicornis Ticks Collected in SFTSV Outbreak Areas in the Republic of Korea, 2013
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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne infectious disease that is endemic to China, Japan, and Republic of Korea (ROK), which is caused by a novel phlebovirus in the hantaiviridae family, SFTSV virus (SFTSV). In the ROK, no studies have focused on the detection of SFTS in ticks collected from areas within the ROK, in which human cases have occurred. In this study, we aimed to investigate the prevalence of SFTSV in ticks collected from the SFTS outbreak areas in the ROK in 2013 and attempted to isolate SFTSV from positive tick pools. A total of 8,313 ticks collected from SFTS outbreak areas in the ROK in 2013 were used to detect SFTSV. To identify the isolated virus, we examined the presence of the SFTSV M gene in passaged cell supernatants using one-step RT-PCR and confirmed the results by indirect immunofluorescent antibody assay (IFA), immunoblotting using a monoclonal SFTSV nucleocapsid protein antibody, and TEM analysis for morphological identification. A single SFTSV was isolated in cell culture from 1 pool of Haemaphysalis longicornis ticks collected from Samcheok-si, Gangwon Province in the ROK. Phylogenetic analysis showed that the SFTSV isolate was clustered with the SFTSV strain from Japan, which was isolated from humans. To the best of our knowledge, this is the first survey of SFTSV prevalence in ticks collected from SFTS outbreak areas in the ROK, and is the first study to isolate SFTSV from these ticks.

Keywords: SFTSV, isolation, Haemaphysalis longicornis, Republic of Korea

Influenza A Virus

F. Infection and Pathogenesis

Cholera Toxin Production

Department of Microbiology and Immunology, Yonsei University College of Medicine

Medicine

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne infectious disease that is endemic to China, Japan, and Republic of Korea (ROK), which is caused by a novel phlebovirus in the hantaiviridae family, SFTSV virus (SFTSV). In the ROK, no studies have focused on the detection of SFTS in ticks collected from areas within the ROK, in which human cases have occurred. In this study, we aimed to investigate the prevalence of SFTSV in ticks collected from the SFTS outbreak areas in the ROK in 2013 and attempted to isolate SFTSV from positive tick pools. A total of 8,313 ticks collected from SFTS outbreak areas in the ROK in 2013 were used to detect SFTSV. To identify the isolated virus, we examined the presence of the SFTSV M gene in passaged cell supernatants using one-step RT-PCR and confirmed the results by indirect immunofluorescent antibody assay (IFA), immunoblotting using a monoclonal SFTSV nucleocapsid protein antibody, and TEM analysis for morphological identification. A single SFTSV was isolated in cell culture from 1 pool of Haemaphysalis longicornis ticks collected from Samcheok-si, Gangwon Province in the ROK. Phylogenetic analysis showed that the SFTSV isolate was clustered with the SFTSV strain from Japan, which was isolated from humans. To the best of our knowledge, this is the first survey of SFTSV prevalence in ticks collected from SFTS outbreak areas in the ROK, and is the first study to isolate SFTSV from these ticks.

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Keywords: SFTSV, isolation, Haemaphysalis longicornis, Republic of Korea

Influenza A Virus

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Keywords: SFTSV, isolation, Haemaphysalis longicornis, Republic of Korea
F-37
Molecular Detection of Tick-borne Viruses from Ticks Collected in the Republic of Korea, 2014
Sook-Min Yoon1, Ye-Ji Lee1, WoorYoung Choi1, Seok-Chul Kim2, Seong-Tae Chung1, Terry A. Klein3, and Won-Ja Lee4
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Ticks play a role in transmission of arboviruses responsible for emerging infectious diseases, which have a significant impact on public health. In the Republic of Korea (ROK), little is known about the information regarding the presence of tick-borne viruses and their vectors, our aim in this study was to screen for Severe fever with thrombocytopenia syndrome virus (SFTSV), Tick-borne encephalitis virus (TBEV), Powassan virus (POWV), Omsk hemorrhagic fever virus (OHBFV), and Langat virus (LGTV) in ticks collected from the ROK in 2014. A total of 21,158 ticks belonging to 3 genera and 6 species were collected throughout the nation from March-October in 2014 and were tested by one-step RT-PCR or nested RT-PCR to detect the target viruses. The most abundant ticks was Haemaphysalis longicornis (83%), followed by I. flavescens (17.6%), I. ricinus (15.7%), H. longicornis (12.4%), Amblyomma testudinarium (10.5%), H. phasiana (9.0%), I. persulcatus (7.4%), and I. turdae (0.01%). The minimum infection rates in ticks with the target viruses were as follows: 0.1% for SFTSV and 0.04% for TBEV. The presence of POWV, OHBFV, and LGTV genomic RNAs could not be detected. Our findings show that tick-borne viruses-infected ticks have been distributed in the ROK. These results will aid understanding of the epidemiology of tick-borne viral diseases in the ROK and emphasize the need for continuous tick-based arbovirus surveillance to monitor the virus emergence.

Keywords: Tick-borne viruses, Republic of Korea

F-38
Application of Detergents to Plasmin Assay of Biological Samples
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Plasmin activity is involved in many physiological processes such as fibrinolysis, liver repair. Plasmin is also proposed to play a role in bacterial and viral infections. Plasmin can be used as a common screening assay for these diseases because changes in the plasmin activity are directly associated. In this communication, we report that detergent molecules can enhancing or suppressing plasmin activity in vitro. Non-ionic detergents, such as NP-40, sodium deoxycholate(DOC), Triton X-100, and Tween-20, were capable of increasing plasmin enzyme activities. However, an ionic detergent sodium dodecyl sulfate inhibited enzymatic activities. When these detergents were applied to the biological samples (brain extract of prion-infected mouse brains), sensitivity of plasmin activity measurement was significantly enhanced. These results suggest that addition of these detergents in the plasmin assay seems to be useful in improving assay sensitivity.

Keywords: Non-ionic Detergents, Plasmin Activity

F-39
Increase of Plasmin Activity During the Progress of Prion Disease in Animal Models
Soyoung Park1, Muhammad Waqas1, Jihyun Lee1, Hye-Mi Lee1, Dae-Inwan Kim1, and Chongsik Ryu2
1Department of Pharmacy, College of Pharmacy, Hanyang University, Ansan, 2Institute of Pharmaceutical Science and Technology, Hanyang University, Ansan

Mechanism underlying prion propagation is yet an enigmatic biochemical event. Conformational conversion of prion protein (PrP) isoforms is believed to be the major event that explains prion propagation. Cellular prion protein (PrPc) undergoes conformational conversion to a infectious prion protein (PrPsc). Plasmin is a cellular protease that cleaves a number of proteins found in the plasma membranes and extracellular matrix. PrPc appears to be cleaved by plasmin, which results in depletion of PrPc in a proper cleavage state for PrPsc generation from PrPc. Based on these facts, we hypothesized that the plasmin activity is altered during the progress of prion disease. To test this hypothesis, we measured plasmin activity of the brain samples collected from RML prion-infected and control mice at the terminal stage of disease. Ten % brain homogenate samples did not show the difference in plasmin activity. However, the membrane fraction further prepared from 10% brain homogenate showed significantly increased plasmin activity in the prion-infected group. Time course experiment demonstrated that plasmin activity in the membrane fraction gradually increased as disease progressed toward the terminal stage. Our study indicates that changes in plasmin activity of the membrane fraction is correlated with PrPc processing and PrPsc generation.

Keywords: Prion disease, Brain homogenate, Plasmin

F-40
LC3/Atg8 Contributes to HCMV Growth by Facilitating Virion Release in an Autophagy-independent Manner
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Autophagy is an important component of innate immunity to remove intracellular pathogens. During HCMV infection, autophagy is induced at early times and subsequently inhibited later by TRS1-Beclin-1 interaction. Recently, it has been demonstrated that LC3/Atg8 plays a role in vesicular trafficking in an autophagy-independent manner during coronavirus infection. In the present study, we investigated whether LC3 plays a role in HCMV growth. HCMV infection substantially increased the level of nonlipidated LC3 (LC3-I) at the late stages of infection, whereas UV-inactivated HCMV infection did not, indicating that this accumulation of LC3-I requires viral gene expression. Depletion of LC3 slightly reduced accumulation of viral proteins. Importantly, LC3 knockdown significantly inhibited the release of progeny virus. However, depletion of Atg7, an E1-like enzyme for LC3 and Atg12 modification, did not result in any apparent defect in both accumulation of viral proteins and virion release, indicating that the LC3 function in progeny virion release is autophagy-independent. Our results suggest that HCMV infection leads to the accumulation of nonlipidated LC3 at the late stages of infection and that LC3 contributes to viral growth by facilitating virion release in an autophagy-independent manner. Work is underway to investigate whether LC3 is functionally associated with the virion assembly complex during HCMV infection.

Keywords: HCMV, Autophagy, LC3
Interaction of HCMV pUL26 with the Cellular ISGylation System

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Interferon-stimulated gene (ISG) 15 encodes a ubiquitin-like protein of which expression is inhibitory for replication of many viruses. We recently found that replication of human cytomegalovirus (HCMV) is also inhibited by ISG15 modification (ISGylation). However, whether HCMV-encoded proteins are modified by ISG15 or regulate ISGylation has not been elucidated. In this study, we show that the HCMV pUL26 tegument protein is a target and a regulator of ISGylation. We found that pUL26 interacts with ISG15, UBE1L, and His5 and is conjugated by ISG15 at two lysine residues, K136 and K169. ISGylation of pUL26 was found to negate its ubiquitination. Analysis using the UL26-ISG15 fusion protein as a surrogate for ISGylated pUL26 revealed that ISGylation of pUL26 is inhibitory for its activity to promote viral growth. Moreover, pUL26 was found to suppress virus-induced ISGylation in a manner independent of its own ISGylation. Consistently, UL26-deleted mutant virus infection provoked more ISGylation and its growth was more sensitive to IFNβ treatment, compared to wide-type virus. Considering that HCMV-induced ISGylation is reduced by IE1, a viral inhibitor of type I interferon signaling, our results demonstrate that HCMV contains countermeasures to suppress both ISG15 transcription and protein ISGylation, highlighting the interplay between virus and ISGylation for productive viral infection.

Keywords: HCMV, pUL26, ISG15, ISGylation

Distinct Commensals Induce Interleukin-1β via NLRP3 Inflammasome in Inflammatory Monocytes to Promote Intestinal Inflammation in Response to Injury

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The microbiota stimulates inflammation, but the signaling pathways and the members of the microbiota involved remain poorly understood. We found that the microbiota induces interleukin-1β (IL-1β) release upon intestinal injury and that this is mediated via the NLRP3 inflammasome. Enterobacteriaceae and in particular the pathobiont Proteus mirabilis, induced robust IL-1β release that was comparable to that induced by the pathogen Salmonella. Upon epithelial injury, production of IL-1β in the intestine was largely mediated by intestinal Ly6C(high) monocytes, required chemokine receptor CCR2 and was abolished by deletion of IL-1β in CCR2(−) blood monocytes. Furthermore, colonization with P. mirabilis promoted intestinal inflammation upon intestinal injury via the production of hemolysin, which required NLRP3 and IL-1 receptor signaling in vivo. Thus, upon intestinal injury, selective members of the microbiota stimulate newly recruited monocytes to induce NLRP3-dependent IL-1β release, which promotes inflammation in the intestine.

Keywords: Commensal Bacteria, NLRP3 inflammasome, Inflammatory monocyte, Interleukin-1 beta, Inflammatory bowel disease

The Prevalence and Characteristics of Bacillus cereus Isolated in Korea, 2012-2014

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Bacillus cereus is an important cause of foodborne disease worldwide. Stool specimens from 57,050 patients with diarrhea were collected to identify the pathogenic bacteria from 2012 to 2014 in Korea. A total of 667 strains of B. cereus isolated from 57,050 clinical samples (6.9% of isolation rate). B. cereus had two distinct foodborne disease types, diarrheal and emetic symptom. In this study, we assessed toxin gene profiling by PCR and analyzed according to isolated region, patients’ age and sex. The presence of five enterotoxin genes (hblC, entFM, hleA, cytK and bceT) and one emetic toxin gene (cereulide; cer) were examined. B. cereus have emetic toxin gene was present in 20.2% of 667 isolates. The rate of entFM, hleA, cytK, bceT and bceT enterotoxin genes among strains have emetic gene was 77.8%, 59.3%, 17.9%, 11.9% and 12.6%, respectively. Also, 86.8%, 83.5%, 51.3%, 42.9% and 34.0% in strains do not have emetic toxin gene. Only three strains were detected have six toxin genes (five enterotoxin genes and one emetic toxin gene). Isolation rate trend showed the highest ratio in the summer season from June to September. Also isolation rate of B. cereus by patients’ age showed highest ratio in children under age of 10, age 60-69. This study demonstrates the distribution of B. cereus toxins, isolated region, patients’ age and sex in clinical specimens. Hygiene education should be addressed on diarrheal disease susceptible groups, such as age under 10.

Keywords: Bacillus cereus, emetic toxin, Enter-Net Korea.

Enteric Bacteria Isolated from Diarrheal Disease in Korea, 2012-2014

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This study was performed to determine the characteristics of the diarrheal causing pathogens according to season, isolated regions, patient’s age and sex and to provide useful data for the prevention of diarrheal disease. Stool specimens from 57,050 patients with diarrhea were collected to identify the pathogenic bacteria from 2012 to 2014 in Korea. And 4,538 pathogenic bacteria were isolated and analyzed according to season, isolated regions, patients’ age and sex. The proportions of isolated pathogenic bacteria were Salmonella spp. 1,414 (31.1%), pathogenic E. coli 2,481 (54.7%), V. parahaemolyticus 80 (1.8%), Shigella spp. 49 (1.1%), Campylobacter spp. 514 (11.3%). Isolation rate showed highest ratio in summer season, from June to September for most of pathogenic bacteria. Isolation rate of pathogenic bacteria by patients’ age showed highest ratio at 0 to 19 year for most of pathogenic bacteria. And Isolation rate by region, 61.3% isolated from cities and 38.7% isolated from rural provinces. Hygiene education should be addressed on diarrheal disease susceptible groups, such as age under 10, age of 10-19, and more than 70 years old, and ongoing monitoring for the pathogens is still required.

In addition, efficient information system and surveillance project for infection prevention should be continued.

Keywords: Surveillance, diarrhea-causing bacteria, Enter-Net
Characterization and Trans-complementation Study of a Recombinant Virus Harboring Point Mutations in ORF49 of Marine Gammaherpesvirus 68

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Gammaherpesviruses such as Epstein-Barr virus and Kaposi’s sarcoma-associated herpesvirus, and marine gammaherpesvirus 68 (MHV-68) are important human pathogens as they establish latency mainly in host B lymphocytes and are associated with various tumors. One of virus proteins, ORF49 is translated as a late gene in lytic replication and is conserved among gammaherpesviruses and shown to cooperate with replication and transcription activator (RTA) in regulating virus lytic replication. Our previous results showed that ORF49 directly bound to RTA as well as its negative regulator, poly (ADP-ribose) polymerase-1 (PARP-1), disrupting the interaction between RTA and PARP-1. Here we generated its multiple mutant constructs, based on its X-ray crystal structure information and analyzed their effects on its function and interaction. We identified specific domain which is important for virus replication and virion production and analyzed their effects on its function and interaction. We identified specific domain which is important for virus replication and virion production and analyzed their effects on its function and interaction. We identified specific domain which is important for virus replication and virion production and analyzed their effects on its function and interaction.

Keywords: gammaherpesvirus, RTA, PARP-1, RED lambda recombination

Molecular Characterization of Viral Pathogens Causing Sporadic Acute Gastroenteritis in Children <5 Years of Age in Korea, 2013-2014

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Acute gastroenteritis is prevalent in a child less than 5 years old, and most important etiological agents are norovirus (NoV), group A rotavirus (RoV), enteric adenovirus (AdV), astrovirus (AsV) and sapovirus (SaV). In this study, we analyzed the prevalence and characteristics of enteric virus in acute diarrhea by viral pathogens with laboratory surveillance in Korea, 2013-2014. Viral infection was revealed 30.76% (4,366) of specimens from tested samples (14,195). The most prevalent group was 0-12 months-old age group (59.8%) and the lowest was 49-60 months (4.7%). NoV (14.4%) was most prevalent most pathogens and RoV (12.1%), AdV (2.3%), AsV (1.3%), and SaV (0.7%) were followed. NoV was predominant in winter season (Nov-Feb), while RoV was peaked in spring season (Jan-May). NoV GI4 was predominant genotype which comprised 55.3% of the NoV GII strains. Type41, type1a and GI1 were the most prevalent genotype of AdV, AsV and SaV, respectively.NoV GI4 was major cause of viral acute gastroenteritis in children. However, GI17 was sharply increased from December 2014, recently. The comprehensive and continuous surveillance is needed to identify the prevalence of different acute gastroenteritis pathogens.

Keywords: Acute gastroenteritis, enteric virus, laboratory surveillance
Keywords: Mycobacterium massiliense Type II genotype, colony forming units (CFUs), TNF-α secretion

F-51

The Activity of Protease IV, a Major Virulence Factor of Pseudomonas aeruginosa is Affected by Quorum Sensing System
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Pseudomonas aeruginosa is an ubiquitous multi-host pathogen, infect plant and insect, nematodes, mammal, and so on. Among many virulence factors of P. aeruginosa, Protease IV has been suggested to be involved in some Pseudomonas infections such as corneal infection. While previous studies of our laboratory suggested that Protease IV is a key virulence factor against insect, secreted protease, regulated by quorum sensing system. Tenchirio molitor, an insect depends on innate immunity for defense against pathogenic bacteria, causing melanization. CFCS from the Protease IV-overexpressing PAO1 strongly induced the melanization of T. molitor larvae. But, CFCS from the Protease IV-overexpressing MWI(ΔrhlI/Δg486 double mutant of PAO1) rarely induced the melanization of that. Interestingly, when checking the protein profile of Protease IV overexpressed PAO1 and MW1, MW1 can overexpress the Protease IV. Protease IV is the lysyl endopeptidase, so we compare the activity of Protease IV overexpressed in PAO1 and MW1 respectively using chromogenic substrate N-(p-Tosyl)-Gly-Pro-Lys 4-nitroanilide acetate salt, Protease IV overexpressed in PAO1 shows strong activity, but Protease IV overexpressed in MW1 is not. So we hypothesize that there is some quorum sensing dependent factor, which can determine the Protease IV’s activity.

Keywords: Quorum sensing, Pseudomonas aeruginosa, Protease IV, Virulence

F-50

NADH Dehydrogenase of Vibrio vulnificus CMCP6 Contributes to Energy Production by Providing Proton Motive Force to ATP Synthase I
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Vibrio vulnificus is a halophilic estuarine bacterium that causes opportunistic infection to susceptible hosts. We had reported that a robust metabolism reprogramming of V. vulnificus after infection. Based on our analysis, we found that NADH dehydrogenase (Vv1_2074) was essential for survival in vivo and ATP synthase I insertion mutants showed significant reduction of [ATP]intracellular after infection. It was known that the proton motive force (PMF) provides a charge to ATP synthase I for generating [ATP]intracellular. We wondered the source of PMF and hypothesized that Vv1_2074 may contribute to PMF generation. We constructed a deletion mutant of Vv1_2074. The in vitro growth of mutant was indistinguishable from its isogenic wild type. However, we observed a significant in vivo growth retardation of mutant by using a rat peritoneal infection model, which was recovered after reversion of mutated gene. Moreover, the [ATP]intracellular in mutant was also significantly reduced under aerobic, microaerobic, and anaerobic conditions, especially; most severe reduction under in vivo growth condition. These results suggest that NADH dehydrogenase provides PMF for generating ATP molecules through ATP synthase I under various growth conditions, especially during V. vulnificus established infection in vivo. With these results, we could add one more experimental evidence to the ‘metabolic reprogramming after infection’ and the accuracy of our systems biological analysis.

Keywords: Vibrio vulnificus, NADH dehydrogenase, Metabolic reprogramming,
Keywords: NLRP10, HOK-16B Cells, Periodontitis, periodontopathogens, Inflammation

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Even influenza B viruses are considered to cause milder clinical disease in mammalian hosts than influenza A viruses, the pathogenic determinants of influenza B viruses are not fully evaluated. In this study, we provide insight into how influenza B viruses can adapt to a mammalian host through molecular modifications that subsequently increase pathogenicity. To investigate the molecular characteristics by which influenza B viruses adapt to mammalian hosts, B/Florida/04/2006 was serially passaged in mice until it was highly virulent, and the biological properties were investigated in mice and ferrets. Wild type (P0), three intermediate-passaged viruses (P5, P9, P12), and the lethal mouse-adapted virus (P17) each obtained one to five amino acid substitutions in HA, M, NP, and PA segments. The P17 virus showed significantly enhanced replication compared to the P0 virus both in vitro and in vivo with highly virulence, while the P0, P5, and P9 viruses did not kill any of the infected mice. Additionally, the P17 virus-infected ferrets demonstrated greater viral titers in the upper respiratory tract compared with infection with P0 or intermediate viruses. To our knowledge, this is the first successful ferret-to-ferret transmission study of type B influenza virus with delineate potential transmission factors.

Keywords: Influenza B virus, Mouse-adaptation, virulence

F-53
Molecular Characterization of Hepatitis A Virus Causing an Outbreak at a Residential Facility for the Disabled, Republic of Korea

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In April, 2015, an outbreak of symptomatic hepatitis A virus (HAV) infections occurred at a residential facility for the severe disabled, Incheon, Republic of Korea. We investigated the source of outbreak and performed phylogenetic analysis of HAV strains. The HAV-RNA positive specimens were analyzed by sequencing. HAV VP3-VP1 junction region (186bp) sequences were aligned with reference genotype from GenBank and were analyzed by sequencing. HAV VP3-VP1 junction region (186bp) sequences were aligned with reference genotype from GenBank and were analyzed by sequencing. The same sequence of subgenotype IA was represented in samples. The same sequence of subgenotype IA was represented both in clinical and environmental samples. Other assigned subgenotype IA and IB strains of each branches were shared a high sequence identity of ≥ 94.1% with previously reported strain in Korea. In this study, phylogenetic analysis showed that subgenotype IA and IB were co-circulated, major causes are subgenotype IA. Causative strain is similar to HAV that occurred before in Korea. The results suggest that prevalence of domestic HAV rather than imported HAV from high-endemic countries were recently occurred in Korea.

Keywords: hepatitis A, hepatitis A virus, residential facility, outbreak, genotype

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Periodontitis is caused by subgingival biofilms that are composed of multi-species oral bacteria, mostly of Gram-negative anaerobes, along with over-aggressive immune response. Quorum sensing (QS) is a cell-to-cell signaling mechanism comprising signaling molecules secreted by various microbial species, affecting phenotypes including virulence and biofilm formation. Autoinducer type 2 (AI-2) is an universal QS molecule that plays an important role in dental biofilm formation. The aim of this study was to investigate the effect of the QSIs on AI-2 activity was assessed using AI-2 reporter strain Vibrio harveyi BB170 and semi-purified Fusobacterium nucleatum. Biofilm formation of periodontopathogens was evaluated by crystal violet staining and confocal scanning laser microscopy. QSIs significantly inhibited AI-2 activity of F. nucleatum, the major bridge organism in dental biofilms. They also inhibited biofilm formation of periodontopathogens including F. nucleatum, Porphyromonas gingivalis, Tannerella forsythia and Aggregatibacter actinomycetemcomitans. AI-2 of periodontopathogens may be a target for the inhibition of pathogenic dental biofilm formation, and QSIs can be applied as preventive agents against biofilm formation of periodontopathogens.

Keywords: Quorum Sensing, Autoinducer, Biofilm, Periodontopathogen, Periodontitis

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Periodontopathogens

Quorum Sensing Inhibitors to Prevent Biofilm Formation of Periodontopathogens

In this study, we provide insight into how influenza B viruses can adapt to a mammalian host through molecular modifications that subsequently increase pathogenicity. To investigate the molecular characteristics by which influenza B viruses adapt to mammalian hosts, B/Florida/04/2006 was serially passaged in mice until it was highly virulent, and the biological properties were investigated in mice and ferrets. Wild type (P0), three intermediate-passaged viruses (P5, P9, P12), and the lethal mouse-adapted virus (P17) each obtained one to five amino acid substitutions in HA, M, NP, and PA segments. The P17 virus showed significantly enhanced replication compared to the P0 virus both in vitro and in vivo with highly virulence, while the P0, P5, and P9 viruses did not kill any of the infected mice. Additionally, the P17 virus-infected ferrets demonstrated greater viral titers in the upper respiratory tract compared with infection with P0 or intermediate viruses. To our knowledge, this is the first successful ferret-to-ferret transmission study of type B influenza virus with delineate potential transmission factors.

Keywords: Influenza B virus, Mouse-adaptation, virulence

F-55
Functional Role of NLRP10 in HOK-16B Cells Infected with Tannerella forsythia and Fusobacterium nucleatum

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This study is to reveal the role of NLRP10 in order to better understand the pathogenesis of periodontitis. The mRNA and protein level of NLRP10 were measured in HOK-16B cells under the infection of Tannerella forsythia or Fusobacterium nucleatum. The mRNA level of NLRP10 was increased in T. forsythia infected HOK-16B cells. The protein level of NLRP10 was augmented in the cells which were infected by T. forsythia, but not by F. nucleatum. And, T. forsythia infection resulted in MAPKs activation, but not NF-κB activation. It was further shown that MAPKs activation was significantly decreased when NLRP10 was downregulated. Also, T. forsythia could invade into HOK-16B cells in a MOI-dependent manner. Our results suggest the further research to unravel the role of NLRP10 on MAPKs activation and bacterial invasiveness.

Keywords: NLRP10, HOK-16B Cells, Periodontitis, periodontopathogens, Inflammation

F-56
Characterization of Mouse-adaption of Yamagata Lineage B Virus

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Even influenza B viruses are considered to cause milder clinical disease in mammalian hosts than influenza A viruses, the pathogenic determinants of influenza B viruses are not fully evaluated. In this study, we provide insight into how influenza B viruses can adapt to a mammalian host through molecular modifications that subsequently increase pathogenicity. To investigate the molecular characteristics by which influenza B viruses adapt to mammalian hosts, B/Florida/04/2006 was serially passaged in mice until it was highly virulent, and the biological properties were investigated in mice and ferrets. Wild type (P0), three intermediate-passaged viruses (P5, P9, P12), and the lethal mouse-adapted virus (P17) each obtained one to five amino acid substitutions in HA, M, NP, and PA segments. The P17 virus showed significantly enhanced replication compared to the P0 virus both in vitro and in vivo with highly virulence, while the P0, P5, and P9 viruses did not kill any of the infected mice. Additionally, the P17 virus-infected ferrets demonstrated greater viral titers in the upper respiratory tract compared with infection with P0 or intermediate viruses. To our knowledge, this is the first successful ferret-to-ferret transmission study of type B influenza virus with delineate potential transmission factors.

Keywords: Influenza B virus, Mouse-adaptation, virulence

F-54
Quorum Sensing Inhibitors to Prevent Biofilm Formation of Periodontopathogens

To our knowledge, this is the first successful ferret-to-ferret transmission study of type B influenza virus with delineate potential transmission factors.
**F-57**

**Roles of Ornithine Lipid in Pseudomonas aeruginosa Infection to Host Cells**

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The ornithine lipids (OLs) are the most representative bacterial phosphate-free lipids. *Pseudomonas aeruginosa* has *olsBA* operon encoding acyltransferases that functions to synthesize OLs. The *olsBA* overexpression attenuated the virulence of *P. aeruginosa* to animal hosts and affected some virulence-related phenotypes. Since the acyl-homoserine lactone (AHL) production and activity of quorum sensing (QS) regulators were repressed by the *olsBA* overexpression, we suggest that these effects are caused by the reduced activity of QS. The *olsBA*-overexpressing *P. aeruginosa* cells and purified OLs were able to increase calcium release and reduce the expression of NOs, and COX-2 in animal cells, implying that OLs may directly modulate the inflammation-related physiology of host cells. Another important virulence phenotype, the biofilm formation was also affected by OL in *P. aeruginosa* in which OLs directly enhanced the biofilm formation of *P. aeruginosa*. In conclusion, the *olsBA* overexpression is likely to attenuate the virulence of *P. aeruginosa* by sequestering the significant portion of the cellular acyl-group pool to the OL synthesis and as a consequence, by reducing the synthesis of AHLs and QS activity. Differently from this, the effects of OLs on the expression of inflammatory factors of host cells and biofilm formation may be directly exerted.

Keywords: *Pseudomonas aeruginosa*, Ornithine Lipid, Virulence, Acyl-Homoserine Lactone

**F-58**

**Development of Capture ELISA Using Human Anti-BoNT/B Monoclonal Antibodies**

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Botulinum neurotoxins (BoNTs) are the most potent toxins on earth and are produced from anaerobic and spore-forming Clostridium species. Intoxication with BoNTs can cause the flaccid paralysis of muscles and failure of breath results in death. Thus effective diagnosis for confirming the presence of BoNTs within samples is highly important. Although mouse bioassay is the standard method for serological diagnosis of botulism, relatively long assay time and the use of live animals are restricting points of this assay. In this study, we compared binding characteristics of anti-BoNT/B human antibodies which were derived from the screening of phage library screening. Each antibody was also tested as pair with rabbit polyclonal antibody for sensitive detection of BoNT/B. Among monoclonal antibodies, we found that one human antibody F-10C most effectively binds to BoNT/B. Using this antibody we have established capture ELISA methods for BoNT/B detection. Our results can be used as an auxiliary test method for laboratory diagnosis of naturally occurred or bioterror-induced botulism in the future.

Keywords: BoNT/B, polyclonal antibody, monoclonal antibodies, capture ELISA

**F-59**

**Enhanced Antibody Neutralization Against Botulism by Apoptotic Cell Targeting Peptide Conjugates**

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Botulinum neurotoxins (BoNTs) are known as the most toxic substances on earth and categorized as potential biowares. Therefore, developing practicable therapy is pivotal in terms of biodefense field. In this study, we established therapeutic fusion protein consisting of the apopotic peptide of PSP-1 (Peptide-based phosphatidylserine) and Apopep-1 (Apoptosis-targeting-peptide-1) to core streptavidin respectively. Both Apopep-1 and PSP-1 were reported to home to apoptotic cells, while only little binding to live cells was observed. As streptavidin specifically binds to biotins which were present on anti-BoNT monoclonal antibody, the toxin-antibody-streptavidin complex were expected to be bound to apoptotic cells. We generated a toxin neutralizing anti-BoNT/A monoclonal mouse antibody, H9-6 which was bound to BoNT/A in western blot analysis. And we also showed that neutralizing potency of H9-6 against BoNT/A was enhanced by introducing apoptotic cell targeting conjugates consisting of Apopep-1 or PSP-1 with streptavidin. Therefore, we expect that this technology might be relevant for developing efficient therapy against botulism.

Keywords: Botulinum neurotoxins A, Apopep-1, PSP-1, Monoclonal antibody, Neutralization

**F-60**

**Novel Mutation in an 8-bp Deletion of the Hepatitis B Virus X Gene Leads to Occult Infection in Korean Vaccinated Individuals**

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Background: Universal infantile hepatitis B virus (HBV) vaccination may lead to an increase in vaccine escape mutation, which may pose a potential threat to the long-term success of massive vaccination.

Methods: To check the prevalence and mutation patterns of vaccine escape variants in South Korea, the vaccinated 87 subjects, which was HBV surface antigen (HBsAg) negative and anti-HBs positive, were screened for the presence of HBV DNAs via both the nested PCR protocol and VERSANT HBV DNA 3.0 assay.

Results: The presence of HBV DNAs were confirmed from a total of 6 subjects (6.9%). All the six variants had a common mutation type (X8Del), 8-bp deletion in C terminal region of HBV X gene (HBxAg) known to have transacting capacity, leading to truncated HBxAg, although there were also some different mutations between them. Our transient transfection analysis using full HBV genome for the presence of HBV DNAs via both the nested PCR protocol and VERSANT HBV DNA 3.0 assay. Results: The presence of HBV DNAs were confirmed from a total of 6 subjects (6.9%). All the six variants had a common mutation type (X8Del), 8-bp deletion in C terminal region of HBV X gene (HBxAg) known to have transacting capacity, leading to truncated HBxAg, although there were also some different mutations between them. Our transient transfection analysis using full HBV genome for the presence of HBV DNAs via both the nested PCR protocol and VERSANT HBV DNA 3.0 assay.

Summary: In conclusion, our data suggest that a novel vaccine escape mutation, X8Del may contribute to the HBV vaccine escape via reduced secretion of HBsAg and virion, maybe by compromised HBsAg transacting capacity. To our knowledge, it is the first HBsAg mutation associated with HBV vaccine escape.

Keywords: Hepatitis B virus, Vaccine escape mutation, HBsAg, Hydrodynamic method

www.fkms.kr | 301
Gender Disparity Induction by Male-Specific Hepatitis B Virus Large Surface Protein Variant W4P in Hepatocarcinogenesis
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Hepatitis B virus-related HCC occurs more frequently in men. However, the underlying mechanisms of carcinogenesis and gender disparity in HBV-induced HCC remain unclear. We reported a novel HCC-related W4P mutation in the large surface protein of HBV genotype C in male HCC patients. This study investigates the physiological role of the variant in carcinogenesis and gender disparity. Although both WT and W4P variant LHBs enhanced cell proliferation by regulating the cell cycle and facilitated the NIH3T3 cell colony formation, the W4P variant demonstrated higher activity compared to the WT. Cells expressing W4P LHB induced tumor masses in a nude mice allograft model. Tumor masses produced by variant LHB were significantly larger in male mice than in female mice, and the tumor masses were significantly reduced by administration of estrogen. The IL-6 was elevated in male mice harboring W4P-induced tumor, and the level was reduced by estrogen treatment. IL-6 levels of HCC patients with the W4P variant were higher than those of patients with WT LHB. The W4P LHB induced a higher production of IL-6 than WT LHB, and the level could be reduced by estrogen. The ability to reduce cell proliferation and colony formation of W4P LHB was hampered by the inhibition of IL-6 signaling. This study suggests that the W4P mutation during the natural course of chronic hepatitis B infection may contribute to HCC development in patients, particularly in the male patients, in an IL-6-dependent manner.

Keywords: PreS1 mutation, Hepatocellular carcinoma, estrogen, interleukin-6, chronic hepatitis B infection

Anti-sepsis Therapeutic Effects of HY-209 on LPS Induced Mouse Liver by Mass Spectrometry-based Label Free Quantitative Analysis
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HY-209 protect septic mice by controlling lipid mediators partly. In severe cases, HY-209 might be crucial mode of action. These results suggest that HY209-treated groups after LPS injection. Among them, two clusters of proteins (35 proteins) were up-regulated in the liver after HY-209 treatment in septic mice. Interestingly, CFTR formed crucial node in protein networks controlled by HY209 in liver. As CFT, related to the fatty acid metabolism systematically, several proteins controlling lipid metabolism formed major networks controlled by HY-209. Considering that lipid mediators are crucial in proinflammatory responses, control of lipid metabolism in liver of septic mice by HY209 might be crucial mode of action. These results suggest that HY-209 protect septic mice by controlling lipid mediators partly.

Keywords: Sepsis, Inflammatory response, HY-209, Quantitative proteomics, CFTR

Construction of an EGFP-expressing Replication Competent Porcine Circovirus Type 2
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There are two type of porcine circoviruses (PCV1 and PCV2) based on pathogenicity and nucleotide sequences. PCV1 is nonpathogenic in swine and isolated as a persistent contaminant of PK-15 cell line, whereas PCV2 is considered to be associated with post-weaning multisystemic wasting syndrome (PMWS), newly emerged economically important swine disease. PCV2 is the smallest virus among animal viruses (17nm), non-enveloped, single-stranded, ambisense DNA virus. The PCV2 genome has two major open reading frames (ORFs). ORF1 encodes the Rep proteins associated with virus replication (35.7KDa) and ORF2 encodes the immunogenic capsid (Cap) protein approximately 27.8 kDa in size. Cloning of the full length PCV2 genome in a plasmid allows the construction of infectious clones. The small size of the PCV2 genome (1.7kb) easily allows construction of full length molecular clone by PCR. In this study, an EGFP-expressing replication-competent molecular clone that allows direct titer measurement was constructed. The EGFP PCR product from pEGFP-N1 plasmid was cloned into pBluescript II SK+(pgS). The viral DNA of PCV2 was extracted from the supernatant of infected PK-15 cells. The PCR products of full length PCV2 genome was then cloned into EGFPpSK(pgS-PCV2-EGFP). After transfection, pSK-PCV2-EGFP showed green fluorescent signals in the nuclei of PK-15 cells. EGFP-fused PCV2 will be more useful for understanding PCV2 replication and pathogenicity. 

Cell-type Specific Regulation of KSHV Replication by OX40-OX40L Interaction
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Kaposi’s sarcoma-associated herpesvirus (KSHV) belongs to the human gammaherpesvirus subfamily, and is associated with malignancies of endothermal origin (Kaposi’s sarcoma, [KS]) and B cell origin (primary effusion lymphoma [PEL] and multicentric Castleman’s disease [MCD]). Viral lytic replication is known to be required for KS and MCD. As KSHV-related tumors mostly develop in immuno-compromised human subjects, immune responses are believed to be important in the control of KSHV replication. Recently, cortical OX40 deficiency, as determined by genome-wide exome sequencing, was shown to be associated with aggressive childhood KS in a patient, suggesting that disrupted OX40–OX40L interactions might be implicated in disease development. Here, we report that interaction of recombinant OX40 protein with OX40L expressed on endothelial cells severely impaired KSHV lytic replication. Furthermore, 4-1BB-4-1BBL interactions were also capable of efficiently inhibiting viral replication in B cells and endothelial cells. To our knowledge, this is the first direct evidence that ligation of tumor necrosis factor superfamily (TNFSF) members and their cognate receptors (TNFRSF) is important for the control of viral lytic replication. These data will likely pave the way for the development of KSHV-specific therapies for KS and MCD, in which viral lytic replication is a disease determining factor.

Keywords: KSHV, OX40, immune regulation
Kaposi’s sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi’s sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman’s disease (MCD). KS is a tumor in the endothelium and the most common cancer in AIDS patients. In the early stage of KS, KS lesions are infiltrated with a large number of immune cells, which produce a wide array of cytokines and chemokines, including interferon-γ, roles of which in the pathogenesis of KS are not known. Here we report IFN-γ inhibits KSHV reactivation from latently infected cells as well as viral replication in de novo infections in endothelial cells while that in B cells is not affected. As IFN-γ treatment after viral reactivation (48 hr post-reactivation) is still capable of significantly down-regulating viral progeny production, it seems that IFN-γ may act on delayed-early, rather than immediate early, phase of viral replication. Taken together, these data imply that inflammatory cytokines, including IFN-γ, may help KSHV enter latency, thus evading immune responses against actively replicating viruses. 

Keywords: KSHV, interferon-gamma, viral replication

Kaposi’s sarcoma-associated herpesvirus (KSHV) is causally associated with 3 human malignancies, including Kaposi’s sarcoma (KS), some forms of multicentric Castleman’s disease (MCD), and primary effusion lymphoma (PEL). Kaposi’s sarcoma is a tumor of endothelial origin with interesting features such as unusually extensive angiogenesis, massive immune cell infiltration, and inflammation. Many cytokines and chemokines are detected at high levels in KS lesions, the roles of most of which are not known. IL-6 is a proinflammatory cytokine and a tumor-inducing factor and it is expressed abundantly in KS and MCD lesions. However, its role in KSHV replication and/or reactivation, thus in KSHV-induced pathogenesis, remain elusive. Of note, IL-6 treatment on latently infected cells with KSHV induced viral reactivation in terms of lytic promoter activation and viral progeny production. Not only IL-6 but also oncostatin M (OSM) and ciliary neurotropic factor (CNTF) had the similar effects, most likely due to the fact that those 3 cytokines share the same signaling receptor, gp130, suggesting that Jak2-STAT3 pathway is involved in KSHV reactivation. This implies that IL-6 may play a critical role in viral reactivation in vivo, thus acting as a physiological stimulus for KSHV reactivation.

Keywords: KSHV, reactivation, IL-6

Kaposi’s sarcoma-associated herpesvirus (KSHV) is the latest addition to human herpesvirus family: human herpesvirus 8 (HHV-8). KSHV is known to be required for the development of B cell lymphomas as well as an endothelial tumor: primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD) are of B cell origin, however, the mechanisms of tumorigenesis seem to differ in that PEL tumors are mostly monoclonal while MCD’s are polyclonal. Viral replication is known to be required for the development of MCD and IL-6, among other cytokines, plays a critical role in the pathogenesis. IL-6 is the canonical cytokine that activates STAT3. IL-6 is abundantly expressed in MCD and Kaposi’s sarcoma lesions and is known to be required for the survival and proliferation of KSHV-infected cells. Although KSHV replication is required for MCD development, it is not known whether IL-6 is directly involved in KSHV replication. To investigate the role of IL-6 in viral replication, IL-6 was treated on ISK219 cells, lysically induced by doxycycline. Compared to the medium-treated cells, IL-6-treated cells produced higher levels of viral progeny. When STAT3, the main target of IL-6 signaling, is knocked down by specific siRNA’s, KSHV replication was significantly down-regulated. Inversely, when a constitutively active STAT3 was expressed, viral replication was augmented by 2-3 fold. Taken together, these data clearly suggest that STAT3 is required for efficient replication of KSHV.

Keywords: KSHV, IL-6, replication

Kaposi’s sarcoma-associated herpesvirus (KSHV) is a large DNA virus which is known to induce at least 3 human cancers. Primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD) are the two tumors of B cell origin. PEL tumors are latently infected with KSHV, thus mostly monoclonal while roughly half of infected B cells found in MCD lesions are lytically activated, thus in nature MCD is a polyclonal tumor. It is interesting to note that KSHV infection in B cells produces two very different tumors. To establish an in vitro infection model for B cells, we employed tonsillar homogenates, which are composed of roughly 30% T cells and 70% B cells. At MOI 1, ca. 5% B cells were infected by KSHV and without lytic induction with sodium butyrate, infected B cells produce a large quantity of viral progeny in the culture supernatant, suggesting that KSHV infection in tonsillar B cells may not establish an efficient latency, rather be spontaneously lytic. Viral replication was efficiently blocked by the addition of activated CD4+ T cells, implying T cells mediated the inhibition. Infected B cells were enriched at d13 post-infection, thus suggesting KSHV-derived survival advantage. The nature of viral factors is now being investigated.

Keywords: KSHV, tonsillar B cells, lytic replication
**F-69**

**Mycobacteria-dependent ROS Production is Mutually Dependent on the Dectin-1 Expression in Human Airway Epithelial Cells**

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The dectin-1 is expressed primarily in myeloid lineage cells, including macrophages and dendritic cells, it has not been revealed whether dectin-1 is actively induced in human airway epithelial cells after infection with mycobacteria. In this study, we investigated whether Mycobacterium tuberculosis (Mtb) actively induced the expression of Dectin-1 mRNA and protein in A549 cells in a toll-like receptor (TLR) 2-dependent manner. In addition, Mtb-mediated generation of reactive oxygen species and Dectin-1 induction were mutually dependent. Moreover, Dectin-1 was required for pro-inflammatory cytokine release and antimicrobial effects on intracellular mycobacterial growth in A549 cells. Collectively, our findings demonstrate the novel induction of Dectin-1 in type II airway epithelial cells and its critical role in the innate immune response against Mtb in non-phagocytic cells.

**Keywords:** Mycobacterium tuberculosis, human airway epithelial cells, Dectin-1, Reactive oxygen species, Toll-like receptor 2

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**F-70**

**Expression of the Global Regulator LeuO is Self-Regulated by Coordinated Binding of H-NSS, ToxR, and LeuO on Cognate cis-Acting Elements**


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**Vibrio vulnificus** produces cyclo(L-Phe-L-Pro) (cFP). This signal molecule induces the expression of sets of genes including ompU. The expression of ompU requires ToxR, a transmembrane transcriptional activator. By using DNA microarray and NGS analyses, we found that cFP also induces the expression of transcriptional activator LeuO in V. vulnificus. Activation of the expression of the LeuO by cFP also requires ToxR. The periplasmic (C-terminal) domain of ToxR was physically bound by cFP as demonstrated by the isothermal titration calorimetry (ITC) assay. The purified ToxR physically binds to the upstream region of LeuO. We also found that LeuO regulates the expression of itself by binding to the region upstream of its cognate gene. H-NSS has been shown to repress a variant of genes in response to environmental signals in a number of enteric bacterial species. We found that the binding site of ToxR on the leuO upstream region overlaps with H-NSS and LeuO binding sites. Furthermore, ToxR outcompetes with H-NSS at the same concentration for the binding. These suggest that auto-regulation of LeuO is modulated by two additional DNA-binding proteins, ToxR and H-NSS. Taken together, cFP signaling is transduced to LeuO via the membrane sensor ToxR. Expression of the global regulator is fine-tuned by balance among LeuO itself, ToxR, and H-NSS in V. vulnificus.

**Keywords:** leuO, ToxR, HNS, *Vibrio vulnificus*

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**F-71**

**Cyclo(L-Phe-L-Pro) Facilitates the Survival of Vibrio vulnificus under Oxidative Stress**

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Pathogens have evolved numerous defense mechanisms against oxidative stress. *Vibrio vulnificus* is an opportunistic pathogen that has an ability to cause severe sepsis and wound infection in human. In previous studies, *V. vulnificus* as well as other *Vibrio* spp. such as *V. cholerae* and *V. harveyi* produces Cyclo(L-Phe-L-Pro) (cFP), which is a signal molecule controlling the expression of genes including those required for the pathogenicity. cFP also plays an ambivalent role in interactions between host and the pathogen. *V. vulnificus* produces several enzymes to overcome the attack of ROS such as superoxide anion and hydrogen peroxide. In this present study, we found that cFP enhances the survivability of *V. vulnificus* under ROS-rich condition. Survivability of the wild type *V. vulnificus* pre-treated with menadione, a compound generating superoxide anion, at 0.1 mM was enhanced by at least three times by 1 mM cFP. However, survivability of mutant with a deletion in soxR, encoding a redox sensing global regulator, or katG, encoding a catalase, was not recovered by the treatment of cFP. However, cFP did not affect the expression of MrSOD or KatG. We found that ToxU, which has been known to be the cognate regulator of cFP, positively regulates soxR. We also demonstrated that purified ToxU binds to the upstream region of soxR. These suggested that cFP enhances the expression of soxR via ToxU, which consequently affects the resistance to ROS but not by SOD or KatG.

**Keywords:** *Vibrio vulnificus*, Oxidative stress, Cyclo(L-Phe-L-Pro)

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**F-72**

**Host Restriction Mechanisms Against HIV-1 Replication and AIDS Pathogenesis**

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Tumor suppressor p53 has been suggested to be a host restriction factor against HIV-1 replication, but the detailed molecular mechanism has remained elusive for decades. Here, we demonstrate that p53-mediated HIV-1 suppression is attributed to PKR-mediated HIV-1 Tat phosphorylation and inactivation. p53-silencing significantly enhanced HIV-1 replication in infected cells. Ectopic expression of p53 suppressed Tat activity, which was rescued by PKR-silencing. In addition, ectopic expression of PKR abolished Tat activity in p53- and eIF2αSε cells. Finally, we found that HIV-1 infection activates p53, followed by the induction and activation of PKR. PKR directly interacted with HIV-1 Tat and phosphorylates the first exon of Tat exclusively at five Ser/Thr residues, which inhibits Tat-mediated provirus transcription in three critical steps: i) Tat translocation into the nucleus, ii) Tat-TAR binding, and iii) Tat-cyclin T1 interaction. These five Ser/Thr sites on Tat were highly conserved in HIV-1 strains prevalent in Europe and the United. Taken together, our findings indicate that p53-derived host restriction of HIV-1 replication is likely achieved at least in part, to a non-canonical p53/PKR/Tat phosphorylation and inactivation pathway in HIV-1 infection and AIDS pathogenesis.

**Keywords:** HIV-1, p53, host restriction, PKR, HIV-1 Tat
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Serotype-independent Protection from Group B A Synthetic Latch Domain Peptide Vaccine Confers T cell-dependent protection of GBS infection than CheGBS.
Keywords: Group B Streptococci, Streptococcus agalactiae, radiation, vaccine, humoral immune response

Irradiated Group B Streptococcal Whole Cell Vaccine Induces Protective Cellular as Well as Humoral Immune Responses
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The Gram-positive bacterium group B Streptococcus (GBS), known as Streptococcus agalactiae, is the most common cause of meningitis in human newborns and pregnant women. GBS. These infections are often associated with a high mortality incidence, despite effective antibiotics and other aggressive therapeutic interventions. Since previous several studies showed that a killed GBS whole cell vaccine (WCV) can confer effective protection against GBS challenge animal model, we investigated the efficacy of an irradiated killed WCV (RadGBS) as compared with a chemically killed WCV (CheGBS). WCV were prepared by treating gamma-irradiation at 9Gy/hr or 99% formalin for 2 hrs. Two dose intraperitoneal immunization with RadGBS or CheGBS in the absence of adjuvant elicited a significant level of IgG and IgM antibodies in the serum, but no statistic different was observed between two groups. Nevertheless, RadGBS immunization was provided significantly higher rate of protection than CheGBS in GBS challenge model. In addition, we found that the total number of CD3+CD4+ T cells and the level of CD4+ T cell-associated cytokine (TNF-α, IL-6 and IL-10) and chemokine (MCP-1 and MIP-1) in the spleen was significantly increased in the RadGBS immunized group as compared with the CheGBS immunized group. These findings strongly suggest that irradiated vaccine can elicit both cellular and humoral immune response and provide effective CD4+ T cell-dependent protection of GBS infection than CheGBS.
Keywords: Group B Streptococci, Streptococcus agalactiae, radiation, vaccine, humoral immune response

F-75
Humanized Virus Suppressing Factor (hzVSF), a humanized Monoclonal Antibody, against Influenza A (H1N1) Virus Infection Suppresses Viral Replication and Inflammatory Response
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We investigated that hzVSF, a humanized VSF, has antiviral and anti-inflammatory effects against influenza virus (H1N1). MATERIAL AND METHODS: hzVSF was produced in Chinese hamster ovary (CHO) cell lines and identified as an IgG4 subtype humanized monoclonal antibody by SDS-PAGE and SE-HPLC. Influenza viruses A/Puerto Rico/8/34 (H1N1) were used in these experiments. In vivo, the virus titers in murine lung were measured by TCID50 assay. In vitro, the effect of hzVSF treatment on viral replication was assayed by western blot analysis. The infiltration of immune cells in murine lung was analyzed by immunohistochemistry by anti-CD4 and anti-F4/80 antibody. 6 cytokines (IL-6, MCP-1, TNF-α, IFN-β, IL-1β, IL-10) in lung of mouse treated with PR8 only or PR8 plus hzVSF treatment were measured simultaneously by cytokine bead array. RESULTS: This study found that hzVSF inhibits the influenza virus replication in vitro and in vivo, suppressing viral pneumonia against infiltration of immune cells into lung. Furthermore, inflammatory cytokines production was reduced markedly in hzVSF-treated mice. CONCLUSION: hzVSF suppress viral replication and lung inflammation for influenza virus infection. These findings suggest that, if developed as new anti-influenza therapeutic, hzVSF may provide to be useful in the treating of influenza-mediated inflammatory diseases.

F-76
Virus Suppressing Factor (VSF) Inhibits Hyperglycemia and Pancreatic Islet Inflammation in Diabetes Mellitus Induced by Encephalomyocarditis Virus
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Virus suppressing factor (VSF) has antiviral activity against several types of viruses and is released from a hybridoma cells fused with splenocytes of BALB/c mice infected with EMC-DV. This study investigated that VSF has efficacy against viral diabetes and its antiviral mechanisms mediating the antiviral effects and anti-inflammatory function of VSF. This study found that VSF inhibited virus replication in vitro and prevented the destruction of pancreatic islets from the infiltration of immune cells in vivo. This study showed that the treatment of VSF into EMC-D-infected mice offered protection from diabetes mellitus (DM), and islets in mice treated with VSF produced less viral proteins and expressed MHC class I molecule. As a result, EMC-D virus rarely induces destruction of islets, decreasing the infiltration of immune cells into the mice’s pancreas. These results are similar to identical with the results after treatment with interferons (IFNs). In addition, these effects may be activated through cellular surface molecule, which allows binding with VSF after EMC-D infection. These findings suggest that VSF induces endogenous antiviral replication cascade and may provide to be useful in the treating of virus-mediated inflammatory diseases.
**F-77**

**Anti-viral Effects of Hyrtios sp. and Haliclona sp. on Rotavirus-infected Caco-2 Cells**

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_Hyrtios_ sp. and _Haliclona_ sp. are species of the marine sponge. The sponge produces secondary metabolites with bioactivity. We studied anti-rotavirus effects of _Hyrtios_ sp. and _Haliclona_ sp. extracts. The cytotoxicity of _Hyrtios_ sp. and _Haliclona_ sp. extracts was assessed using MTT assay. Antiviral assay was performed on Caco-2 cells. Results show that none of the _Hyrtios_ sp. and _Haliclona_ sp. extracts examined had cytotoxicity on Raw 264.7, HT-29, and Caco-2 cells. These _Hyrtios_ sp. and _Haliclona_ sp. extracts displayed rotavirus inhibitory effects on caco-2 cell infected rotavirus. In conclusion, our findings suggest that these _Hyrtios_ sp. and _Haliclona_ sp. extracts may have potential to be developed into anti-rotavirus treatments.

**Keywords:** sponge, _Hyrtios_ sp., _Haliclona_ sp., antiviral, rotavirus

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**F-78**

**Post-translational Modification of TANK-binding Protein 1 is Regulated by a Viral Immune Modulator of Gammaherpesvirus**

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_Gammaherpesviruses including Epstein-Barr virus (EBV) and Kaposi’s sarcoma-associated herpesvirus (KSHV), and marine gammaherpesvirus 68 (MHV-68) are important as they are causative pathogens in various types of malignancies. They are also characterized by the ability to establish life-long persistent infection in the host by evading immune system._

_Previously, we reported that open reading frame 11 (ORF11) of MHV-68 functions as a viral immune modulator that suppresses TANK-binding kinase 1 (TBK1) mediated IFN-β signaling by sequestering TBK1. ORF11 binding to TBK1 disrupts the interaction between TBK1 and IRF3. By performing sequence alignment, we identified sequences which are highly conserved in the motifs of a cellular enzyme involved in post-translational modification of proteins. Using deletion and single amino acid mutants, we demonstrated the enzymatic activity of ORF11 in TBK1 modification. These results suggest a novel function of ORF11 in regulating the post-translational modification of TBK1._

**Keywords:** Gammaherpesvirus, MHV-68, ORF11, TBK1

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**F-79**

**Introduction of Chonbuk, Gyeongsang, and Kyungpook National University Hospital Branches of National Culture Collection for Pathogens**

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_Nationwide culture collection of pathogens is an essential part of the infrastructure for the controls of infectious diseases, the advancement of the medical research and the life science, and the development of the biotechnology and the health industry. Therefore, the Branches of National Culture Collection for Pathogens were established in Chonbuk (CNUH, Jeonju), Gyeongsang (GNUH, Jinju), and Kyungpook (KNUH, Daegu) National University Hospital by the support of the Ministry of Health & Welfare (The Korea Centers for Disease Control and Prevention). The Branches aim for collection, deposition, preservation, and distribution of the bacteria which are associated with the hospital-acquired infections, community-acquired infections, and chronic infections. Since 2008, 3 Branches have collected and registered 11,240 isolates of 253 species with clinical, epidemiological, and bacteriological information. In additions of general isolates, GNUH includes the pathogenic fungi, KNUH the reckettsia, and GNUH the antibiotic resistant pathogens. The repository isolates of pathogens could be requested through Branches (GNUH, tel 063-259-3085, fax 063-259-3086; KNUH, tel 055-750-9256, fax 055-750-9260; KNUH, tel 053-200-3381, fax 053-200-3389) without any payment. The Branches are attempting to play a resource repository for clinical isolates of pathogens which are useful to develop standard and reference strains for education, investigation, and industry._

**Keywords:** Culture Collection

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**F-80**

**Host Transcriptional Analysis of Mycobacterium avium subsp. paratuberculosis Infection in Animal Model**

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_Paratuberculosis (PTB), or John’s disease, is a chronic granulomatous enteropathy of ruminants caused by infection with Mycobacterium avium subsp. paratuberculosis (MAP). A number of researches have proposed that the principal infective agent of Cohn’s disease, a chronic enteropathy in humans, is MAP. PTB has very long latent periods and continues to excrete the infective bacteria in feces before development of the disease. These feral shepherds might act as sources of infection to other animals, the development of a diagnostic method that is useful in the early stage is very important to eradicate or control the disease. The identification of biomarkers in specific stage of PTB will provide advanced knowledge of pathogenesis and will enable especially their use in early diagnosis of slow-growing bacterial infection. In the current study, we applied a microarray-based approach to discover genes related to potential immune responses in a model of canine MAP infection and naturally MAP-infected cattle. The differentially expressed genes, which were statistically significant with log2-fold change >1.5 and p <0.05, were characterized by functional, network, and pathway analysis using Ingenuity Pathway Analysis (IPA). These results provide information which further the understanding of the immunopathologic response to MAP infection in mice, thereby providing insights valuable for research into the pathogenesis for MAP infection._

**Keywords:** mycobacterial infection, John’s disease, Cohn’s disease, Transcriptional profiles, Animal model
The Effect of Branched-chain Amino Acids Supplementation on the Replication of Salmonella Typhimurium in Nitric Oxide-producing Raw 264.7 Macrophages

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Bacterial proliferation can be restricted by nitric oxide (NO) produced in phagocytic host cells as a component of innate immune response. NO can target Fe-S cluster containing enzymes to cause bacteriosis. In the branched-chain amino acids (BCAAs; isoleucine, leucine, valine) biosynthesis pathway of Salmonella enterica serovar Typhimurium, dihydroxy acid dehydratase (IlvD) and isopropylmalate isomerase complex (LevCD) are essential Fe-S enzymes for BCAAs synthesis, and their inactivation by NO has been implicated in the NO-mediated BCAAs auxotrophy. In this study, we present that the replication of mutant S. Typhimurium lacking IlvD or LevCD is impaired in NO-containing cultures and inside NO-producing RAW 264.7 macrophages, but exogenous supplementation of BCAAs can recover their replication under both conditions. BCAAs supplementation did not affect the NO consumption rate of S. Typhimurium, suggesting the BCAAs-promoted NO resistance independent of NO metabolism. BCAAs supplementation induced intracellular survival of IlvD and LevCD mutants at wild-type levels inside RAW 264.7 macrophages that produce constant amounts of NO regardless of varied supplemental BCAAs concentrations. Our results suggest that the NO-induced BCAAs auxotrophy of Salmonella, due to inactivation of iron-sulfur enzymes for BCAAs biosynthesis, could be rescued by bacterial taking up exogenous BCAAs.[Supported by NRF grant (2008-0062283)]

Keywords: Branched-chain amino acids, iron-sulfur cluster enzyme, Nitric oxide, Salmonella Typhimurium

Rapid Detection of blaKPC-2 and blaNDM-1 type Carbapenemase-producing Enterobacteriaceae from Clinical Isolates by Loop-mediated Isothermal Amplification

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Carbapenem-resistant Enterobacteriaceae (CRE) is the leading causes of nosocomial infection at intensive care units. Rapid and sensitive detection of CRE infections is required to provide appropriate antibiotic treatment. In this study, loop-mediated isothermal amplification (LAMP) was optimized as a reliable diagnostic technique for detection of the presence of blaKPC-2 and blaNDM-1 genes, critical components for carbapenem resistance. Reactions were most efficiently performed at 62 °C with 2 mM MgSO4. Positive LAMP reactions were achieved with only using 100 pg template DNA for blaKPC-2 and 10 pg for blaNDM-1, which represent significantly low limit of detection, when compared with conventional PCR. Furthermore, positive LAMP reactions were accomplished with reaction times of 40 min for both blaKPC-2 and blaNDM-1, respectively. Finally, 100 CRE patient isolates were successfully tested for the presence of blaKPC-2 or blaNDM-1 gene by LAMP, which was determined to be more sensitive than conventional PCR. Together, our results clearly demonstrate the usefulness of LAMP assay for the diagnosis of CRE.

Keywords: LAMP, carbapenem-resistance, Enterobacteriaceae

The Role of the Spy (spheroplast protein y) Gene in Response of Salmonella Typhimurium to Reactive Oxygen/nitrogen Species

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Antimicrobial actions of reactive oxygen/nitrogen species (ROS/RNS) derived from in host phagocytes inactivate various bacterial macromolecules. In response to these stresses, bacteria also induce expression of molecular chaperones to aid in ameliorating protein misfolding. In this study, we explored the function of an extracytoplasmic chaperone Spy, that is localized exclusively in the periplasm when bacteria exposed to conditions causing spheroplast formation, in the resistance of Salmonella enterica serovar Typhimurium to ROS/RNS. A spy deletion mutant was constructed in S. Typhimurium and subjected to ROS/RNS stresses. The spy mutant Salmonella showed a modest decrease in growth rate in NO-producing cultures, and no detectable difference of growth rate in H2O-containing cultures, compared with that of wild type Salmonella. Quantitative RT-PCR analysis showed that spy mRNA levels were similar regardless of both stresses, but were increased considerably in S. Typhimurium mutants lacking the flavohemoglobin Him, which are incapable of NO detoxification, and lacking an alternative sigma factor 8O, conferring hypersusceptibility to H2O2, respectively. Results demonstrate that Spy expression can be induced under extreme conditions of both stresses, and suggest that the protein may have supportive roles in maintaining proteostasis in the periplasm where various chaperones may act in concert with Spy, thereby protecting bacteria against toxicities of ROS/RNS.

Keywords: chaperone, reactive nitrogen species, reactiveoxygen species, Salmonella, Spy
**Poster Session**

### F-85

**Development of a Functional Diaper by Using Phellodendron Amurense RUPR**

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Our team used pajansas for newborn and cloth for baby diaper which are dyed and could use antibiotic and antiinflammatory because Phellodendron amurense RUPR have antibiotic and antiinflammatory to protect newborn babies. Therefore, we will graduate and apply them to the diapers for adults. We conducted susceptibility test through Phellodendron amurense RUPR extracts and confirmed antibiotic relating to bacterial infection. Measurement on vitality of antiinflammatory (nitrite) showed that when we concentrated Phellodendron amurense RUPR extracts to 25 μg/ml, 50 μg/ml, 100μg/ml, the final results was effective on 61% of antiinflammatory in 100μg/ml Measurement on cytotoxicity showed that we couldn't find cytotoxicity in the concentration rate of 5 μg/ml, 50 μg/ml, 100μg/ml. We sprinkled mixed liquid into the fabric, passed it through dry zone and dried it. The experiments indicated that the diapers have an effectiveness on antiinflammatory in 3% of low concentrated rate via specified Phellodendron amurense RUPR extracts powder. Through this experiments and results, We think we can apply these diapers to various human-friendly functional products.

**Keywords:** Phellodendron amurense RUPR, antiinflammatory, antibiotics, diaper

### F-86

**In Vitro Studies on the Inhibition of Mycobacterial Cell Wall Mycolic Acid and Promoting Intracellular Killing by DPG-5: Promising Anti-Mycobacterial Activity**

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Due to emergence of multi-drug resistant (MDR) and extremely drug resistant (XDR) strains of *Mycobacterium tuberculosis* (MTB), current treatment regimen often fail. In order to combat and restrict MTB growth and spreading, therefore novel anti-mycobacterial agents either from natural source or synthetic origin should be screened against MTB for novel antibiotic potency. Deoxypergularinine-5 (DPG-5), a synthetic analogue of Deoxypergularinine (DPG), was evaluated for its biological activity against both susceptible and resistant strains of MTB. DPG-5 showed MIC (minimum inhibitory concentration) against all strains of MTB ranging from 3.125 to 6.25. We also demonstrated the effect of DPG-5 on MTB cell wall mycolic acid and found that at elevated concentration DPG-5 inhibited mycolic acid specifically. Later we demonstrated the intracellular killing activity of DPG-5 using GFP activated H37Ra in mouse macrophage followed by CFU counting. We found that DPG-5 actively reduced the bacillary count in infected macrophage. Beside cytotoxic evaluation of DPG-5 were also corroborated and a minimal cytotoxicity of DPG-5 in different eukaryotic cell lines was observed. Thus DPG-5 holds the promising antinococcal activity for future drug development.

**Keywords:** MDR, XDR, MTB, DPG-5, CFU
**G-1**

Apo-9'-fucoxanthinone, Isolated from *Sargassum muticum*, Suppress Pro-inflammatory Cytokine Production in CpG-stimulated Immune Cells by Down Regulating Mitogen-activated Protein Kinase Pathway

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This study was designed to investigate the inhibitory effect of apo-9'-fucoxanthinone (APO-9') isolated from *S. muticum* on the production of pro-inflammatory cytokines. *S. muticum* extract (SME) showed significant inhibitory effects on pro-inflammatory cytokine production in immune cells. Bone marrow-derived macrophages (BMDMs) and dendritic cells (BMDCs) pre-treated with APO-9'and then stimulated with CpG DNA exhibited a strong dose-dependent inhibition of interleukin (IL)-12 p40, IL-6 and tumor necrosis factor (TNF)-α production. It displayed a strong inhibitory effect on the phosphorylation of ERK1/2 and on activator protein (AP)-1 reporter activity. The findings herein reveal that SME and APO-9' have a significant anti-inflammatory property and warrant further studies concerning the potentials of SME and APO-9' for medicinal use.

Keywords: Pro-inflammatory cytokine, Apo-9'-fucoxanthinone, ERK1/2, *Sargassum muticum*, Inflammation

**G-2**

HCMV Regulation of Receptor-Interacting Protein Kinase 1 (RIP1)-Mediated NF-κB Signaling

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The human cytomegalovirus (HCMV) UL45 protein is a tegument protein and is an inactive ribonucleotide reductase R1 subunit (R1R) homolog. Since murine cytomegalovirus R1R homolog M45 interacted with murine receptor-interacting protein kinase 1 (mRIP1) and inhibited mRIP1-mediated signalings, we investigated the effect of UL45 on human RIP1-mediated signaling. UL45 interacted with RIP1 in a manner independent of the RHIM and ubiquitination of RIP1. Overexpression of UL45 decreased the RIP1-mediated NF-κB activation. We produced a recombinant virus encoding HA-tagged UL45 and found that HA-UL45 colocalized with another tegument protein UL48. UL48, a viral deubiquitinating protease (DUB), interacted with both UL45 and RIP1 in cotransfection assays. Consistently, HA-UL45 formed a complex with UL48 and RIP1 in recombinant virus-infected cells. Like UL45, UL48 also inhibited the RIP1-mediated NF-κB activation. Overexpression of UL45 and UL48 synergistically decreased the RIP1-mediated NF-κB activation. At early times of HCMV infection, tegument UL48 inhibited the TNFα-mediated NF-κB activation by modulating RIP1 ubiquitination. At late times of HCMV infection, both UL45 and UL48 inhibited the TNFα-mediated NF-κB activation. Collectively, our data demonstrate that HCMV UL45 and UL48 tegument proteins play a role in the regulation of RIP1-mediated NF-κB signaling.

Keywords: HCMV, UL45, UL48, RIP1, NF-κB

**G-3**

Characterization of Surface Binding Protein to Signal Molecules Related to Quorum Sensing of *E. coli* SE15 Isolated form Indwelling CA-UTI Patients

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Catheter-associated urinary tract infection (CA UTI) is one of the most common care-associated infections around the world. The pathogenesis of CA UTI is related to the susceptibility of inert catheter material to microbial colonization and has been attributed to its ability to express various virulence factors by quorum sensing (QS) of colonized bacteria. Since the QS signal is transduced into an intracellular via bacteria membrane, they can recognize each other by QS and also, altered gene expression (related to virulence genes and biofilm) in the target bacteria. Thus due to the role of surface proteins as interfaces between the cell and the environment, they are important candidates for developing novel drugs to protect against bacterial pathogens. Therefore, the purpose of this study is to investigate autoinducer (Acyl homoserine lactones; AHLs, AI-2) binding receptor proteins from surface proteins of *E. coli* SE15. In our result, we were able to identify 10 proteins from the surface protein bound to AHL by using MALDI-TOF and all peptides identified were from the membrane proteins. Among these identified proteins, we found receptor protein related to quorum sensing signal molecule (AHLs) such as Signal recognition particle protein, Long-chain fatty acid transport protein. These proteins are considered as a putative role related to fatty acid transport and also, will be helpful to fully understand QS mechanism.

Keywords: Quorum sensing, CA UTI, Proteomics

**G-4**

*Enterococcus faecium* Isolated from Chicken Cecum Stimulates Immunomodulating Activity and Promotes Longevity in *Caenorhabditis elegans*

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The aim of this study was to investigate the effect of a lactic acid bacteria (LAB) strain isolated from healthy chicken cecum on immunomodulatory activity and lifespan extension. The isolate was identified as *Enterococcus faecium* by 16S rDNA gene sequence analysis and was designated as *E. faecium* L11. The effect of artificial digestive fluid on the viability of *E. faecium* L11 was investigated. *E. faecium* L11 showed >66% and >62% of survival in artificial gastric juices (pH 2.5 and 1% peptic) and 0.5% bile salt, respectively. The heat-killed *E. faecium* L11 cells significantly (p <0.05) increased immune cell proliferation compared with the control, and stimulated the production of IL-6 and TNF-α in mouse macrophages. In addition, *E. faecium* L11 showed the preventive or protective effect against *Salmonella typhimurium* in *Caenorhabditis elegans*. To evaluate the effect of *E. faecium* L11 on lifespan extension in *C. elegans*, the longevity assay was performed by feeding worms on a lawn of *E. faecium* L11 or *E. coli* O50. *E. faecium* L11 significantly (p <0.05) extended the lifespan of *C. elegans* compared with *Ecoli* OP50 used as a control. These results suggest that *E. faecium* L11 can be considered as a potent probiotic supplement for livestock.

Keywords: *Enterococcus faecium*, Immunomodulatory activity, Longevity, Probiotic supplement


**G-5**

Peptide H Suppresses TNFα Expression in Human Breast Cancer MDA-MB-231 Cells

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Chungkookjang, fermented soybeans contain diverse peptides produced during fermentation. Human breast cancer MDA-MB-231 cells were treated with peptide H derived from Chungkookjang, and TNFα expression was conspicuously reduced, explaining IL6 reduction since IL6 is induced by TNFα. The structure of peptide H was different from those of glucocorticoid and dexamethasone, suggesting different mechanism of TNFα expression suppression. Lipoxigenase also reduces TNFα and IL6 expressions. Interaction of peptide H and lipoxigenase was predicted using PepSite. The model was obtained. However, p value was 0.26 suggesting it is unlikely that they interact. Peptide H having the reduction effect of TNFα expression is expected to be developed as drugs for rheumatoid arthritis and Crohn’s disease.

Keywords: MDA-MB-231, TNF, Chungkookjang, IL-6

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**G-6**

Inhibitory Effect of Sargassum micracanthum on Collagenase Activity

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This study was conducted to investigate the collagenase inhibitory activity of Sargassum micracanthum ethanol extract (SMEE) as the ingredients of cosmetics. The SMEE was fractionated with n-hexane, chloroform, ethyl acetate, n-butanol, and water. The n-hexane fraction of SMEE showed a greater inhibitory effect with an IC50 value of 51.08 μg/mL than other fractions. The n-hexane fraction was separated using silica gel column chromatography and the fraction eluted with chloroform/methanol (100:0) showed the highest inhibition of collagenase activity. Subsequently, the active compound was separated using Sephadex LH-20 column chromatography, and an octadecyl silica (ODS) Sepak cartridge. In conclusion, fraction 2-1 and 2-2 was separated from SMEE. Particularly, the fraction 2-1 showed a strong collagenase inhibitory activity with an IC50 value of 38.67 μg/mL. These results suggest that Sargassum micracanthum and its fractions may prevent collagen breakdown by inhibiting collagenase and also might be applicable as a natural ingredient for collagenase inhibitor.

Keywords: Sargassum micracanthum, collagenase activity, hexane fraction

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**G-7**

The Inactive *Vibrio vulnificus* Cysteine Protease Domain as Strong Immunogen

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Infection with *Vibrio vulnificus*, an important opportunistic human pathogen, frequently progress to severe skin lesions and septicaemia with a mortality rate exceeding 50%, despite aggressive antibiotic therapy and supportive care. Of the several virulent factors, *V. vulnificus* RtxA1/MARTXv contains putative domains that may be related to its specific toxin activity. Although recent studies have been well characterized several domains, the functions of some domains still remain to be more investigated. In this study, we expressed and purified the putative full-length cysteine protease domain (CPD) of *V. vulnificus* and its catalytically inactive version, CPD(C3725S), of the CPD lacking cysteine protease activity by mutating the conserved Cys residue at position 3725 to a Ser residue. As predicted, the C3725S mutation in CPD completely abolished the cysteine protease activity. Furthermore, when added exogenously, the purified CPD(C3725S) did not induce potential cytotoxicity through *in vitro* and *in vivo* assays and stimulated strong immune responses. These results suggest that the conserved Cys is functionally important residue for the cysteine protease activity of *V. vulnificus* CPD and the mutant CPD(C3725S) may be more suitable immunogen for vaccination against *V. vulnificus* infection.

Keywords: *V. vulnificus*, MDA-MB-231, TNF, Chungkookjang, IL-6

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**G-8**

Sea Lettuce (*Ulva fasciata*) Extract Inhibits the CpG-induced Inflammatory Response by Attenuating the Mitogen-activated Protein Kinase and NF-κB Pathways

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This research was conducted to study the inhibitory effect of sea lettuce (*Ulva fasciata*) extract (UFE) on pro-inflammatory cytokine production in immune cells. UFE (0-50 μg/mL) pre-treatment resulted in a dose dependent inhibitory effect against interleukin (IL)-12 p40, IL-6, and tumor necrosis factor (TNF)-α productions in CpG-stimulated bone marrow-derived macrophages (BMDM) and dendritic cells (BMDC). UFE pre-treatment showed strong inhibitory effect on the phosphorylation of p38 mitogen-activated protein kinase (MAPK) while it inhibited moderate inhibition on nuclear factor (NF)-κB activation as evaluated using degradation of IκBα. In activator protein (AP)-1 and NF-κB reporter gene assay, UFE pre-treatment displayed moderate inhibitory effect on both AP-1- and NF-κB dependent reporter gene activities. These results suggest that UFE has significant anti-inflammatory properties and warrants further study regarding potential use as a medicinal food.

Keywords: *U. fasciata*, Pro-inflammatory cytokines, Inflammation, NF-κB
of apoptosis-specific anti-tumor agent. Taken together, BS can be considered as a promising specific tumor targeting anti-tumor therapeutic effects, giving a positive evaluation that the bacterium might have optimistic effects on the lung cancer incidence rate.

Findings in this study direct us that BS has lung cancer specific targeting effects. Further studies are needed to confirm the precise effects of BS on lung cancer cells.

**Keywords:** Lung cancer, anti-inflammatory, MTT assay, Cytotoxicity

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**Anti-inflammatory Activity of Grifola frondosa Ethanol Extract in LPS-induced RAW 264.7 Cells and Mouse Models**

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Nowadays, many studies are conducted to use various active compounds extracted from herbal medicines and plants as natural sources in clinical application. In this study, the anti-inflammatory activity of *Grifola frondosa* ethanol extract (GFEE) was examined using LPS-induced RAW 264.7 cells and mouse models. As a result, NO and pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) were inhibited up to over 50% with increasing concentration of GFEE without any cytotoxicity. In addition, GFEE suppressed the expression of inducible nitric oxide, cyclooxygenase, and nuclear factor kappa B in a dose-dependent manner. Moreover, GFEE inhibited mitogen-activated protein kinases, including extracellular signal-regulated kinase 1/2, p38, and c-Jun N-terminal kinase signaling. In mice ear edema test, the formation of ear edema was reduced at the highest dose compared to the control and reduction of ear thickness and the number of mast cells were observed in histological analysis. In acute toxicity test, no mortalities occurred in mice administered 5,000 mg/kg body weight of GFEE over 2-week observation period. These results indicate that GFEE exhibits anti-inflammatory effects and can be used as a material to treat inflammatory diseases.

**Keywords:** *Grifola frondosa*, MAPKs, nuclear factor kappa B, ear edema

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**Host Immune Responses to Mycobacterium tuberculosis Antigen Rv34xx**

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Macrophages are involved both in initiation of immune responses as APCs and the effector stages of immunity as inflammatory. During the early stages of infection with *Mtb*, macrophages have several antimicrobial mechanisms that control the intracellular bacilli. Identification of the mycobacterial proteins modulating macrophage function are essential for understanding TB pathogenesis and for developing new TB eradication strategies. We searched for novel protein Rv34xx that induce macrophage activation from *Mtb* CFPs. Rv34xx protein was identified by this process, expressed in E. coli, and evaluated for its immunostimulatory potential on mouse BMDMs. We found that Rv34xx more activates MAPKs and PI(3)Ks in infected macrophages than without antigens. Also, we compared the *Mtb* grown within non-activated BMDM and activated BMDM by Rv34xx. Interestingly, Rv34xx induced phagosome-lysosome fusion cause that *Mtb* growth inhibition in infected macrophages. Consequently, activated BMDMs by Rv34xx were more efficient than non-activated BMDMs at eliminating the infection. We also showed that immunizing Rv34xx significantly increased the secretion of proinflammatory cytokines and the expression of surface molecules in BMDMs. We found that Rv34xx more activates MAPKs and PI(3)Ks in infected macrophages than without antigens. Also, we compared the *Mtb* grown within non-activated BMDM and activated BMDM by Rv34xx. Interestingly, Rv34xx induced phagosome-lysosome fusion cause that *Mtb* growth inhibition in infected macrophages. Consequently, activated BMDMs by Rv34xx were more efficient than non-activated BMDMs at eliminating the infection. We also showed that immunizing Rv34xx significantly increased the secretion of IL-2, IL-17A and IFN-γ in murine model using avirulent *Mtb* H77Rv. These results suggest that Rv34xx induces protective immune response against *Mtb* infection superior than other mycobacterial antigens.

**Keywords:** Mycobacterium tuberculosis, Culture filtrate protein, host immune response

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**Crystal Structure of NleB2, an Enteropathogenic Escherichia coli Type III Effector Protein**

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Type III secretion systems (T3SSs) in gram-negative pathogens are multi-protein complexes that allow the injection of bacterial effector proteins into the host cell cytoplasm without being affected by the extracellular matrix. For example, enteropathogenic *Escherichia coli* (EPEC) expresses NleB protein as a T3SS effector and disrupts the host immune system. Recently it is discovered that NleB, as a glycosyltransferase, does unique and unappreciated protein posttranslational modification, arginine glycosylation. Here we describe crystal structure of NleB2, one of the NleB homologs at 2.6Å resolution. The structure shows that NleB2 belongs to the GT-B class glycosyltransferase and the active site is well conserved. NleB2 has an characterized extended helix-loop-helix arm to the outward and the active site is located at lower position from the center while the active sites of other GT-B family enzymes positions at the center of the whole structures. Canonical D-X-D motif is well conserved and the first Asp residue carries a magnesium metal ion and the latter Asp residue plays a role as a nucleophile. Even though complex structure with UDP-GlcNAc was not defined yet but NleB2 would take a UDP-GlcNAc as a substrate like other glycosyltransferase because key residues for binding with UDP-GlcNAc is well conserved.

**Keywords:** NleB2, Effector protein, Immune evasion
G-13  Lactic Acid Bacteria Polarize Mouse Splenocytes and Bone Marrow Derived Macrophage (BMDM) to a M-2 Macrophages

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Macrophages play a central role in both innate and adaptive immune responses and are known to differentiate into pro-inflammatory M1 or anti-inflammatory M2 phenotypes following activation. The M1 killer type response and the M2 repair type response are best known, and are two extreme examples. Among other markers, inducible nitric oxide synthase and type-1 arginase, the enzymes that are involved in L-arginine/nitric oxide metabolism, are associated with the M1 and M2 phenotype, respectively, and therefore widely used as the markers for characterization of the two macrophage phenotypes. Different lactic acid bacteria strains are one of many commensal microbes and are known to play important roles in maintaining the health of their host. In this study, we are interested in knowing whether LAB can induce macrophages polarization. To generate mouse splenic mature macrophages, mouse splenocytes were cultured in M-CSF for 7 days. For activation macrophages were treated with either LPS, or LAB for an additional 24 h. To assess macrophage polarization, iNOS and arginase were analyzed by RT-PCR, and also the secreted cytokines were determined by ELISA. Treatment with LAB significantly increased the arginase expression and IL-10 production. Together, these findings indicated that LAB facilitated the polarization of M-2 macrophages. The results expanded our knowledge about functions of LAB-involved the macrophage polarization.

Keywords: Lactic acid bacteria, Macrophages polarization, Arg-1, iNOS

G-15  The Anti-Influenza Activity of Wogonin Involves Modulation of Interferon (IFN) and AMPK Signaling Pathways

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Wogonin, which is a flavonoid compound isolated from Scutellaria baicalensis, has been used as an anti-tumor agent. Despite wogonin well-described activities in diseases associated with cancer or chronic inflammation, its role during viral infection is largely unexplored. In this study, we investigated anti-viral effect of wogonin using PR8 virus (Influenza A virus). Wogonin treatment effectively suppressed influenza virus replication in Madin-Darby Canine Kidney (MDCK) cells and Human Lung Epithelial (A549) cells. In addition to reducing viral replication efficiency and viral protein expression, wogonin reduced the host inflammatory response to influenza virus infection in a dose-dependent manner. In contrast, wogonin treatment following influenza A virus infection led to up-regulation of interferon (IFN)-induced antiviral signaling. Additionally, influenza A virus infection induced 5'-adenosine monophosphate-activated protein kinase (AMPK) phosphorylation and activation in a time-dependent manner. These data suggest that wogonin possesses a potent anti-influenza activity mediated by regulation of AMPK activation, suggesting that wogonin has the potential to be developed as an anti-influenza drug.

Keywords: Wogonin, Influenza A virus, Interferon, AMPK, Anti-viral response

G-14  Positive Role of Promyelocytic Leukemia Protein in Type I Interferon Response and its Regulation by Human Cytomegalovirus

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PML, a major component of PML nuclear bodies, is involved in diverse cellular processes such as cell proliferation, apoptosis, gene regulation, and DNA damage response. PML also acts as a restriction factor that suppresses incoming viral genomes, therefore playing an important role in intrinsic defense. Here, we show that PML positively regulates type I interferon response by promoting transcription of ISGs, and that this regulation by PML is counteracted by HCMV IE1 protein. Small hairpin RNA-mediated PML knockdown in human fibroblasts reduced ISG induction by treatment of interferon-β or infection with UV-inactivated HCMV, PML was required for accumulation of activated STAT1 and STAT2, interacted with them and HDAC1 and HDAC2, and was associated with ISG promoters after HCMV infection. During HCMV infection, viral IE1 protein interacted with PML, STAT1, STAT2, and HDACs. Analysis of IE1 mutant viruses revealed that, in addition to the STAT2-binding domain, the PML-binding domain of IE1 is necessary for suppression of interferon-β-mediated ISG transcription, and that IE1 inhibited ISG transcription by ISGF3 in a manner requiring its binding of PML and STAT2, but not of HDACs. In conclusion, our results demonstrate that PML participates in type 1 interferon-induced ISG expression by regulating ISGF3, and that this regulation by PML is counteracted by HCMV IE1, highlighting a widely shared viral strategy targeting PML to evade intrinsic and innate defense mechanisms.

Keywords: PML, IFNbeta, IE1

G-16  Improvement of Tight Junction Integrity in Caco2 Cell Monolayers by Oxyresveratrol

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Oxyresveratrol (OXY) is a hydroxyl-substituted stilbene found in the roots, leaves, stem, and fruit of many plants. In this study, we purified OXY from the ethanol extract of Ramulus mori and investigated its effect on tight junctions (TJs) in the human intestinal Caco2-2 cell. Improvement of TJ integrity is important to recover inflammatory bowel disease. Cell viability was measured by the MTT assay. To investigate the effect of OXY on improvement of TJ, transepithelial electric resistance (TEER) was measured using ohm/volt meter after treatment of OXY. Monolayer permeability was evaluated by the paracellular transport of FITC-dextran 4-kDa (FD4). The expression levels of TJ genes and proteins (Claudin-1, Occludin, and ZO-1) were assessed by quantitative real-time PCR and western-blot analysis, respectively. In addition, the formation of TJ by OXY was visualized by confocal microscopy. The results showed that OXY increased TJ integrity and reduced permeability compared with the control. OXY significantly (p < 0.05) increased the mRNA and protein expression levels of Claudin-1, Occludin, and ZO-1 in a dose-dependent manner, which enhanced barrier function. Therefore, OXY might be used as a therapeutic agent to restore barrier function in inflammatory bowel disease.

Keywords: Caco2, Tight junction, Oxyresveratrol
Crystal Structure of *Toxascaris leonina* Galectin with Two Carbohydrate Recognition Domains

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The full-length crystal structure of *Toxascaris leonina* (Tl)-galectin, a galectin-9 homologue protein, was determined at a resolution of 2.0 Å. Galectin-9 exhibits a variety of biological functions, including cell aggregation, eosinophil chemotraction, activation, and apoptosis of mouse thymocytes, T cells and human melanoma cells. Similar to the galectin, Tl-galectin may function as a regulatory molecule in the host immune system. There are two molecules of Tl-galectin in *P2_2_2_1* per asymmetric unit, and each N-terminal and C-terminal carbohydrate recognition domain (CRD) of Tl-galectin is composed of six-stranded β-sheets and five-stranded β-sheets with a short α-helix. The NCRD and CCRD of Tl-galectin resembles human galectin-7 NCRD and its CCRD to human galectin-9, but the residues on the interface and loop regions of the NCRD and CCRD are flexible and related to interaction. Engagement of the T cell immunoglobulin mucin (Tim)-3 CRD of IgV domain by galectin-9 ligand is known to be important for appropriate termination of T helper 1-immune responses. To investigate the binding site of Tl-galectin protein, we modeled the interaction between Tl-galectin and Tim-3 proteins. Tim-3 is docked to a major groove of the Tl-galectin structure, which is larger and deeper than the minor groove. The structural information presented herein will provide insights into the development of novel anti-inflammatory agents or selective modulators of immune response.

Keywords: Crystal structure, Carbohydrate recognition, *Toxascaris leonina*, Galectin

Oral Mucosal Vaccine Against HIV-1 Using Poliovirus-derived CTL Vaccine Vector

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We developed a CTL vaccine vector by modification of the RPS-Vax system, a mucosal vaccine vector derived from a poliovirus Sabin 1 strain, and generated an oral CTL vaccine against HIV-1. A DNA fragment encoding a cytoplasmic transduction peptide (CTP) was integrated into the RPS-Vax system to generate RPS-CTP, a CTL vaccine vector. An HIV-1 p24 cDNA fragment was introduced into the RPS-CTP vector system and a recombinant poliovirus (rec-PV) named vRPS-CTP/p24 was produced. vRPS-CTP/p24 was genetically stable and efficiently induced Th1 immunity and p24-specific CTLs in immunized poliovirus receptor-transgenic (PVR-Tg) mice. In challenge experiments, PVR-Tg mice that were pre-immunized orally with vRPS-CTP/p24 were resistant to challenge with a lethal dose of p24-expressing recombinant vaccinia virus (rMVA-p24). These results suggested that the RPS-CTP vector system had potential for developing oral CTL vaccines against infectious diseases.

Keywords: Poliovirus Sabin 1, mucosal vaccine, viral vector, HIV-1 p24, CTL

Diverse Innate Signaling is Required for the Activation of *Mycobacterium chelonae*-mediated CCL2 and CCL5 Expression in Macrophages

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*Mycobacterium chelonae* (Mch) is an atypical rapidly growing mycobacterium that belongs to the *M. chelonae* complex, which can cause a variety of human infections. During this type of mycobacterial infection, chemokines play an important role in the mediation of intracellular communication and immune surveillance by which they orchestrate cellular immunity. However, the intracellular signaling pathways involved in the macrophage-induced chemokine production during Mch infections remain unknown. Thus, the present study aimed to determine the molecular mechanisms by which Mch activates the genes expressions of chemokine (C-C motif) ligand 2 (CCL2) and CCL5 in bone marrow-derived macrophages (BMDMs). The results showed that Mch triggered the expression of CCL2 and CCL5 in BMDMs via toll-like receptor 2 (TLR2) and myeloid differentiation primary response gene 88 (MyD88) signaling and that it rapidly activated nuclear factor (NF)-κB signaling, which is required for the Mch-induced gene expressions of CCL2 and CCL5 in BMDMs. Moreover, while the innate receptor Dectin-1 was partly involved in the Mch-induced expression of the CCL2 and CCL5 in BMDMs, the generation of intracellular reactive oxygen species (ROS) was an important contributor to these processes. Taken together, the present data indicate that the TLR2, MyD88, and NF-κB pathways, Dectin-1 signaling, and intracellular ROS generation contribute to the Mch-mediated expression of chemokine genes in BMDMs.

Keywords: Mycobacterium chelonae, Chemokine, TLR2, Dectin-1, ROS

*Mycobacterium tuberculosis* RpfE Promotes Simultaneous Th1- and Th17-type T-Cell Immunity via TLR4-dependent Maturation of Dendritic Cells

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Reciprocal induction of the Th1 and Th17 immune responses is essential for optimal protection against *Mycobacterium tuberculosis* (MtB); however, only a few MtB antigens are known to fulfill this task. A functional role for resuscitation-promoting factor (Rpf) E, a latency-associated member of the Rpf family, in promoting naive CD4+ T-cell differentiation toward both Th1- and Th17-cell fates through interaction with dendritic cells (DCs) was identified in this study. RpfE induces DC maturation by increasing expression of surface molecules and the production of IL-6, IL-1β, IL-23p19, IL-12p70 and TNF-α but not IL-10. This induction is mediated through TLR4 binding and subsequent activation of ERK, p38 MAPKs and NF-κB signaling. RpfE-treated DCs effectively caused naive CD4+ T cells to secrete IFN-γ, IL-2 and IL-17A, which resulted in reciprocal expansions of the Th1- and Th17-cell response along with activation of T-bet and RORγt but not GATA-3. Furthermore, lung and spleen cells from MtB-infected WT mice but not from TLR4−/− mice exhibited Th1 and Th17 polarization upon RpfE-stimulation. Taken together, our data suggest that RpfE has the potential to be an effective MtB vaccine because of its ability to activate DCs that simultaneously induce both Th1- and Th17-polarized T-cell expansion.

Keywords: Mycobacterium tuberculosis, Dendritic cell, RpfE
**G-21**

**Mycobacterium avium Complex Protein MAV20XX Induces Macrophage Apoptosis through Toll-Like Receptor 4**

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Apoptosis is an important process in macrophage innate immune defence mechanism for limiting bacterial viability. Recent studies have shown that *Mycobacterium avium* complex (MAC) infection of macrophages in vitro leads to apoptosis of significant numbers of infected cells and their sonic extracts induce a macrophage apoptosis. However, any components of MAC that are involved in inhibiting or triggering apoptosis are not identified. Recently we identified the MAV20XX protein fractionation of *M. avium* culture filtrate protein using multistep chromatography. In this study, we investigated the biological effects of MAV20XX on murine macrophages. MAV20XX protein-induced apoptosis is associated with mitochondrial pathway, down regulation of Bcl-2, dissipation mitochondrial transmembrane potential (ΔΨm) and release of cytochrome c from mitochondria as well as increase in caspase 3, caspase 9 and poly (ADP-ribose) polymerase (PARP) cleavage. However, MAV20XX protein-induced apoptosis was significantly reduced in TLR4-deficient macrophages but not TLR2. Enhanced ROS production and Apoptosis signal-regulating kinase 1 (ASK1), JNK activation were essential of MAV20XX-mediated apoptosis and induced inflammatory cytokines production. Taken together, our data suggest that MAV20XX cause apoptosis of murine macrophages.

Keywords: apoptosis, *Mycobacterium avium*, Macrophage

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**G-22**

**Vaccine Development by Using Outer Membrane Vesicles Released from a Detoxified Mutant of Enterotoxigenic Escherichia coli**

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ETEC strains can produce heat-labile (LT) toxin, the major virulence factor leading to diarrhea, which is secreted via association with outer membrane vesicles (OMVs) that consists of the ETEC cell-surface antigens, including lipopolysaccharide (LPS) that are also referred as endotoxin. In order to achieve a detoxified (LT-deficient and a less-endotoxic) ETEC-OMV as a vaccine, genes of *lt-A* and *msbB* were inactivated in ETEC H10407 strain, and the resulting Δlt-A/ΔmsbB mutant was used as the detoxified ETEC-OMV (vaccine) producer. After characterization of the OMVs, they were utilized in the prime-booster vaccine experiments to evaluate the efficacy of the OMV vaccine against ETEC infections in mice with the H10407 strain and a clinical isolate in Korea. The mice that received OMV by intramuscular injections at two-week interval showed a high IgG antibody titer towards the outer membrane proteins (OMPs) of ETEC, which confers the vaccinated mice full protections against the challenges with the H10407 and clinical ETEC isolate, respectively, compared to the (non-vaccinated) control mice.

Keywords: ETEC, outer membrane vesicles

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**G-23**

**The Production of Thymic Stromal Lymphopoietin (TSLP) is Regulated by Synergistic Effects of Toll-like Receptor Agonists in Bone Marrow-derived Dendritic Cells**

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Thymic stromal lymphopoietin (TSLP) is primarily produced by a various cells such as epithelial cells, keratinocyte, and stromal cells. It is one of the crucial pathogenic factors in allergic inflammation. TSLP expression is responsible for the development of inflammatory Th2 cell responses. However, it is not well known how TSLP play roles in Th2 polarization in allergic inflammation. Since dendritic cells (DCs) are important in initiation of inflammatory immune responses, we explored the production of TSLP by DCs after treatment with various ligands for toll-like receptors (TLRs). The TLR2 ligand or TLR4 ligand significantly induced TSLP expression by bone marrow derived-DCs. We also found that TLR9 ligand + TLR2 ligand or TLR9 ligand + TLR4 ligand induced TSLP expression by DCs synergistically. Synergistic effects of TLR ligands on TSLP expression was dependent on the MyD88. Interestingly, stimulation of DCs with the combination of TLR ligands enhanced Th2-polarizing capacity of DCs and sustained it longer. Taken together, our data suggest that combination of TLR ligands enhanced TSLP production by BM-DCs synergistically and also potentiated Th2 polarization of T cells by DCs. Considering that Th2 polarization in allergic inflammation is partly caused by TSLP-secreting DCs, the signal transducing molecules bridging between TLRs and TSLP expression in DCs might be good target molecules for the control of allergic inflammation.

Keywords: allergy, TLR, Inflammation, Dendritic cells, TH2 cells
Establishment of Escherichia coli Strain for Production of 5-Aminolevulinic Acid through Metabolic Engineering
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Escherichia coli JW01 strain was metabolically engineered to produce 5-aminolevulinic acid (ALA). C5 non-standard amino acid is widely used in agricultural and medical industries. First, Rhodobacter sphaeroides hemH gene catalyzing the condensation of succinyl-CoA and glycine to make ALA was codon-optimized and cloned into high copy number plasmid pKE112. Second, plasmid pKE112hemA was introduced into the in silico deleted WL3110 strain; the WL3110 (pKE112hemA) strain produced 0.249 ± 0.027 g/L of ALA. Third, in silico knockout simulation was carried out to identify additional gene knock-out targets to further improve ALA production. The gsvTHP genes (glycine cleavage system) were predicted as knockout targets. The JW01 strain (WL3110 gsvTHP harboring pKE112hemA) produced 1.17 ± 0.035 g/L of ALA, which was 4.7 times higher than that obtained with the base strain. Finally, in order to increase the succinyl-CoA pool, the glyoxylate shunt flux was enhanced by the deletion of the icd and sldAB genes, while the TCA cycle flux was reinforced by the deletion of pdcA gene. The JW03 strain (JW01 icdΔ sldAB pdcAΔ) harboring pKE112hemA was able to produce 1.72 ± 0.001 g/L of ALA. Fed-batch culture of the JW03 strain resulted in the production of 5.77 g/L of ALA in 41 h. (Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A202656 (funded by the Ministry of Education, Science and Technology))

Keywords: 5-Aminolevulinic acid, Systems metabolic engineering, Escherichia coli, Succinyl-CoA, Glycine

Establishing a Synthetic Pathway for Production of Gamma-butyrolactone in Mannheimia succiniciproducens
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γ-butyrolactone (GBL) is an important four carbon (C4) chemical, which has a wide range of industrial applications. GBL can be produced by acid treatment of 4-hydroxybutyric acid (4-HB), which is a derivative of succinic acid. Heterologous metabolic pathways were designed and established in succinic acid overproducing M. succiniciproducens LPK7 (p3S4CD) or succinate semialdehyde reductase in LPK7 (p3SYCD). Fed-batch cultures of LPK7 (p3SYCD) and LPK7 (p34CD) resulted in the production of 6.37 and 6.34 g/L of 4-HB, respectively. Finally, GBL was produced by acid treatment of the 4-HB obtained from the fermentation broth. This study demonstrates that 4-HB, and potentially other four carbon platform chemicals, can be produced by the engineered rumen bacterium M. succiniciproducens. (Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A202656 (funded by the Ministry of Education, Science and Technology))

Keywords: Mannheimia succiniciproducens, Metabolic engineering, 4-Hydroxybutyric acid, γ-Butyrolactone, Succinic acid

Establishing a Synthetic Pathway for Production of Putrescine from Escherichia coli
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Putrescine, 1,4-diaminobutane, is an important chemical which is used as a monomer of nylon in chemical industry. Biosynthesis of putrescine from renewable biomass is able to be a good substitute of petroleum based putrescine production from an environmental point of view. There was a trial of development of putrescine producing Escherichia coli strain through construction of biosynthesis pathway using gene knockout and overexpression. We expected that productivity of this strain is able to be increased by use of recently developed knockdown method, small regulatory RNA system. Using this system we repressed competitive branch pathways and increased flux to putrescine. Additionally, culture condition was modified to support the increased putrescine productivity by adding higher amount of nitrogen source and dissolved oxygen. The fed-batch cultivation of finally engineered strain and modified culture condition resulted in dramatically increase of productivity and yield. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012-2C1AAA001-2012M1A2A202656); the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea)]

Keywords: metabolic engineering, sRNA, small RNA, putrescine
H-5 Recombinant Immunogenic Influenza Hemagglutinin Glycoproteins
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Influenza virus hemagglutinin (HA) is expressed as a precursor protein, HA0, which is then proteolytically cleaved to create two disulfide-linked polypeptide chains: HA1 after the removal of the signal peptide and HA2. HA2 contains two membrane-interacting hydrophobic sequences, the N-terminal fusion peptide and the C-terminal transmembrane domain (TMD). Primarily, rHA proteins for use as vaccine antigens have been expressed as HA0 or HA1 derivatives using the baculovirus/insect cell expression system. Yet only a few studies on the expression of rHA in E. coli have used HA0, HA1 derivatives, or HA2 derivatives. A novel rHA subunit protein-based influenza fluid vaccine contains two soluble rHA proteins and is a nonfusogenic antigen mimic of the naturally processed and disulfide-linked HA1/HA2, not as a HA0 precursor form. We also report here the immunogenicity of the recombinant influenza vaccine in mice in order to briefly evaluate rHA specific antibody responses after single or booster subcutaneous injections, dually administrated with two soluble rHA proteins of influenza virus A strain X31 (H1N2) HA. Antibody responses induced by two soluble rHA antigens were evaluated by ELISA assays to detect rHA antigens injected and to validate both anti-HA1 and anti-HA2 antibodies produced in the mice sera. Antigenic rHA proteins also elicited neutralizing antibodies against homologous H1N2 influenza virus in the immunized mice.

Keywords: Antigen mimic, Fusion peptide, Influenza hemagglutinin, Nonfusogenic, Recombinant vaccine

H-6 Expression and Characterization of Dimeric Single Chain Variable Domain Fragment(ScFv) Fused to E. coli Alkaline Phosphatase and Its Application for a Sensitive Direct Immunoassay
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In immunoassay, newly developed primary antibody is added and followed by addition of a secondary antibody coupled to enzyme to monitor antigen binding activity of antibody. During this process, non-specific binding activity caused by secondary antibody should be considered and eliminated. In addition, additional incubation time for binding of secondary antibody could be time-consuming and tedious step. We cloned alkaline phosphatase (AP) isolated from E. coli chromosome and linked to a target-specific ScFv to bypass problems mentioned above. Single-chain variable domain fragment(ScFv) fused to E. coli alkaline phosphatase(AP) was expressed and purified by using Ni2+-NTA-agarose. As expected, ScFv-AP successfully bound to specific antigen in ELISA, western and immunocytochemistry in which its binding activity was measured by color development of substrate catalyzed by AP directly coupled to ScFv without using secondary antibody. In addition, natural dimerization of AP by non-covalent association induced bivalent ScFv production which was confirmed by size exclusion chromatography (SEC). Taken these data together, it was concluded that ScFv-AP fusion protein was successfully produced as a soluble form in E. coli and showed clear antigen binding activity in several immunoassays without addition of secondary antibody which sometimes causes time-consuming, expensive and non-specific false binding.

Keywords: alkaline phosphatase, singlechain variable fragment, Nif2+-NTA-agarose, Dimerization, ScFv-AP fusion protein

H-7 Development of High Copy Plasmid for the Enhanced Production of Recombinant Proteins in Leuconostoc citreum
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Leuconostoc is a group of hetero-fermentative lactic acid bacteria that plays important roles in fermentation of kimchi, cheese and sauerkraut. It is also regarded as a promising host strain for the production of recombinant proteins (particularly protein therapeutics such as vaccine, antibody, etc) due to their GRAS (Generally Recognized as Safe) status. For the production of recombinant proteins, it is generally required to use the efficient gene expression system which can be stably replicated in the host with high copy number. In Leuconostoc sp., a few expression vectors have been developed but, they have relatively low copy number and are not suitable for high-level protein production. Here, we sought to develop a high-copy-number plasmid suitable for high-level gene expression in Leuconostoc citreum. By using the FACS-based high-throughput screening of plasmid random library, the high-fluorescent cells were selectively sorted. With the isolated clones, the plasmid copy numbers were determined by quantitative PCR (qPCR), and we successfully isolated one plasmid which exhibited an incredible increase in the copy number. Also, with the engineered plasmid, the production of recombinant proteins in Leuconostoc citreum was examined and, we could achieve the significant increase in production yield.

Keywords: Lactic Acid Bacteria, Leuconostoc, High copy plasmid, Plasmid engineering, FACS

H-8 Fruit and Citrus Peel Waste as a Valorization Biomass for the Bioethanol Production
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Large quantities of fruit waste are generated from agricultural processes worldwide. This waste is often simply dumped into landfills or the ocean. Fruit waste has high levels of sugars, including sucrose, glucose, and fructose, that can be fermented for bioethanol production. However, some fruit wastes, such as citrus peel waste (CPW), contain compounds which can act as an inhibitor to the fermentation process, and must be removed for efficient bioethanol production. We developed a novel approach for converting single-source CPW (i.e., orange, mandarin, grapefruit, lemon, or lime) or CPW in combination with other fruit waste (i.e., banana peel, apple pomace, and pear waste) to produce bioethanol. In addition, two in-house enzymes were produced from Avicel and CPW as carbon source, and were tested with fruit waste at 12-15% (w/v) solid loading. The enzymatic conversion rates of fruit waste to fermentable sugars were approximately 90% for all feedstocks after 48 h. We also designed a d-limonene removal column (LRC) that successfully removed this inhibitor from the fruit waste. When the LRC was coupled with an immobilized cell reactor (ICR), yeast fermentation resulted in ethanol concentrations and yields that were 12-fold greater than products from ICR fermentation alone.

Keywords: Bioethanol, Biomass, Fruit waste, Fermentable sugars, Limonene
Developing Production System of Cellulase and Laccase Derived from Brown-Rot Fungus Tyromyces Palustris in Pichia pastoris X-33

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Brown-rot fungi contribute to biomass recycling by decomposition of wood and generation of wood decay. It is known that brown-rot fungi have cellulytic enzyme; endoglucanase, exoglucanase and beta-glucosidase. Ligninolytic capacity is also observed. Among lignocellulolytic enzymes, cellulase and lignase have a huge biotechnological application including biomass production, pulp and paper industry, soil bioremediation, starch processing, and grain alcohol fermentation. In the bioconversion of lignocellulose, an efficient lignocellulose hydrolysis requires high amount of enzyme. So, we tried to develop the production of cellulase and laccase in Pichia pastoris. To obtain ligninolytic enzymes, cellulase and lignase, Tyromyces palustris was selected. The cDNA of TpCell2 and TpLac were cloned into pGEM-T easy vector. Then, these genes were again cloned into Pichia expression vector, pPICZα-C. TpCell2 integration into the Pichia genome typically occurs. In the case of TpLac, the integration into Pichia genome is being tried. In further study, the expression and activity of TpCell2 and TpLac in P. pastoris is investigated.

Keywords: Brown-rot fungi, Cellulase, Laccase, Lignocellulolytic Enzyme, Pichia pastoris

Antimicrobial Effect of Plant Endophytic Fungi Isolated from Morus alba L.

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Endophytic fungi are microorganisms inhabiting living plant tissues without causing apparent symptoms on the host. They are drawing increasing attention due to their ability to produce various bioactive compounds as well as their effects on host growth and resistance to biotic and abiotic stresses. In this study, antimicrobial effects of endophytic fungi isolated from Morus alba L. were evaluated. Since M. alba is well-known for high antibacterial effect by itself, antimicrobial effect of the endophytic fungi from this tree was highly expected. The crude ethyl acetate extract of five strains showed antimicrobial activity in a dose dependent manner against human pathogenic microorganisms including Candida albicans, Candida glabrata, Cryptococcus neoformans, Staphylococcus aureus, and Streptococcus mutans. The effects were dose dependent and each fungal strain showed specific activity against different pathogens. These strains are identified as two Phomopsis sp. (JS169, JS171), one Fusarium sp. (JS170), and two Colletotrichum sp. (JS361, JS367) by ITS sequencing. These data will serve as a valuable resources to develop novel antifungal or antibiotic materials.

Keywords: endophyte, Phomopsis sp., Fusarium sp., Colletotrichum sp., antimicrobial effect

Development of Actinobacillus pleuropneumoniae ApxI, II, III, and IV-specific Diagnostic ELISA Methods Using Recombinant Apx Antigens

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Actinobacillus pleuropneumoniae (A. pleuropneumoniae) is the etiologic agent of porcine pleuropneumoniae. As Apx toxins of this pathogen have been considered as major virulence and immunogenic factors, ELISA tests based on the Apx toxin antigens could be diagnostic methods for A. pleuropneumoniae infection. In this study, full regions of Apx toxins were divided by N-terminal, middle part, and C-terminal and then analysis of sequence alignment and similarity was carried out to find more specific regions in genes of ApxI, ApxIIA, and ApxIIIA. The identified specific gene regions were amplified and their recombinant proteins expressed in E. coli M15 cells. Antigenicity of purified proteins were carried out by SDS-PAGE and Western blot using sera of guinea pigs primed by each of Apx vaccines. Genes of ApxI, ApxIIA, and ApxIIIA showed percent identity of 40-50% each other. Western blot analysis showed the antigenicities rather than specific reactions in the purified proteins from parted regions of each Apx toxin. The diagnostic ELISA methods are being optimized using sera of vaccinated guinea pigs and rabbit polyclonal antibodies against each recombinant protein. Taken together, these ELISA methods may be useful for diagnosis of A. pleuropneumoniae: ApxI, II, III, and IV-specific antibodies.

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Keywords: A. pleuropneumoniae, Apx toxins, ELISA, Diagnosis, Recombinant proteins

Functional Analysis of Recombinant Human and Putative Fungal O-GlcNAc Transferases Expressed in Saccharomyces cerevisiae

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O-GlcNAcylation is an important post-translational modification for many cellular processes, which is mediated by O-GlcNAc transferases (OGTs) catalyzing the addition of O-D-N-acetylglucosamine (GlcNAc) to the Ser/Thr residues of target proteins. Bioinformatics analysis indicated the presence of putative OGTs in fungal species including Magnaporthe grisea and Yarrowia lipolytica. Sequence analysis showed that M. grisea OGT (MgOGT) and Y. lipolytica OGT (YOGT) have the well-conserved catalytic domain, suggesting they may have the catalytic activity. These fungal OGTs, along with a human OGT, were expressed as recombinant proteins in Saccharomyces cerevisiae for functional analysis. Immunoblotting with an antibody against O-GlcNAc showed that the recombinant MgOGT and YOGT were not subjected to auto-O-GlcNAc modification. Moreover, the recombinant OGTs did not show activity in the in vitro assay using casein kinase II as a substrate. On the other hand, the recombinant human OGT showed auto-O-GlcNAcylation and the in vitro catalytic activity. Interestingly, overexpression of human OGT affected negatively the growth of S. cerevisiae. However, the chimeric human-fungal OGTs, in which the catalytic domains of fungal OGTs were fused with the TPR domain of human OGT, did not show enzyme activity. The information and resources of recombinant OGTs obtained in this study would facilitate further functional analysis of various fungal OGTs to elucidate their physiological roles.

Keywords: O-GlcNAc transferase (OGT), Magnaporthe grisea, Yarrowia lipolytica
**H-13**

**Development of 9E10 Single-Domain Nanobody (9E10 VHH) Against c-myc Peptide through Camelization of Murine Heavy Chain Variable Domain to Increase Solubility**

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These studies are classified into two parts. The first part was to make 9E10 VHH nanobody by grafting the CDR region of the variable heavy chain (VH) in anti-c-myc 9E10 antibody onto FR backbone in a camel’s VHH nanobody. The second part was to develop dimeric and trimeric 9E10 VHHs by using the leucine and isoleucine zippers to increase antigen binding activity. Dimeric and trimeric 9E10 VH were expressed in E. coli and purified with Ni²⁺-NTA-agarose. Purified dimeric and trimeric 9E10 VHH were migrated as 35 kDa in SDS-PAGE and the yield of expressed 9E10 VHHs were higher than that of a murine 9E10 ScFv. In addition, due to avidity effect based on d/trimerization, the binding affinity between trimers and antigens was higher than affinity between dimers and antigens in ELISA. In addition, avidity effect of trimeric 9E10 VHH to the c-myc peptide was also confirmed by western blot.

Keywords: VHH nanobody, camel, dimeric, trimeric, c-myc peptide

**H-14**

**Functional Expression and Characterization of Target-specific Single-Chain Variable Domain Fragment (ScFv) Conjugated to Streptavidin**

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Numerous recombinant antibody such as single-chain variable domain fragment (ScFv) were rapidly and efficiently isolated from either immunized or non-immunized antibody libraries by using in vivo/in vitro screening. However, selected ScFvs are usually monomeric suggesting antigen binding affinity may not be good enough for analytical, diagnostic and therapeutic purposes. In this work, we describe a simple way to tetramerize a ScFv antibody by fusion with streptavidin (STR). Firstly, streptavidin (STR) gene was assembled and cloned into pET-22b expression vector which contains T7 promoter for strong transcription and pelB leader for efficient secretion. Streptavidin (STR) was successfully expressed. Tetramerization of STR by non-covalent association was confirmed by size-exclusion chromatography. Biotin binding activity of STR was also shown in outherlony immunosassay. Secondly, anti-DRA hAY4 ScFv fused to STR (hAY4 streptabody) was constructed in pUC 119 expression vector to use lacZ promoter for transcription and pelB leader. hAY4 streptabody was successfully expressed and tetramerized as 174 kDa in size-exclusion chromatography (SEC). Due to tetramerization effect, hAY4 streptabody was migrated as 43 kDa in heated SDS-PAGE. However, in non-heated SDS-PAGE, hAY4 streptabody was shifted up to 174 kDa which corresponds to tetrameric size. As in case of STR, hAY4 streptabody could bind to biotinylated protein in outherlony immunosassay.

Keywords: ScFv, streptabody, tetramerization, outherlony, size-exclusion chromatography

**H-15**

**Enhancement of Hexane Tolerance in Pseudomonas sp. BCNU 106 by Addition of Trehalose**

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The main obstacle in the practical application of organic solvent tolerant microorganisms seems to be that they always exist in comparatively lower growth in the presence of organic solvents. Especially when using whole cells as catalysts, solving of the growth problem is a prerequisite for application. During cultivation of solvent tolerant bacterium Pseudomonas sp. BCNU 106 in LB broth both with 1% hexane and 0.05 M trehalose, the uptake of trehalose was determined together with the measurement of growth. Exogenous trehalose was transported into the cells and conferred protection against hexane stress. The strategy of this study was to enhance the cell growth of the solvent tolerant bacterium BCNU 106 to achieve higher activity. This is the first study suggesting that trehalose supplementation is the best method for a solvent tolerant strain to overcome the weak tolerance to solvents, allowing growth to high cell densities.

Keywords: Exogenous trehalose, Pseudomonas sp., Stress tolerance, hexane tolerance, Trehalose

**H-16**

**A Novel Psychrophilic Alkaline Lipase from Metagenome of Dokdo Sediment**

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Using deep-sea sediments at ‘Dokdo’ Island, we have constructed a metagenomic library that consisted of 60,672 fosmid clones. Functional screen of a “Dokdo” deep-sea sediment metagenomic library identified a gene LipES1, whose product displayed cold-active and alkaliphilic lipase. LipES1 sequence encoded a 552 amino acid protein with a predicted molecular weight of 55.6 kDa. LipES1 exhibited the highest sequence similarity (44%) to Haliangium ochraceum DSM 14365 carboxylesterase. Phylogenetic analysis indicated that LipES1 belongs to a currently uncharacterized family of lipases. Within the conserved domain, LipES1 retained the typical catalytic triad that consists of the consensus pentapeptide motif GESAG. LipES1 demonstrated a broad substrate specificity toward the long acyl group of ethyl esters (C2-C12) with maximal activity recorded toward p-nitrophenyl butyrate (C4) at pH 9.0 and 40 °C (specific activity of 255.4 U/mg). The enzyme remained stable in the range of 0–30 °C, and pH 9.0–10.5. LipES1 displayed 40% residual activity at 0 °C. LipES1 also exhibited no significant loss of activities in the presence of various organic solvents (methanol, ethanol, and isopropanol), and detergents (SDS (1%), CTAB (0.5%), and DMSO).

Therefore, LipES1 has the excellent potential for many industrial applications performed in cold temperature and alkaline conditions.

Keywords: Lipase, Cold-active, Dokdo, Metagenome
Expression Analysis of Codon-optimized NNV Capsid Protein in the Yeast Yarrowia lipolytica for Recombinant Vaccine Development
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Yarrowia lipolytica is a dimorphic non-pathogenic yeast with significant capacities for high molecular weight protein secretion and efficient degradation of hydrophobic substrates. Here, we employed Y. lipolytica as host to express the codon-optimized intact and N-terminal truncated capsid proteins of a Nervous Necrosis Virus (NNV) causing major infectious diseases of fishes. The codon-optimized gene encoding NNV capsid protein was expressed under the control of a strong constitutive Y. lipolytica TEF1 promoter using two ARS-based vectors pNHATX2 and pMR, carrying different selection markers. The intact recombinant NNV capsid proteins were detected by anti-NNV antibody as monomer (37 kDa) and oligomer assuming trimer, which is a basic unit for formation of NNV virus-like particles. On the other hand, the N-terminal truncated forms showed lower expression levels and decreased antigenicity. We further constructed a multiple integrative plasmid carrying 26S rDNA fragment as selection marker to increase integrated copy numbers of the NNV expression cassette. Oral immunization of mice with the whole recombinant Y. lipolytica expressing the intact NNV capsid induced highly the formation of IgG against NNV capsid protein. Our results strongly support that the potential of whole recombinant Y. lipolytica expressing recombinant NNV capsid protein as oral vaccine to prevent NNV infection of fishes.

Keywords: NNV capsid protein, Yarrowia lipolytica, vaccine, 26s rDNA

Oxidative Stress Response in Pseudomonas Strains Exposed to Toluene
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Activities of oxidative stress enzymes, catalase, superoxide dismutase, total anti-oxidative capacity and glutathione S-transferase activities, in two toluene-tolerant bacteria and one non toluene-tolerant bacteria were tested for response to oxidative stress caused by toluene. Pseudomonas sp. BCNU 106 and BCNU 171 was distinguishable from other Pseudomonas strains on the basis of its ability to grow on high concentration toluene. All bacteria possessed a basal level of antioxidant enzymes activity prior to being exposed to toluene. Enzyme activities changed in a toluene concentration (100-400 ppm) dependent manner after exposure to toluene for 6h. Compared to non-toluene-tolerant P. putida, toluene-tolerant bacteria had relatively high tolerance to toluene stress, especially Pseudomonas sp. BCNU 106. Therefore, the investigation of their antioxidant properties will be useful for further study on toluene-tolerance of bacteria and the defense mechanism of antioxidant enzymes against toluene or other organic solvents.

Keywords: Pseudomonas sp. strains, Solvent-tolerant bacterium, Antioxidant defense, Toluene adaptation

Flux Optimization of TCA Cycle for Efficient Production of Fumaric Acid
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Fumaric acid is an important C4-dicarboxylic acid widely used in chemical, food, and pharmaceutical industries. Rational metabolic engineering together with flux optimization were performed for the development of an Escherichia coli strain capable of efficiently producing fumaric acid. The initial engineered strain, CWF4N overexpressing phosphoenolpyruvate carboxylase (PPC), produced 5.30 g/L of fumaric acid. Optimization of PPC flux by examining 24 types of synthetic PPC expression vectors further increased the titer up to 5.72 g/L with a yield of 0.432 g/g/glucose. Overexpression of the succinate dehydrogenase complex (sdhCDA) led to an increase in carbon yield up to 0.493 g/g/glucose. Based on this mutant strain, citrate synthase(CS) was combinatorially overexpressed and balanced with PPC using 48 types of synthetic expression vectors. As a result, 6.24 g/L of fumaric acid was produced with a yield of 0.500 g/g/glucose. Feed-batch culture of this final strain allowed production of 25.5 g/L of fumaric acid with a yield of 0.366 g/g/glucose. Deletion of the aspA gene encoding aspartate aminotransferase and supplementation of aspartic acid further increased the fumaric acid titer to 35.1 g/L with a yield of 0.490 g/g/glucose. (Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1A9A1001-2012M1A2A2026556)

Keywords: Fumaric Acid, Escherichia coli, Flux Optimization, Metabolic Engineering
Development of a Metabolically Engineered Strain for the Production of Beta Alanine, a Precursor for Nylon-3 Synthesis
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Metabolic engineering strategies were taken to produce 3-amino propionic acid (3-AP), an important platform chemical for manufacturing acrylicamide and acrylonitrile, in Escherichia coli. Using a frumaric-acid producing E. coli strain as a host, the aspA gene (encoding L-aspartate-α-carboxylase) was overexpressed and the native promoter of the aspA gene was replaced with the strong trc promoter. Additional overexpression of the aspA and phospholipomynovate carboxylase (ppc) genes, and the supplementation of ammonium sulfate in the medium allowed production of 3.49 g/L 3-AP. Fed-batch culture of the final strain yielded 17.9 g/L 3-AP in 89 h, with an overall yield and productivity of 0.186 g 3-AP/g glucose and 0.200 g/L/h, respectively. (Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for BioRefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AA001-2012M1A2A2026556))
Keywords: 3-Aminopropionic acid, Beta Alanine, Aspartase, Frumaric Acid

Optimal Strain and Knockdown Target Screening Method Using Synthetic Small Regulatory RNA for Enhanced Production of Tyrosine and Cadaverine
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Small regulatory RNAs (sRNAs) are gene expression regulators which act on post-transcriptional phase of bacterial gene expression. Here, we developed synthetic small regulatory RNA system to down-regulate the gene expression. The target mRNAs binding domain of MicC, one of the well-studied sRNAs in Escherichia coli, was replaced to translation initiation sequence of our target genes. We found that MicC scaffold based synthetic sRNA is properly repressed expression of DrrD2, a fluorescence protein. Synthetic sRNAs was used for metabolic engineering to enhance the production of tyrosine and cadaverine. Through screening of 14 different strains which harboring one or combination of the synthetic sRNAs targeting ppc, tyrA, csrA, and pgi, we isolated a tyrosine producer producing 2 g per liter of tyrosine. Using a library of 130 synthetic sRNAs, we screened knockdown targets that increase cadaverine productivity substantially. Repression of mscI led to a 55% increase in cadaverine production compared to the base strain. This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for BioRefineries (NRF-2012-C1AA001-2012M1A2A2026556), the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea
Keywords: small RNA, metabolic engineering, tyrosine, cadaverine

Aminovalerate and Glutarate Production Pathway Introduced into Escherichia coli
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5-Aminovalerate (SAVA) is an important C5 platform chemical. Escherichia coli was metabolically engineered for the production of 5-aminovalerate (SAVA) and glutarate. When the recombinant E. coli WL3110 strain expressing the Pseudomonas putidabaTD genes encoding delta-aminomaleralidase and lysine 2-monooxygenase, respectively, were cultured and produced 3.6 g/L of SAVA by converting 7 g/L of L-lysine. When the dkaTB genes were introduced into recombinant E. coli strain XQ56 allowing enhanced L-lysine synthesis, 0.27 and 0.5 g/L of SAVA were produced directly from glucose by batch and fed-batch cultures, respectively. Further conversion of SAVA into glutarate demonstrated by expression of the P. putida gdhTD genes encoding SAVA aminotransferase and glutarate semialdehyde dehydrogenase. When recombinant E. coli WL3110 strain expressing the dkaTB and gdhTD genes was cultured in a medium containing 10 g/L L-lysine and 10 g/L α-ketoglutarate, 1.7 g/L of glutarate was produced.
Keywords: L-lysine, 5-aminovalerate, Glutarate, Escherichia coli, Metabolic engineering

Optimization of a Static Acid-Binding Lectin Purification Procedure from a Mushroom Fruiting Body
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Although a mushroom fruiting body contains many interesting proteins, it is difficult to obtain the reproducible quality and quantity of them. In this study, I optimized the purification procedure for glycan-specific binding lectins from a mushroom fruiting body. The one step purification showed at least three main proteins containing other minor proteins on SDS-PAGE. To optimize the purification of the glycan-binding proteins, ion exchange chromatography using DEAE-Sepharose, affinity chromatography using a glycoprotein-immobilized column and size exclusion chromatography were sequentially applied. The conditions on the purification procedure of the mushroom lectins including NaCl conc. in ion exchange chromatography and Cal conc. in affinity chromatography were optimized. Finally, two distinct proteins, L1 and L2, were separately isolated. Interestingly, L2 indicated strong binding properties to ion-exchange column and fetuin-agarose column rather than L1. The purified two lectins showed hemagglutination activities against porcine erythrocytes. Interestingly, L2 showed protease activity, however L1 did not show any catalytic activity. A hemagglutination inhibition assay indicates that these lectins showed different binding specificities. These optimized purification procedures could be useful to separate an individual lectin bound to specific glycan structures in a glycoprotein (This work was supported by Rural Development Administration, Project No. PJ900793).
Keywords: mushroom, Lectin, purification, Glycan, mushroom fruiting body
H-25

Antigen-Specific Chicken Antibody Selection from Non-immunized Antibody Gene Library using β-lactamase-based Protein Fragment Complementation Assay (PCA)

Dong Woom Park1, Seong-Hee Han1, Jeong-Hye Lee1, Min-Jin Choi1, Rachelle Canete1, Dong Woon Park1, Seung-Hee Han1, Jeong-Hye Lee1, Jin-Hee Kang1, Pro-r6-331-lactamase–based Antigen-Specific Chicken Antibody Selection from epimerization of D-fructose to D-psicose by whole-cell using biotechnological means has been explored. In this study, the production of psicose by natural and chemical means has been challenging and so its production highly soluble in water (4% w/w at 25 °C). However, production of psicose Furthermore, it has only 0.3% of the calories of sucrose and it is also substitute, considering that it has 70% sweetness relative to sucrose. An antioxidant and has an insulin resistance effect and is a good sucrose sugar which is a C-3 epimer of D-fructose, is hypolipidemic, neuroprotective, and important in several applications including the food industry, pharmaceutical and cosmetic industry. D-psicose, a rare sugar which is a C-3 epimer of D-fructose, is hypolipidemic, neuroprotective, and has an insulin resistance effect and is a good sucrose substitute, considering that it has 70% sweetness relative to sucrose. Furthermore, it has only 0.3% of the calories of sucrose and it is also highly soluble in water (4% w/w at 25 °C). However, production of psicose by natural and chemical means has been challenged and so its production using biotechnological means has been explored. In this study, the epimerization of D-fructose to D-psicose by whole-cell biocconversion reaction using recombinant cells of Corynebacterium glutamicum containing D-psicose epimerase requires great influx of the enzyme’s substrate (D-fructose). However, the yield has been restricted by the uptake of D-fructose in its free form. So, transporters which uptake D-fructose inside the cell need to be over-expressed. This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant #: PJ011062), RDA, Korea.

Keywords: Psicose, Fructose, Sugar transport, Corynebacterium glutamicum, D-Psicose 3-epimerase

H-27

SpiE Interacts with Corynebacterium glutamicum WhcE and is Involved in Heat and Oxidative Stress Responses

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The gene whcE in Corynebacterium glutamicum positively responds to oxidative and heat stress. To search for proteins that interact with WhcE, we employed a two-hybrid system with WhcE as the bait. Sequencing analysis of the isolated clones revealed peptide sequences, one of which showed high sequence identity to a hydrophobe/amphiphile efflux-1 family transporter encoded by NCgl1497. To verify the interaction of the NCgl1497-encoded protein with WhcE in vitro, we assayed reporter gene expression by RT-qPCR. The WhcE protein strongly interacted with the NCgl1497-encoded protein in the presence of oxidative and heat stress. Furthermore, purified WhcE and NCgl1497-encoded proteins interacted in vitro, especially in the presence of the oxidant diamide, and the protein-protein interaction was found to be disrupted in the presence of the reductant dithiothreitol. Besides, NCgl1497-deleted mutant strain showed decreased survivability against diamide and heat stress. The mutant strain also exhibited reduced transcription of the thioredoxin reductase gene, which is known to be regulated by whcE. Based on these data, NCgl1497 was named spiE (stress protein interacting with WhcE). Collectively, these data suggest that spiE is involved in the whcE-mediated oxidative stress response pathway of C. glutamicum. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A01004556).]

Keywords: whcE, oxidative stress
Enhancement of Cyclohexane Tolerance in *Pseudomonas* sp. BCNU 106 by Addition of Trehalose to Culture Media
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Solvent hyper-tolerant *Pseudomonas* sp. BCNU 106 has limited potential for its use in biotransformation and biodegradation, because of limited growth with solvents. Therefore, a strategy is needed to allow better growth to broaden its performance in biotechnological applications. *Pseudomonas* sp. BCNU 106 was cultivated in a medium supplemented with 50 mM trehalose, and cell survival was then monitored during all cultivation times with 1% (v/v) cyclohexane. BCNU 106 grown in the presence of exogenous trehalose showed higher tolerance to cyclohexane, indicating that trehalose might be imported into the cells and also metabolized as a carbon source for survival. It can thus have more potential for biotransformation and biodegradation, especially for biotransformation in the presence of cyclohexane.

Keywords: Exogenous trehalose, *Pseudomonas* sp., Stress tolerance, Cyclohexane tolerance, Trehalose
Intra Species Variation of *Bjerkandera adusta* Concerning Decolorization and Oxidation of Organic Pollutant

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White rot fungi can be extensively applied to biotechnological procedures in terms of mineralization of organic pollutant. Among a number of white rot fungi, *Bjerkandera adusta* inhabiting on hardwood in various forest areas, is commonly known as a smoky polypore fungus belonging to Basidiomycota. In addition, *B. adusta* is an attractive fungus due to their ability to decolorize waste water and degrade organic pollutants. However, not all the *B. adusta* is able to oxidize organic pollutants by their ligninolytic enzymes. Accordingly, a total of 49 isolates of *B. adusta* were collected from various areas such as national parks, mountains, and some local areas in Korea. And their characterization were carried out in terms of decolorizing dye, reactive blue 4 (RB4) and oxidizing gallic acid by classification aligned with phylogenetic tree. In the results of the study, 100 mg L⁻¹ of RB4 was decolorized by *B. adusta* within 10 days, and abilities to decolorize RB4 were significantly similar according to aligned group of phylogeny. Interestingly the results were within 10 days, and abilities to decolorize RB4 were significantly similar according to aligned group of phylogeny. Consequently, phylogenetic classification of *B. adusta* is responsible for intra species variation in characterization of decolorization and oxidation of organic pollutant according to the same clusters.

**Keywords:** *Bjerkandera adusta*, Decolorization, Gallic acid reaction, Reactive Blue 4, White rot fungi

Characteristics of a Recombinant FSH and LH of Japanese Eel with and without CTP of Equin Produced in Silkworm Using BmNPV Baculovirus

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In the Japanese eel *Anguilla japonica*, the administration of exogenous gonadotropin (GTH) is necessary for the artificial induction and completion of gonadal maturation due to its GTH deficiency under captive conditions. Here, we report on the production of biologically active recombinant follicle stimulating hormone (rFSH) and Luteinizing hormone (rLH) in Japanese eel using silkworm expression system. The two compositive FSH and LH, i.e. common glycoprotein, alpha polypeptide (CGa) and hormone-specific beta polypeptides (FSH/b533 and LH/b533), were produced with (AfFSH/b533 and JdLH/b533) and without (AfFSH and JdLH) carboxyl-terminal peptide (CTP) of equine chorionic GTH/b533, and were proven to be glycosylated and secreted as the mature peptides. The rFSHs and rLHs of the silkworm hemolymph were separated and a band showed positive reaction with anti-His. In addition, the activity of rFSHs and rLHs were confirmed via cAMP level in CHO cells with FSH and LH receptor gene of Japanese eel. The availability of each two type rFSHs and rLHs have allowed us to study the biological function of this interesting factor in detail.

**Keywords:** Baculovirus, Silkworm, recombinant protein, gonadotrophin, Japanese eel

Isolation of *Chlorella vulgaris* Mutants Producing High Lipid and their Characterization

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Micro-algae *Chlorella vulgaris* (C. vulgaris) is important source for bio-diesel because of high content of neutral lipids. In this study, we want to obtain the mutants induced by UV-B irradiation. We were exposure to UV-B of C. vulgaris for 1, 2, 3, 4, 5 min. It was that UV-B exposure time is proportionate to decreased cell viability and pigment content. We induced mutants of C. vulgaris through ultraviolet irradiation and selected two strains by lipid contents. We named mutant ‘UM10’, ‘UM15’, and ‘UM18’, and they were cultivated in the same way with the wild type. After 21 days cultivation, cell growth, dry cell weight, pigment content, and lipid content were measured for characteristics of mutants. As a result, cell growth and dry cell weight of mutants were increased about 1.4, 1.5 times compared with wild type, respectively. Chlorophyll and carotenoid contents were measured in order to investigate pigment content in micro-algae through photosynthesis. It was shown that chlorophyll and carotenoid contents of mutants were decreased about 10% compared with wild type. Lipid contents were increased about 1.2, 1.5 times compared with wild type.

**Keywords:** Chlorella vulgaris, Ultra-violet, Chlorophyll, Carotenoid, Lipid

Development of Novel SRP Machinery-engineered *E. coli* Mutant for the High-level Secretory Production of Antibodies and Therapeutic Proteins

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Signal recognition particle (SRP)-dependent secretion pathway of *E. coli* is an analogue of eukaryotic SRP pathway, which is featured by co-translational translocation. Even though this pathway offers great benefit of preventing cytoplasmic aggregation of proteins before secretion, limited capacity was major hurdle for utilizing the pathway as an alternative of recombinant protein secretion. To overcome this problem, we developed a novel *E. coli* mutant, of which SRP machinery was dramatically increased. For the engineering of *E. coli* strain, we firstly designed a new periplasmic fluorescent reporter system by using a special fluorescent dye, FITAEDT-F. With this reporter system, we screened transposon-generated *E. coli* mutant library through the several rounds of FACs, after then a novel *E. coli* strain, of which SRP machinery was fairly improved, was isolated. It was identified as single gene-deficient strain, and the general effect of this mutation for SRP pathway-mediated protein secretion was demonstrated by different kinds of model proteins. In addition, very high production yields of human full-length IgG and GPCR mutant were achieved in the fed-batch cultivations with the isolated *E. coli* mutant.

**Keywords:** Escherichia coli, SRP pathway, FACS, IgG, GPCR
Prevention of bacterial adhesion and biofilm formation on material surfaces is a challenging task with societal importance. Biofilm formation on medical devices causes persistent infections that associated with major morbidity and mortality among patients. Biofilm is a complex impermeable shield and highly resistant to chemicals, and antibiotics. As a result, development of innovative antimicrobial strategies with novel therapeutic solutions is in urgent need. Nanotechnology based approaches are being explored recently to reduce bacterial resistance with promising antibiotic properties. Most metal oxide nanoparticles (NPs) exhibits excellent antimicrobial activity through different mechanisms against pathogenic bacteria. The larger surface-to-volume ratios and defined crystallographic structures, facilitates mass exchange between NP and the surrounding medium, resulting in high surface accessibility as well as faster dissolution of bactericidal components attached with NPs. Nanofabrication allows the development of abiotic surfaces that prevent infections on medical devices. This anti-biofilm technology focuses on biomaterials with different bioactive substances for medical devices to make them resistant against biofilm formation that are superior to current antibiotic treatments.

Keywords: biofilms, nanoparticles, surface coating, antibacterial, anti-biofilm

Biofunctionality and Bioconversion Activities of KA6(Bacillus licheniformis), KA84(Enterococcus faecium), KA89(Bacillus licheniformis), KA107(Bacillus licheniformis)

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In this study, we conducted the studies on the biofunctionalities and bioconversion activities of the four strains. In order to find out new functional resources and to elucidate new biofunctionalities and bioconversion activities of four kinds of microorganisms, the following experiments were carried out. These biofunctional resources are involved in physio-biochemically important amino acids such as ornithine(Orn), citrulline(Cit) and r-aminobutyric acid(GABA). We isolated these strains(KA6, KA84, KA89 and KA107), which were tested and analyzed bioconversion activities transforming Arg(arginine) into Orn and Cit. As the results, all four strains(KA6, KA84, KA89 and KA107) showed bioconversion activities converting Arg into Orn and Cit directly or indirectly.

Keywords: Biofunctionality, Bioconversion, Enterococcus faecium, Bacillus licheniformis

Bioconversion Efficiency in Accordance with the Substrate(Arg;Arginine) Concentrations

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In this study, we conducted experiments related with bioconversion efficiency according to the higher concentrations of substrate (Arg;Arginine). Of the biofunctional resources, emphases are placed on physio-biochemically important amino acids such as ornithine(Orn), citrulline(Cit) and r-aminobutyric acid(GABA). We isolated four strains(KA6, KA84, KA89 and KA107), which were tested and analyzed bioconversion activities under changing the concentrations of Arg at the higher levels of 3X and 5X. As the results, when we applied high concentration of Arg with 3X or 5X that is higher than the normal condition(control group in the media), KA6, KA89 and KA107 did convert Arg into Orn and/or Cit to the extent of 100%. On the other hand, KA84 showed approximately 70% of bioconversion efficiency under 3X and 5X concentrations of Arg in the complex medium and did not in the minimal medium. Finally, multi-biofunctional microbes (KA6, KA84, KA89 and KA107) were related to biodegradability and bioconversion activity, antibacterial-antifungal microbes (KLEK10, KLEK14) against Staphylococcus, Vibrio, Salmonella, and E. coli. Accordingly our results, we suggest that our identified multi-biofunctional microbes can be contributed to the healthy non-antibiotic poultry production and the bioremediation and regeneration of polluted waste materials.

Keywords: Bioconversion, Efficiency, Substrate, Arginine, Concentration
**H-41**

**Novel Bacteria-Phage Pairs Showing Phage-Antibiotic Synergy (PAS) and Study on Its Mechanism**

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Bacteriophages-mediated therapy becomes increasingly recognized as a novel treatment strategy for many diseases, and high phage productivity is crucial for the phage-drug development. Phage-Antibiotic Synergy (PAS), a term describing the phenomenon that sub-lethal dosage of antibiotics (abx) increases phage productivity of the host bacteria, offers an efficient method to increase phage production. Here we report several novel sets of bacteria and abx showing the PAS effect. In Gram-positive bacteria, like *Staphylococcus aureus* and *Bacillus cereus* and Gram-negative bacteria, like *Escherichia coli* (*E.coli*) and *Pseudomonas aeruginosa*, sub-lethal doses of ampicillin, cefotaxime, ciprofloxacin, or mitomycin C induced PAS effect; larger plaques, burst size increases, and enhanced phage production via filamentation were observed. The increased burst size was resulted from increase of phage DNA replication, transcription, translation, and prolonged phage assembly. *In vivo* PAS was also observed in *C. elegans*. Under the PAS-inducing condition in *E.coli*, recA and FtsZ were upregulated and downregulated, respectively. recA overexpression in *E.coli* caused 2-4 fold longer length than wild type and showed phage burst comparable to PAS condition. However, recA-deletion mutant *E.coli* also showed weak PAS, so recA pathway might not be the only way causing PAS. Through recA modification and application of novel sets of bacteria and abx, effective phage-drug production could be achieved.

Keywords: Phage-Antibiotic Synergy, PAS mechanism, Bacteriophage, recA

**H-42**

**Monoclonal Antibodies Specific for the NS 1 Protein of Japanese Encephalitis Virus Suitable for Diagnostic Purpose**

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Japanese encephalitis virus (JEV) belongs to the genus *Flavivirus* of the family *Flaviviridae*. Laboratory diagnosis of JEV is mainly based on the detection of virus specific IgM antibodies in serum and CSF. Now, an IgM capture ELISA is the first choice of testing method in most diagnostic laboratories. While envelope gene has been normally used for diagnosis, NS1 protein is considered as an alternative target. The objective of the study is developing monoclonal antibodies specific for the NS 1 protein of JEV suitable for diagnostic purpose.

Genotype 1 strain of JEV, K05S5, isolated from mosquito in Korea, was used in this study. The full-length NS1 was cloned into a drosophila expression vector pMT/Bip/V5-His A. Then, the stable cell line (S2) expressing NS1 protein was constructed and expressed recombinant protein was used for antibody production. After immunization of BALB/c mice with the recombinant protein, four clones of hybridoma (2C11, 4E5, 5H7, and 6C5) were selected by using ELISA and Western blotting. The resultant antibodies were reactive with the major genotypes (1 and 3) of JEV, as well as other flaviviruses (Yellow fever virus, West Nile virus lineages 1 and 2) in immunofluorescence assay. Thus, these antibodies may be useful reagents for JEV and related flavivirus detection in several formats.

Keywords: Japanese encephalitis virus, nonstructural protein 1, monoclonal antibody, flavivirus

**H-43**

**Microbial Network Analysis in a Pilot-Scale MBR**

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Network analysis was applied to examine complex microbial interactions on the membrane of a pilot-scale membrane bioreactor (MBR) for wastewater treatment. The MBR with flat-sheet membrane (a pore size of 0.4 μm) was independently operated for 4 and 8 days at a hydraulic retention time of 5.2 h. Membranes were collected and cut into 18 tile-pieces. Bacterial and fungal communities were analyzed using next generation sequencing platforms. Microbial network analysis was applied to identify associations among the relative abundances of operational taxonomic units (OTU) using extended local similarity analysis (eLSA). Network analysis results indicated that there were significant inter-domain and intra-domain interactions. For instance, the most abundant bacterial OTU representing *Acinetobacter* that acted as a network hub was associated with many bacterial and fungal OTUs at day 4, and two different fungal OTUs (unidentified Fungi) hubs were associated with a majority of bacteria. Microbial network analysis can provide a clear, comprehensive view to better understand the microbial interaction and association within the MBR.

Keywords: Microbial community, Microbial association, Network analysis

**H-44**

**Effect of Commercialized Chlorine Dioxide to Clinical Bacteria**

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Chlorine dioxide is a chemical compound with the formula ClO₂. As one of several oxides of chlorine, it is a potent and useful oxidizing agent used in water treatment and in bleaching. It is used at moderately acidic pH (3.5 to 6). Sometimes chlorine dioxide is used as a fumigant treatment to 'sanitize' fruits such as blueberries, raspberries, and strawberries that develop molds and yeast. Farm-e Tok stick containing chlorine dioxide gas was already commercialized and kindly was provided by Purgo Farm company. In this study, whether it could inhibit bacterial growth by the stick was analyzed by colony forming unit. Bacteria applied were followed: *Staphylococcus aureus, S. pyogenes, Streptococcus agalactiae, Escherichia coli O157:H7, Klebsiella pneumoniae*. Initial bacterial concentration of *Salmonella typhi* and *Shigella sonnei* was 1.5 x 10⁵ and *Shigella sonnei* had 1.5 x 10⁴ or 1.5 x 10³. When one stick was released to a triple sugar iron (TSI) agar plate, the surface color of the culture plate was changed a little into yellowish color. However, four culture plates did not show any color changes by one gas kit. All bacterial colonies showed less than 10 and their growth was inhibited by chlorine dioxide up to 99.9%.

Keywords: Chlorine dioxide, bacteria
Effect of Monosodium Glutamate to a Mammalian Cell and \textit{Escherichia coli}

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Monosodium glutamate (MSG) is widely used as one of the synthetic flavoring matters. However, it is not well known about its effect to a mammalian cell and bacteria. Moreover, its safety was of interest for food and other usage, e.g., metabolism. In this study, it was analyzed how commercialized natural seasonings and synthetic flavoring matters have an effect on a human microvascular endothelial cell (HBMEC) and a pathogenic bacterium, \textit{Escherichia coli}. Five grams of the selected popular seasonings were dissolved with 40 ml of distilled water (DW) and then mixed bacteria directly in the mouthwash. Several methodologies tested, e.g., mixing bacteria directly in the mouthwash, long term treatment of bacteria with solution, bacterial suspension treated with Cololo solution for 1 hr incubation at room temperature (RT), and then further 4 hr incubation at RT, were more effective than direct inoculation of bacteria after mixing with Cololo solution. Although Hexamedine solution had the strongest bactericidal effects, it would be harmful to continue its usage for even 10 days. Therefore, our data suggests that Cololo solution would be a better alternative to Hexamedine solution.

Keywords: Hexamedine, Cololo
Engineering of Corynebacterium glutamicum for Production of Vanilla Flavors
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Vanilla flavors with a unique sweet fragrance are widely used in food, cosmetics and pharmaceuticals industries. Natural vanilla flavors are mainly composed of vanillin and various minor aromatic compounds such as 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 4-hydroxybenzoic acid, protocatechuic acid (PCA), protocatechual alcohol, 3,4-dihydroxybenzaldehyde, vanillic acid, vanillyl alcohol, and so on. In order to develop a new environmentally friendly process, first, we engineered Corynebacterium glutamicum producing PCA, a precursor of vanillin, by deletions of trpE and csm and replacement of pcaHG with ubiC, resulting in PCA395. To develop vanilla flavor-producing strain, vanA and vanB coding vanillate demethylase were deleted from PCA395, and resulted in VAN401 strain. A car coding carboxylic acid reductase and hcomt coding catechol O-methyltransferase from human were expressed in pCX vectors, respectively. Among them, CAR and HCOMT were expressed very well under the control of PIlvC-M1 promoter by SDS-PAGE analysis. In addition, the expression of HCOMT with PIlvC-M1 with UbiCm in another shuttle vector, pCES208 showed the highest concentration of vanillic acid at flask fermentation. An engineered C. glutamicum VAN4022 expressing CAR and HCOMT under the control of PIlvC-M1 with UbiCm produced 100 mg/l of vanillyl alcohol, 48 mg/l of vanillic acid, and 260 mg/l of PCA.

Keywords: Vanilla flavor, Corynebacterium, Metabolic engineering

Hydrolysis of Codium fragile by Mannanase from Bacillus subtilis R2AL2A
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Bacillus subtilis R2AL2A isolated from cow feces exhibited a hydrolysis activity of Locust bean gum(LBG) and AzCl-galactomannan. A secreted mannanase of Bacillus subtilis R2AL2A was purified to homogeneity from a culture supernatant by ammonium sulfate precipitation and ion-exchange chromatography. The molecular mass of the enzyme was estimated to be 37 kDa by SDS-PAGE. The temperature and pH for maximum activity were 50ºC and pH 6.0, respectively. Although the enzyme exhibited low activity toward mannann(2.7 U/mg), it showed relatively high activity toward glucomanann(617.2 U/mg) and galactomannan(LBG, 460.2 U/mg) which was mannobiose as a final degradation unit. The mannanase activity was increased with the addition of Cu2+ and Ca2+, and strongly inhibited by ethylenediaminetetraacetic acid. Finally, the Bacillus subtilis R2AL2A hydrolyzed Codium fragile, green seaweed with a mannan backbone, showing 50% degradation after 6 days at 25ºC. When polysaccharides-degrading activities of the extract of Codium fragile by Bacillus subtilis R2AL2A were determined by using substrate such as alginic, fucoidan, laminarin, agarose, or carrageenan, it exhibited just only mannanase activity. This indicates that Codium fragile was efficiently hydrolyzed by mannanase of Bacillus subtilis R2AL2A, which means that the extract of Codium fragile has great potential as a supplement in functional food and health-promoting prebiotics.

Keywords: mannanase, Codium fragile, Bacillus subtilis, green seaweed, prebiotics
Production of a Functional Enzyme Food with Bacillus licheniformis and Lactobacillus casei by Two-Step Fermentation

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Isolation of lactic acid bacteria from Baechu kimchi was attempted to study Leuconostoc strains. Isolates named as BL-1A, BL-5 and BS-1 could not grow at 30°C but Leuc. mesenteroides subsp. mesenteroides KCTC 3722 still grew very well at the mesophilic temperature. From the results of hexose fermentation, isolates revealed exactly same patterns of fermentation. It implied that the isolates and the Leuc. mesenteroides subsp. mesenteroides KCTC 3722 had same fermentation mechanism with hexoses. Typically BL-1A could not ferment cellobiose among tested disaccharides as same as a standard strain resulting in final acidity as of 5.8±0.05. The BL-1A could not ferment melitose at all as same as the standard strain (pH 5.7±0.01) but could ferment raffinose (pH 4.7±0.1). This unique pattern of fermentation in trisaccharides was usually found in Leuconostoc species. This kind of similarities were also found in fermentation of 7 carbohydrate derivatives. Therefore the BL-1A from Baechu kimchi can be easily identified as another subspecies of Leuc. mesenteroides.

Keywords: Kimchi, LAB, Leuconostoc mesenteroides

Degradation of Fucoidan by Fucoidan-degrading Enzyme from Formosa sp.

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Fucoidans, polysaccharides containing primarily sulfated L-fucose, are fundamental component of brown algae and marine invertebrates. Fucoidans play crucial roles in bioactivity such as anticoagulant, antiviral, antiinflammatory, antiallergic, antioxidant, antibacterial, antitumor properties. However, the clinical application of fucoidan has been limited by high molecular weight structure. To efficiently solve this problem, fucoidans need to be depolymerized by fucoidanase. In this study, the two types of fucoidanases (Fase1, Fase2) from Formosa sp. were isolated by anion exchange chromatography respectively. Fase1, Fase2 degraded fucoidan from Fucus vesiculosus and produced LMF(Low Molecular weight Fucoidan), VLMF(Very Low Molecular weight Fucoidan), respectively. The formation of LMF, VLMF were determined by thin layer chromatography(TLC) and HPAEC. LM and VLMF were purified by paper chromatography method. To determine the number-average degree of polymerization, purified LMF(PLMF) and VLMF(PVLMF) were analyzed by Phenol-sulfuric acid method, DCA method. The degree of polymerization(DP) values for PVLMF, PLMF and fucoidan(Fucus vesiculosus) were 12.80, 18.74, 22.15, respectively. This result suggested that Fase2 produces lower molecular weight products than Fase1. For determining antibacterial activity, treatment with 50~1000μg/mL PVLMF, PLMF and fucoidan(Fucus vesiculosus) showed inhibition against Ecoli O127, Salmonella enteritidis, Staphylococcus aureus. Keywords: Fucoidan, Fucus vesiculosus, Fucoidanease, Formosa algae, Marine bacteria
I-5
Optimizing Culturing Conditions and Comparative Fibrinolytic Activities of Fibrinolytic Enzymes from Bacillus subtilis Strain
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A new Bacillus strain designated as P3 producing protease has been isolated from the traditional food of Korea. The strain P3 was found to exhibit the good fibrinolytic activity comparing with nattokinase. The 16S rRNA sequence revealed that the P3 was closely related to Bacillus subtilis with 99% homology. The optimal medium composition for production of fibrinolytic enzyme in the B. subtilis P3 were 1% glucose, 1% yeast extract, 0.01% sodium chloride. We compared the fibrinolytic activity during the fermentation of B. subtilis natto and B. subtilis P3. To investigate the possibility of its industrialization, supernatant of B. subtilis P3 was freeze dried and compared fibrinolytic activity with other released products. Result will be utilized for development of the health functional material using Bacillus strain P3.
Keywords: Bacillus subtilis, Fibrinolytic enzyme, Industrialization

I-6
Isolation of γ-Aminobutyric Acid-Producing Enterococcus faecium JK29 from Traditional Fermented Foods and Its Culture Optimization for GABA Production
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In order to develop the fermented foods-derived strain producing γ-amino butyric acid (GABA), 147 lactic acid bacteria (LAB) roughly isolated from different traditional fermented foods were further screened with 1% L-monosodium glutamate (MSG), resulting in 23 GABA-producing strains. After the 16S rRNA gene sequence and glutamate decarboxylase (gad) gene sequence analysis, Enterococcus faecium JK29 producing 1.56mM of GABA was finally selected despite its low GABA production level, because Enterococcus genus strains have not been well reported to be GABA-producer compared to the other LABs. The amino acid sequence of GAD of E. faecium JK29 (Accession num. AJ25608.1) showed 99% identity with the already-reported GAD of E. faecium DO (Accession num. YP_006348671.1). In order to enhance the GABA production by E. faecium JK29, the culture conditions were optimized with the modified MRS medium containing 0.5% sucrose and 2% yeast extract with 1% MSG. The maximum GABA production finally reached 14.8 mM at 30°C for 48 h with the optimized condition. This study was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2014R1A1A1000298).
Keywords: γ-Aminobutyric acid, Glutamate decarboxylase, Enterococcus faecium, Optimization, Fermented foods

I-7
Preparation and Quality Characteristics of the Fermentation Product of Gastrodia Elata Blume Powder by Lactic Acid Bacteria
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This study was performed to reduce unpleasant taste and flavor of Gastrodia elata Blume powder as well as to improve utilization as functional food materials using fermentation. Gastrodia elata Blume belongs to Orchidaceae, which is a perennial parasitic herbaceous plant, grows in the woods of the central provinces of China, Korea and Japan. Its rhizome has been traditionally used as a tonic, a sedative and an antispasmodic. Non-fermented Gastrodia elata Blume powder was used as a control, while fermentation was carried out using Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus brevis and incubated at 37°C for 1~3 day. So it was compared to measuring lactic fermented Gastrodia elata Blume powder that change of pH, total acidity, total reducing sugars etc. The results showed that overall taste and flavor were better in fermented Gastrodia elata Blume powder by Lactobacillus brevis than in raw and fermented other lactic acid bacteria. It is plausible that unpleasant taste and flavor of Gastrodia elata Blume powder was declined by fermentation.
Keywords: Gastrodia elata Blume, Lactic acid bacteria, Lactobacillus brevis

I-8
Evaluation of the Efficiency of Compounds Present in Ingredients of Kimchi against Influenza A Virus Infection Using Proteomic Analysis
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Kimchi, a traditional fermented food regularly consumed in Korea, contains various types of antimicrobial compounds. Among the various tested compounds present in Kimchi ingredients, quercetin showed the highest selectivity index (SI) against influenza A virus (IAV) H1N1. In this study, the effect of pretreatment and periodic treatment of quercetin at the concentration of 20μM on IAV in MDCK cells was compared. Cell viability, apoptosis and viral copy number were calculated using MTT assay, Annexin V staining and reverse transcription-PCR, respectively. Antiviral activity of quercetin was evaluated by a reduction in IAV PA gene expression and plaque forming units. Periodic treatment showed significantly higher relative viability of IAV-infected cells and higher inhibition of IAV PA gene expression in the cells without cytotoxic effects at the concentration compared to pretreatment. Immunofluorescence assay demonstrated that periodic treatment suppressed IAV NP penetration to nucleus. Furthermore, to explain the mechanisms underlying the antiviral effects of quercetin treatment, a comparative proteomic analysis was performed in four samples (M, mock; Q, quercetin-treated; IAV, IAV-infected; and Q+IAV-quercetin-treated IAV-infected). Among 75 proteins detected in the samples, 7 proteins were significantly downregulated in Q+IAV compared to IAV. In conclusion, the periodic treatment of quercetin is effective to reduce severity of IAV infection.
Keywords: Natural compounds, Ingredients of Kimchi, Influenza A Virus, Proteomic analysis, Antiviral Effect
I-9
Enhancement of Conjugated Linoleic Acid and Isoflavone-Aglycone Contents in Different Fermented Soybean Cultivars with Mycelia of *Polyozellus multiplex*

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In this study, changes in conjugated linoleic acid (CLA) and isoflavone contents and biological activities during the solid-state fermentation of four soybean cultivars *Polyozellus multiplex* were investigated. During solid-state fermentation of different soybean cultivars, the total phenolic content of soybean and isoflavone-aglycone (daidzein, genistein, and daidzin) decreased, while radical scavenging activities and α-glucosidase inhibition activity increased.

The highest levels of daidzein, genistein, and daidzin were obtained in the final fermentation day (8 days) with Daepung cultivar. In consequence, the results of DPPH and ABTS radical scavenging activities and α-glucosidase inhibition activity were the highest in Daepung cultivar than other soybean cultivars. The fermented soybeans showed higher CLA contents and biological activities compared to any other unfermented soybeans examined. In particular, the levels of cis-9, trans-11, trans-10, cis-12, and total CLA were detected to be 47.25 μg/g, 4.63 μg/g, and 51.88 μg/g in the final fermentation day (8 days) with Daepung cultivar.

Keywords: Polyozellus multiplex, CLA, Fermentation, Soybean, Isoflavone.

I-10
Production of Ginseng Berry makgeolli and Analysis of its Physicochemical Properties

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Ginseng (the roots of Panax ginseng C.A. Meyer) is frequently taken orally as a traditional herbal medicine in Asian countries, and its use is increasing worldwide. Among the ginseng, ginseng berry can be harvested from 3 or 4-year-old ginseng plant. Ginseng berry contains high amount of ginsenoside Re. When the seed is obtained, ginseng berry flesh has been discarded. Recently, some ginseng berry flesh has been used in the related field of cosmetics.

The concentration of ginsenoside in ginseng berry extract obtained from *Anseong Ginseng Nurgyup* was 3.6 mg/g. When the ginseng berry makgeolli was manufactured, ginseng berry extract was diluted to 5% of total amount of makgeolli. pH values and total acidity of the ginseng berry makgeolli was pH 3.30±0.03 and 1.28±0.0 %, respectively. Sugar content of the ginseng makgeolli was 8.8 ± 0.0 ‘Brix and ethanol content was 14.00 %. Overall, the ginseng berry mash was reused and the new ginseng product as wine is introduced in the market.

Keywords: ginseng, ginseng berry extract, makgeolli.

I-11
Ergothioneine Contents of Shiitake (*Lentinula edodes*) Fruiting Bodies on Sawdust Media with Different Nitrogen Sources

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Ergothioneine is a naturally occurring amino acid analogue and has antioxidant and anti-inflammatory properties. The objective was to investigate the effects of sawdust media with different carbon sources on ergothioneine contents of *Shiitake* (*Lentinula edodes*). To assess the effect of different nitrogen sources on ergothioneine contents, the fungus was grown in sawdust media with four different nitrogen sources. Different nitrogen sources including ammonium nitrate, sodium nitrate, ammonium sulfate and histidine at the concentrations of 0, 0.1, 0.2, 0.3% were used. The contents of ergothioneine in *Shiitake* fruiting bodies were quantified by high performance liquid chromatography (HPLC). As a result, ergothioneine was detected in all samples. The fruit bodies cultivated with 0.2% ammonium sulfate showed the highest ergothioneine content, which was 1.7-fold higher than the control. Our results demonstrate that the cultivation of *Shiitake* mushroom in sawdust medium with suitable ammonium sulfate concentration was effective to enhance ergothioneine contents. There was no significant effect on the enhancement of ergothioneine content in sawdust medium with sodium nitrate and histidine group.

Keywords: *Lentinula edodes*, Ergothioneine, nitrogen source, Shiitake, Antioxidant.

I-12
Taxonomic Characterization and Safety of *Nuruk* Molds Used Industrially in Korea

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We examined taxonomic characteristics and safety of eight *Nuruk* molds that are widely used for making soybean paste, soy sauce and alcoholic beverages in Korea. HK1 from Hakyang Fermentation Co., SW101 from Sosun Fermentation Co., CF1001, CF1002, CF1003 from Chungmoo Fermentation Co. and KACC 93210 are Yellow-*Nuruk* molds, and SW201 from Sosun Fermentation Co. and CF1005 from Chungmoo Fermentation Co. are White-*Nuruk* molds. Six strains of Yellow-*Nuruk* molds were identified as *Aspergillus oryzae*. HK1, SW101, CF1001 and CF1003 of Yellow-*Nuruk* molds have middle length of stipes (711-1121 μm). CF 1002 used for soy sauce has shorter stipes (543 μm). KACC 93210 that is isolated from traditional Korean *Muju* has very short stipes (ave. 270 μm), and it showed velvety colonies although the others showed floccose colonies. The strain has different DNA sequences of endA gene from other strains in NCBI GenBank as well as strains used in Korea, suggesting that it is unique from any other strains published.

SW201 and CF1005 of White-*Nuruk* molds were identified as *Aspergillus luchuensis* or *A. luchuensis* var. *Kawachii* that is known as safe, non-toxigenic fungus. The six strains of Yellow-*Nuruk* molds did not produce any mycotoxins such as aflatoxin, cyclopiazonic acid and sterigmatocystin. Therefore, eight *Nuruk* molds that are used for making soy sauce, soybean paste and alcoholic beverages in Korea were proved to be safe in this study.

Keywords: *Aspergillus luchuensis*, *Aspergillus oryzae*, Nuruk molds, Safety, Taxonomic characterization.
Breed of a New Cultivar Lentinula edodes (Shiitake) Strain “Sanmaru 2ho” and its Characteristics
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I-13

A new Lentinula edodes (shiiitake) strain “Sanmaru 2ho” was bred by the method of Mono-mono crossing. Mycelial growth was the highest at 25°C. Mycelial growth rate was 8.4 mm per one day, which was faster than “Sanmaru 1ho” (7.9 mm per one day) which was developed previously. Sanmaru 2ho showed the highest productivity (4.5 kg per 100 kg media) when it was grown in columnar-shaped sawdust media with 100-days incubation period compared with brick- or cylinder-shaped media of 100-days or 120-days incubation period. Sanmaru 2ho has hemispherical fruitbody, yellow-brown colored and diameter of pileus is ca. 63.8 mm, thickness of pileus is ca. 23.2 mm in thickness. Sanmaru 2ho has hemispherical fruitbody, yellow-brown colored and diameter of pileus is ca. 63.8 mm, thickness of pileus is ca. 23.2 mm. Fruit-body production of “Sanmaru 2ho” was most at summer. Optimal temperature of fruit-body formation was 21~29°C and the fruiting show concentrated occurrence.

Keywords: Breed, Fruit body, Lentinula edodes, New strain, Sawdust Cultivation

Biocontrol Activity of Rice-originated Antagonistic Bacterial Strains against Aspergillus flavus, Aspergillus candidus and Aspergillus fumigatus on Stored Rice
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I-15

Control of Aspergillus spp. contaminating rice is important for their economic impact on the grain quality and aflatoxin production. Several control measures have been applied; however, safe alternatives to chemical control are still needed. Therefore, this research aimed at assessing biocontrol activities of selected bacterial strains against the predominant Aspergillus spp. on stored rice. In this study the previously selected three antagonistic bacterial strains (Bacillus aryabhattai KU143, Microbacterium testaceum KU313 and Pseudomonas protegens AS15) were tested against target Aspergillus spp. and compared with a commercial fungicide, Benlate. Treatment with each of these bacteria significantly (P<0.05) reduced the populations of all tested fungi on inoculated unhulled rice. For assessment of the biocontrol modes of action of these bacteria, all tested bacterial strains showed significantly (P<0.05) better colonization than controls; two of these strains significantly (P<0.05) inhibited the all tested fungi growth in dual culture and on plate. Taken together, the selected antagonistic bacterial strains had broad spectrum biocontrol activities against three Aspergillus spp. on stored rice with ability to colonize the surface of rice grains as well as volatiles and antibiotics productions. Thus, the bacterial strains tested in this study might be potential biocontrol agents against rice storage fungi to serve as safer alternatives to the commercial fungicides.

Keywords: Aspergillus flavus, Aspergillus candidus, Aspergillus fumigatus, Biocontrol, Rice

Persistence of Salmonella enterica and Listeria monocytogenes in Indigenous Microbial Community Formed Soil
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I-16

These days, most people have a great interest for a healthy life, and consequently the consumption of high-quality agricultural products with environment-friendly farming is increasing dramatically. However, there is no enough study for eco-friendly agriculture methods using competition between microorganisms. The objective of this study was to investigate the survival of Salmonella enterica and Listeria monocytogenes in indigenous microbial community formed soil (watermelon and chili cultivated soil) under the green-house environmental conditions. Autoclaved soil and non autoclaved soil were inoculated with S. enterica and L. monocytogenes (about 9~10 log CFU g-1). Samples had been collected during 5 weeks depending on the given conditions. Population of S. enterica and L. monocytogenes decreased during this period. In particular, non autoclaved chili cultivated soil samples observed that the population of L. monocytogenes is sharply reduced in 4~5days (from 5 log CFU g-1 to 0.5 log CFU g-1). In autoclaved and non autoclaved soil the survival of S. enterica and L. monocytogenes show significant change about 7~8 log CFU g-1 during 7 days, then after gradually decreased until 35 days. This study will provide useful and practical guidelines to applicators of soil in deciding appropriate handling and time frames for land application for sustainable agriculture.

Keywords: Salmonella enterica, Listeria monocytogenes, food-borne pathogen
Antioxidant Activity of Cultivation Extracts from Lactobacillus spp on the Modified-MRS Induced by Trichoderma harzianum β-mannanase

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This study was performed to study on purification and structural analysis of hydrolysates of glucomannan by Trichoderma harzianum. β-Mannanase and find out the effect of metabolism activity of Lactobacillus spp (L. casei, L. plantarum, L. reuteri) by hydrolysates. Trichoderma harzianum β-mannanase was purified by sephadex G-100 chromatography. The final preparation thus obtained showed a single band on SDS-PAGE(SDS-poly acrylamide gel electrophoresis). The molecular weight was determined to be 52.5kDa by SDS-PAGE. To investigate of the effects of glucomanno-oligosaccharides on the in vitro growth of Lactobacillus spp were cultivated individually on the modified-MRS medium containing carbon source, such as D.P 2, 3 glucomanno-oligosaccharides hydrolysates by Trichoderma harzianum. Several studies reported the antioxidant activity of Lactobacillus using assays in vitro. The antioxidant activity of Lactobacillus spp was investigated in vitro. Culture supernatant, intact cells, and intracellular cell-free extracts of Lactobacillus spp were involved in this study.

Keywords: Trichoderma harzianum, Lactobacillus spp, glucomannan, β-mannanase

Biological activity of dietary fiber from Cladosiphon novaecaledoniae kylin(Mozuku)
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Cladosiphon novaecaledoniae kylin(Mozuku) belongs to brown seaweeds and has much fucoidan and dietary fiber. In this study, we were extracted of dietary fiber from Mozuku and analysed to bioactivity of dietary fiber. We were extracted to dietary fiber using the acid and sodium salt. As a result, dietary fiber contents of using D.W was higher than dietary fiber extract of using acid and sodium salt. We were analyzed to bioactivity of dietary fiber from Mozuku. For analysis of bioactivity, we were examined to antioxidant and anticancer activity. The result of antioxidant activity, total polyphenol content and free radical scavenging effect of soluble dietary fiber from mozuku was higher than insoluble dietary fiber and fucoidan. We were treated to dietary fiber in HT-29, B16F10 and 3T3-L1 and cultured for 24, 48 h. In the result of antiproliferation in cell line, when dietary fiber and fucoidan from mozuku were treated to cell line, cell viability were decreased to control.

Microbial Contamination Analysis of Commercial Processed Egg Products in Korea
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Food service industry in these days was increased due to expansion of women's economic activity and westernized in diet. This change made food service industry more bigger and it lead increasing needs of processed egg products. Processed egg products were made by separating, drying, freezing, heating from egg. Egg products were easy to decay than other food ingredients because of good for microbial growth owing to high moisture content and rich nutrients environment. Therefore, purpose of this study was to evaluated the contamination level of commercial egg liquid(whole liquid egg, egg yolk, liquid egg white) and egg thermoforming products(fried egg, slice egg). The samples were purchased from a market in Gyeonggi-do between 2012 and 2015. Indicator microbial(aerobic plate counts, coliform) and food poisoning microbial (Staphylococcus aureus, Listeria monocytogenes, Salmonella) were examined based on Korea food standard codes. As a result, whole liquid egg had the highest microbial contamination and liquid egg white was the lowest in egg liquid. And slice egg tend to had highest microbial contamination than fried egg. Because of it had more handling process during slice egg manufacture process than fried egg. Processed egg products were easy to contamination when getting rid of eggshell. Therefore, slice egg that had big portion of maker during making process and whole liquid egg, egg yolk had to be more careful on sterilization, hygiene in order to initial microbial control.

Keywords: Processed Egg Products, Microbial contamination, Egg liquid products, Egg thermoforming products
Antioxidant Activity of Cultivation Extracts from Bifidobacterium spp. on the modified-MRS Medium Induced by Xylogone sphaerospora. β-mannanase

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This study was performed to study on purification and structural analysis of hydrolysates of OGM(Galactosyl glucomannooligosaccharides) by Xylogone sphaerospora. β-mannanase and find out the effect of metabolism activity of Bifidobacterium spp (B. animalis, B. bifidum, B. longum) by hydrolysates. Xylogone sphaerospora. β-mannanase was purified by sephadex G-100 chromatography. The final preparation thus obtained showed a single band on SDS-PAGE(SDS-poly acrylamide gel electrophoresis). The molecular weight was determined to be 42kDa by SDS-PAGE. To investigate of the effects of galactosyl glucomannooligosaccharides on the in vitro growth of Bifidobacterium spp. were cultivated individually on the modified-MRS medium containing carbon source, such as D.P 7, 8, 12, 13 galactosyl glucomannooligosaccharides hydrolysates by Xylogone sphaerospora. The health-promoting Bifidobacterium such as Bifidobacterium animalis and Bifidobacterium bifidum grew more effectively by D.P 7, 8, 12, 13 galactosyl glucomannooligosaccharides hydrolysates by Xylogone sphaerospora. Bifidobacterium longum grew more effectively by whole galactosyl glucomannooligosaccharides hydrolysates by Xylogone sphaerospora. The antioxidative activity of Bifidobacterium spp. was investigated in vitro. Culture supernatant, intact cells, and intracellular cell-free extracts of Bifidobacterium spp. were involved in this study.

Keywords: β-mannanase, GGM, Bifidobacterium spp., Xylogone sphaerospora

Purification of the Bacteriocin Produced by Bacillus tequilensis 10b Isolate

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A bacterial isolate producing bacteriocin was indentified as Bacillus tequilensis 10b by 16S rRNA sequence analysis. The bacteriocin was inactivated by proteolytic enzymes such as α-chymotrypsin, carboxypeptidase A, protease K and subtilisin A. It showed antibacterial activity against Gram-positive bacterial indicators. Antibacterial activity was retained at the pH range from 2.0 to 8.0 but decreased more than at 8.0. The bacteriocin activity was stable up to at 80°C for 40 min but 80% of activity was lost at 100°C for 10 min. The bacteriocin was resistant to solvents such as acetone, methanol, isopropanol up to at 50% concentration but sensitive to butanol. The bacteriocin was purified through ammonium sulfate precipitation, Q-sepharose anion exchange chromatography and reverse-phase high-performance liquid chromatography. The molecular weight was determined to be 3.4 kDa by MALDI-TOF/TOF mass spectrometry. Taken these together, the bacteriocin may be potentially useful for the control of food-spoilage bacteria.

Keywords: Bacteriocin, Bacillus tequilensis 10b, Proteolytic enzymes, RP-HPLC, MALDI-TOF/TOF

Analysis of Microbiota on Ear Shells Collected from Different Regions at Summer and Winter

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Ingestion of ear shells can occur food poisoning, and this food poisoning can be affected microorganisms on ear shell. The analysis of microbiota in ear shells will help to understand food poisoning induced. In this study, we collected ear shells from four different regions at summer and winter, which is known to the maximum production area in Korea, and analyzed their microbiota using high-throughput sequencing. A total of 40 samples were collected from regions, and extracted DNA was amplified with pathogen specific primers. Over 2,700 species were detected in samples, gammaproteobacteria was the predominant group in all samples. The differences of microbiota among regions were analyzed, and differences of microbiota at each season were investigated. Tenericutes is increased in the winter sample, Planctomycetes, Verrucomicrobia increased in the sample of the summer. The microbiota including pathogenic genes, which were amplified by pathogen specific primer, was separated from the microbiota without pathogenic genes in PCoA plot. The potential of food poisoning by pathogenic microbiota will be analyzed by functional gene level in further study. These results can be used to analyze the potential food poisoning ear shells by microbiota information. Acknowledgement This research was supported by a grant (14162MFDS972) from Ministry of Food and Drug Safety in 2015.

Keywords: Food-borne bacteria, Metagenome, Pyrosequencing, 16S rRNA gene, Pathogenic gene
Enhancement of Isoflavone Aglycone Contents and Biological Activities in Different Fermented Soybean Powder Milk with Lactobacillus plantarum P1201

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In this study, we compared the changes of isoflavone-aglycone contents and biological effects, α-glucosidase and pancreatic lipase inhibition activities, soybean powder milk of Korean soybean cultivars, such as Suaedanbaek, Daecon, and Wooram and USA soybean by a potential probiotic Lactobacillus plantarum P1201. In the case of soybean powder milk of Suaedanbaek cultivar, after 72 h of fermentation, the pH decreased from 6.0 of initial fermentation to 3.98, while the acidity, viable cell numbers and protein contents increased to 1.25%, 9.43 log cfu/g and 254.92 mg/g. A one mg/ml of fermented Suaedanbaek from soybean powder milk milk were ABTS (79.77%) and hydroxyl radical scavenging activities (52.26%). In other hand, α-glucosidase (78.72%) and pancreatic lipase inhibition activities (18.51%) results were the highest in fermented Wooram soybean cultivar compared with the other samples examined. Importantly, the total phenolic, flavonoids, and isoflavone-aglycone contents increased, but glycosides and malonylglycosides contents decreased. In particular, the levels of daidzein, glycitein, and genistein were present at concentrations of 584.24 μg/g, 178.34 μg/g, and 370.47 μg/g at the final fermentation day (8 days) of the germinated soybean fermented. From this result, we suggest that the high antioxidant activity of the solid-state fermentation of soybean might soybean fermentation. From this result, we suggest that the high antioxidant activity of the solid-state fermentation of soybean might.

Keywords: Lactobacillus plantarum, Fermented soymilk, Isoflavone, Antioxidant, Enzyme inhibition activity

Change of Phytoestrogen and Biological Activity during Solid-State Fermentation of Soybean by Mycelia of Polypezillus multiplex

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In this study, changes in β-glucosidase activity, total phenolic and isoflavone contents, and biological activities during the solid-state fermentation of soybean (SSB) and germinated soybean (GSB) by Polypezillus multiplex were investigated. The levels of β-glucosidase activity and total phenolic and isoflavone-aglycone contents increased, while antioxidant activity (DPPH, ABTS, and hydroxyl radical scavenging activities and FRAP assays) and α-glucosidase inhibition activity increased, but the isoflavone-aglycone and -malonylglycoside contents decreased after the solid-state fermentation of soybean. In particular, the germinated soybean for 12 days displayed the highest antioxidant activities, compared to those of the soaking soybean and this of soybean fermented. Also, the highest levels of daidzein, glycitein, and genistein were present at concentrations of 584.24 μg/g, 178.34 μg/g, and 370.47 μg/g at the final fermentation day (8 days) of the germinated soybean fermentation. From this result, we suggest that the high antioxidant activity of the solid-state fermentation of soybean might be related to markedly higher levels of total phenolic and isoflavone-aglycone contents achieved during fermentation.

Keywords: Polypezillus multiplex, Germinated soybean, Isoflavone, Biological activity, Antioxidant activity

Antioxidant Activities and Isoflavone Contents in the Produced Cheonggukjang with Different Soybean Cultivars by Bacillus amyloliquefaciens 9-3

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The study was aimed to investigate the changes of total phenolic, flavonoid, and isoflavone contents and biological effects, α-glucosidase and pancreatic lipase inhibition activities during fermentation of soybean (Suaedanbaek, Daecon, Wooram, and USA) fermented Cheonggukjang by Bacillus amyloliquefaciens 9-3. The levels of total phenolic, flavonoids and isoflavone-aglycone contents and DPPH and ABTS radical scavenging activities and FRAP assay results increased, but glycosides contents decreased during Suaedanbaek, Daecon, Wooram, and USA soybean fermentation. Particularly, the Wooram Cheonggukjang showed highest level of total flavonoids and isoflavone contents and DPPH, ABTS and OH radical scavenging activities and FRAP were obtained 0.80 mg/g, 4962.24 μg/g, 88.25%, 95.34%, 64.17% and 0.81%, respectively. Also, α-glucosidase and pancreatic lipase inhibition activities were obtained 87.09% and 29.98%, respectively. In other hands total phenolic content (3.21 mg/g) was the highest in fermented USA soybean cultivar compared with the other samples examined. From this result, we suggest that the high antioxidant activity of Wooram Cheonggukjang might be related to the remarkably higher levels of total flavonoids and isoflavone-aglycone contents achieved during fermentation.

Keywords: Isoflavone, Cheonggukjang, Antioxidant activity, Soybean, Fermentation

Incidence, Antimicrobial Resistance, and Molecular Characteristics of Non-typhoidal Salmonella Including Extended-spectrum β-lactamase Producers in Chicken Carcasses

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The current study was carried out to detect Salmonella spp. contamination on chicken carcasses produced in poultry slaughterhouses in South Korea. To determine the seasonal prevalence, serotypes, and antibiotic resistance patterns of Salmonella, a total of 120 chicken carcasses in six poultry slaughterhouses were analyzed through two individual sampling in summer 2014 and winter 2015. A total of 18 chicken samples (15%) were found contaminated with Salmonella with higher rate during summer (14 isolates, 11.7%) than during winter (4 isolates, 3.3%). Among these isolates, S. enterica serotype Typhimurium was the most prevalent, followed by S. almonella Hadar, S. Bareilly and S. Virchow. All isolates were resistant to at least one antibiotic, of which one remarkable isolate was resistant to ten antibiotics including third-generation cephalosporins. This cephalosporin-resistant strain exhibited the extended-spectrum β-lactamase phenotype and harbored the gene encoding CTX-M-15, the most prevalent ESBL enzyme worldwide. Based on molecular subtyping using an automated rep-PCR system (DiversiLab), all Salmonella isolates except the ESBL-producing strain showed low genetic heterogeneity, with at least 95% similarity in their rep-PCR banding patterns. The ESBL-producing isolate was distinguished by molecular subtyping patterns and distinct antibiotic resistance profiles.

Keywords: Salmonella, chicken carcasses, antimicrobial resistance, molecular characteristics
Enzyme IIA\textsuperscript{Nw} post-translationally Regulates Propionate Metabolism in \textit{Salmonella enterica} Serovar Typhimurium

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Nitrogen-metabolic phosphotransferase system (PTS\textsuperscript{N}) is composed of enzyme I\textsuperscript{N}, NP, and enzyme IIA\textsuperscript{N} (EIA\textsuperscript{N}) encoded by \textit{ptsI}, \textit{ptsO}, and \textit{ptsV}, respectively. EIA\textsuperscript{N} has been reported to have variations of regulations associated with metabolism of carbon and nitrogen, potassium homeostasis, and virulence of several pathogens. In order to understand roles of EIA\textsuperscript{N} further, we analyzed transcriptome of wild-type and a mutant \textit{Salmonella} strain lacking \textit{ptsV} by RNA sequencing. One of the highly down-regulated genes in the \textit{ptsV} mutant was the propionate catabolism operon (prpBCDE). Propionate is one of the intestinal short chain fatty acids and present in high concentrations in the animal intestine. It has been suggested that \textit{Salmonella} uses propionate as an environmental cue as well as a source of carbon and energy. We verified that expression levels of the \textit{prpBCDE} operon decreased more than 3-fold in the \textit{ptsV} mutant compared to wild-type using quantitative Real-time PCR and \textit{β}-galactosidase assay. Moreover, analysis of western blots showed that half-life of PrpR protein in wild-type was three times longer than that of \textit{ptsV} mutant, indicating post-translational regulation of PrpR by \textit{ptsV}. Taken together, these results suggest the possibility that \textit{ptsV} may play important roles in both carbon and nitrogen metabolism. This research was supported by a grant (14162MFDS972) from the Ministry of Food and Drug Safety, Korea in 2015.

Keywords: Nitrogen-related enzyme IIA, propionate, propionate catabolism operon

Survival and their Inactivation Kinetics of \textit{B. cereus} and \textit{E. coli} in Simulated Gastric Fluid

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Foodborne illness associated with pathogens is an important food safety issue in over the world. When foodborne pathogens have been exposed to sub-lethal acid treatment conditions, adapted or resistant strains have developed. Acid resistance causes a food safety problem because it may result in enhanced protection for cells which are subsequently exposed to gastric fluid as well as sanitizers. Gastric fluid is a first barrier about which the objective of the study were to create acid resistance and to analyze the inhibition kinetics of unstressed type cultures and resistant food isolates against simulated gastric fluid (SGF) with various pH for \textit{B. cereus} and \textit{E. coli}. When strains were exposed against SGF (pH2.5) for 1 hour, food isolates showed higher acid resistance, i.e., survival rate of food isolates were higher than type cultures by 19.5% and 25.1% for \textit{B. cereus} and \textit{E. coli}, respectively. The kinetic parameters of the microbial inactivation were estimated using GInaFit tool to find log-linear + tail model can best describe the microbial inactivation in SGF. Parameters \(K_{\text{mic}}\) of the log-linear + tail model for unstressed type strains of \textit{B. cereus} and \textit{E. coli} were up to 30% and 60% higher than for resistant food isolates in SGF with pH 1.5-5.0, respectively. Result on the higher resistance of food isolates than type strains provide useful information in risk assessment of foodborne illness.

Keywords: Bacillus cereus, Escherichia coli, kinetic, simulated gastric fluid, survival

Enzyme Production by Fungal Isolates from the Korean Traditional Starter, Nuruk

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Approximately 140 strains of filamentous fungi and yeasts were isolated from different samples of nuruk, a fermentation starter used in the production of the traditional Korean rice wine, makgeolli. The nuruk samples were collected in 2014 and the strains isolated. The enzyme activities of approximately 130 of these strains were assayed. Glucoamylase, alpha-amylase, protease, and saccharogenic power assays were performed on the samples and high preforming samples were found. The results were typically higher on the wheat koji than on rice for all enzyme assays tested with the mean for alpha-amylase activity 7.66 and 28.82 units/g koji, the mean for glucoamylase activity 132.32 and 162.85 units/g koji, and the mean saccharogenic power 167.08 and 177.2 units/g koji for rice and wheat koji, respectively. Among the strains 31 strains were identified as \textit{Aspergillus oryzae}. Out of the top ten alpha amylase producers 8 were found to be \textit{A. oryzae}. Strains of \textit{A. oryzae} were also found to have high glucoamylase and saccharogenic activity. Among the nuruk samples four strains of \textit{Lichtheimia ramosa} (formally \textit{Absidia}) and two \textit{Syncephalastrum} species had very high protease activity. \textit{Saccaromyces fibuligera} was also a dominant species with 33 isolates among which were some of the top enzyme producers. Based on the high production of enzymes 10 were selected for their possible use in the production of makgeolli.

Keywords: fungi, nuruk, mycology, enzymes, makgeolli

Progress in the Development of a Transgenic Mouse Model Susceptible to Human Norovirus Infection

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Human norovirus (hNoV) is a single-stranded positive-sense RNA virus, belonging to the Calicivirus family. Its genome is \(\sim 7.2\) kb long, encoding 8 structural and non-structural proteins. hNoV infection accounts for millions of cases of acute gastroenteritis, causing more than 200,000 mortalities annually. Human fucosyltransferase 2 (hFUT2) is known to be required for hNoV infection in humans. Since its discovery in 1968, enormous effort has been made to develop in vitro infection models and susceptible small animal models to investigate mechanisms of viral replication and its pathogenesis. Recently, an in vitro infection model has been established which may necessitate further refinement. Here we report a progress in the development of a mouse model which transgenically expresses hFUT2, the known hNoV attachment receptor. The transgenic model is currently being characterized for its susceptibility to hNoV.

Keywords: human norovirus, animal model, transgenic mouse
Changes of Microbial Diversity, Free Amino Acids and Flavor Compounds During Fermentation of *bitter melon* Made of Bitter Melon Ingredient

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The bitter melon- *mulkimchi* made of 30% bitter melon was allowed to fermentation as needed to effect. Based on the 16S rDNA sequence analyses, total 6 different bacterial genuses (Lactococcus, Weisella, Leucobacter, Paenibacillus, Comamonas, Halosporilus) were identified in the bitter melon- *mulkimchi* samples those were fermented at 0, 1, 3, 6, 9 and 12 days. In fact, the nonessential amino acid urea (160.9 mg/100 g) and arginine (233.5 mg/100 g) was the highest of the detected amino acids at 12 days of fermentation. The remaining amino acids especially alanine, proline, aspartic, serine and glutamic acid were shown significant differences in the middle stages of fermentation, but observed highest at the final stages as 54.4, 24.5, 64.7, 33.7, 57.9 mg/100 g, respectively. However, all content of essential amino acids are enhanced in accordance with the fermentation process. During the fermentation of bitter melon- *mulkimchi*, volatile ingredients in a total of 53 species were detected. The content of the main flavor component is gradually increased with the longer fermentation time. The bitter melon- *mulkimchi* made of 30% bitter melon enriched with microbial diversity, flavor ingredients and free amino acids which can be used as a basis to a variety of functional foods.

Keywords: Bitter melon

Antimicrobial Activities of Coagulase-negative Staphylococci Against *Staphylococcus aureus*

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Antibiotic resistant *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA) is difficult to manage therapeutically. Because of these facts, new strategies for controlling multi-drug resistant staphylococci are required. Bacteriocins offer the further prospect of application for the improvement of food preservation because they are generally recognized as “natural” compounds able to influence the safety and quality of foods. We have screened the bacteriocin production of 259 gram-positive bacteria isolated from hospitals, animals and foods to find new alternative agent. Ten multi-drug resistant *S. aureus* strains were selected as indicators for agar diffusion assay. Sixty-six of 259 isolates had antibacterial activities. The isolates having antibacterial activities were identified using selective media and 16S rDNA sequences analysis. Among 66 isolates, CNS such as *saprophyticus, gallinarum, pasteurii, hominis* and *warrari* showed strong antibacterial activities against *S. aureus*. Our results showed that *S. saprophyticus* isolated from bean sprout had broad spectrum to multi-drug resistant *S. aureus* and gram positive bacteria.

Keywords: bacteriocin, MRSA, foodborne pathogens, food safety, *Staphylococcus aureus*

Fermentation Condition of Black Garlic Vinegar with an Addition of Apple Extract

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This study was performed to investigate the quality characteristics and antioxidant activity of black garlic vinegar supplemented with or not-supplemented with apple extract during two-step fermentation. The characteristic of vinegar including total acidity, pH, ethanol, total sugar, brix, polyphenol and total flavonoid were evaluated. Antioxidant activities of black garlic vinegar were then examined by DPPH radical scavenging activity and reducing power. During ethanol fermentation, brix, sugar, brix, polyphenol and total flavonoid were measured. Antioxidant activity of black garlic vinegar supplemented with or not-supplemented with apple extract during two-step fermentation. The antioxidant activity of black garlic vinegar supplemented with or not-supplemented with apple extract was observed to be 55.54 % after 13 day of fermentation. Therefore, this studies suggested the usability of using apple extract as the supplementation for black garlic vinegar fermentation.

Keywords: Black garlic, Vineger, Apple
I. Food Microbiology

Development of an In-frame Reporter System Measuring MARTX<sub>Vv</sub> Production in Vibrio vulnificus

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Almost current antibiotics focus on inhibiting enzymatic activities of essential protein targets, therefore, preventing bacterial growth. This is critical in this time of increasing antibiotic resistance. Thus, it is necessary to create a new paradigm for antibiotics targeting production of virulence factor without inhibition of bacterial growth. In this study, we constructed a novel system that can measure the <i>Vibrio vulnificus</i> growth as well as the production of Multifunctional Autoprocessing RTX toxin (MARTX<sub>Vv</sub>) that is one of the most important virulence factors. Polymerase chain reaction (PCR) was used for amplifying β-lactamase (promoterless bla, reporter) gene and N/C- terminal repeat regions of rtxA1 encoding MARTX<sub>Vv</sub>. An in-frame insertion of bla was carried out between N-terminal and C-terminal region of rtxA1, and those fragments were subcloned with suicide vector to remove effector domain in wild-type rtxA1. Escherichia coli SM10 λ pir was used as a conjugal donor to generate the bla-rtxA1 transcription fusion of <i>V. vulnificus</i> by homologous recombination and <i>V. vulnificus</i> reporter strain was used for cell-based Bla-nitrocefin (substrate of Bla) assay to quantify secreted MARTX<sub>Vv</sub> contained in bacterial supernatant. Therefore, it is suggested that this in-frame reporter system is useful for high throughput screening (HTS) to identify anti-Vibrio agent that inhibit the production of MARTX<sub>Vv</sub> without any growth defects.

Keywords: Antibiotic resistance, <i>Vibrio vulnificus</i>, Exotoxin
Characterization of Salmonella spp. Isolated in Patients with Acute Gastroenteritis in North Gyeonggi-do, Korea
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In this study, we researched the mechanism of antibiotic resistant in Salmonella spp. from diarrheal patients and outbreak in north Gyeonggi-do from 2011 to 2014. We studied serotypes, antimicrobial susceptibility, mechanism of antibiotic resistance such as ESBL, QRDR mutation and PMQR from 113 isolates. Genetic analysis of Salmonella was carried out with PFGE by Xba-I. From 113 isolates, 26 serotypes were identified, with the most common being S. Thompson, S. Typhimurium, S. Enteritidis. Antimicrobial resistance showed most often to Ampicillin, Nalidixic acid and Tetracycline. Also, multidrug-resistant isolates were 32.74%. PFGE showed 47 patterns of clusters with 90% similarity. As a result of PCR, 7 isolates had ESBL CTX-M-15 type gene and 23 isolates had TEM-1 type gene. Sequencing of gyrB gene showed mutation of amino acid. Amino acid Serine to Phenylalanine at position 83(Ser83/g314Phe) in 2 isolates. Asp87/g314Gly or Tyr or Asn in 22 isolates, Ser in 1 isolates. Both gyrB and parC gene were mutated in 1 strain which was resistant to Ciprofloxacin. The result of multiplex PCR showed that gyrS1, gyrR2 were detected in 5 strains which were resistant to Nalidixic-acid. In conclusion, In this study, we showed multidrug-resistant, ESBL, QRDR mutation, PMQR in Salmonella spp.

Therefore, It need to effective use of antibiotics toherapy and continuous monitoring of the antibiotic resistance in north Gyeonggi-do

Keywords: Salmonella, Antibiotic resistance, ESBL, QRDR, PMQR

Set1, the Methyltransferase for H3-K4, is a Negative Regulator of Morphogenesis in Candida albicans
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The Set1 is a sole histone methyltransferase responsible for catalyzing methylation of histone H3 at lysine 4 (H3K4) in Candida albicans. C. albicans is the most common fungal pathogen in human and its ability to grow as a filamentous form is important for pathogenesis of C. albicans. Here, we observed that a deletion of the SET1 gene causes more rapid growth for hyphae under the microscope. To study the molecular mechanism of accelerated hyphal formation in Set1Δ strain, we performed the RNA-sequencing and ChIP-sequencing of wild-type (WT) and Set1 Δ strain in normal condition or hyphal induced condition. In Set1 Δ strain, the expression levels of hypha-induced genes were more increased than those of WT and the levels of hypha-repressed genes were lower than those of WT. Furthermore, the overall gene-expression patterns of Set1 Δ under non-hypha inducing condition are more similar with the expression patterns of WT under the hypha inducing condition rather than those of WT under non-hypha inducing condition. Suprisingly, we found that the H3K4me3 of Set1 Δ-induced genes are not detected in any conditions. These results show that the C. albicans Set1 regulates morphological transition negatively independent with H3K4me3. [This work is supported by NFR-2013R1A1A1008065 and NRF-2014H1A2A1021300]

Keywords: Candida albicans, Set1, H3K4me3, Morphogenesis, Histone modification

Partial Purification and Analysis of Antimicrobial Fatty Acids from Allium hookeri Root
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In this study, we partially purified the ethyl acetate fraction of the root of Allium hookeri. A fractionation process guided by agar diffusion assays with infectious bacteria was carried out to purify and identify the active compounds. After solvent extraction and several chromatographic steps, six antimicrobial fractions were isolated. From Fr-1 and Fr-6, we identified nine compounds that have antimicrobial activity using GC-MS. We evaluated the antimicrobial susceptibility and the MIC (minimum inhibitory concentration) against multidrug-resistant bacteria. This study is a significant contribution to the knowledge of unique compounds from the Allium hookeri root as potential sources for the medical and food industry.

Keywords: Allium hookeri root, Antimicrobial fatty acid, GC-MS

Distribution of Pathogenic Filamentous Fungi Resources for Diagnostic Test and Research
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During the last twenty years, the incidence of fungal infections has increased worldwide along with the increasing population of high risk patients such as immunocompromised patients. Thus Filamentous fungi resources are very important for research of fungal infection. NCCP make an effort to various pathogenic fungi resources including filamentous fungi. 116 filamentous strains of 66 species were collected from Konyang University and Choonnam National University in 2009-2012. NCCP had assured the quality of resources using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry(MALDI-TOF MS) and ITS sequencing. Analysis of data was performed through the integrated MALDI Biotyper software and the web-site(www.boldsystem.org). Also strains have confirmed phenotypic characteristics such as colony morphology, Lactophenol cotton blue(LPCB) stain for cell wall and structures. As a result, 15 Strain of 14 species have been identified (Arthroderma incarnatum, Aspergillus clavatus, A. fumigatus, A. niger, Cunninghamamella bertholtiae, Epidermophyton floccosum, Fusarium dimerum, F. oxysporum, F. solani, Microsporum canis, Penicillium oxalicum, Rhizopus oryzae, Trichophyton interdigitale, and T. rubrum). These pathogenic filamentous fungi resources are opened to the public through the web-site (http://www.cdc.go.kr). It would be expected to be useful resources for diagnostic test and researches.

Keywords: Filamentous fungi, Identification
Expression of Enterovirus71 Virus-like Particles as Vaccine Candidate

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Enterovirus (EV) 71 is the main pathogen associated with hand-foot-mouth disease (HFMD) and can lead to the disease with severe mortality in children. Since 2009, in the Republic of Korea, an outbreak of EV71 C4a infection with neurologic involvement emerged, where in HFMD involvement was identified and central nervous system complications were reported. Furthermore, phase 1 to 3 clinical trials incorporating an EV71 vaccine have been conducted in Asia; however, no approved effective antiviral drugs or vaccines are currently available. To produce vaccine of the virus-like particles (VLPs) platform, we constructed recombinant baculoviruses to co-express EV71 P1 structural protein and 3CD protease using the BacHTS system. The recombinant baculoviruses resulted in P1 cleavage by 3CD and subsequent VLP assembly in infected cells. The production levels were compared between P1 only, P1 and 3CD ratios of 5:1, and P1-3CD as indicated by the strongest band intensity by Western blot. Formation of the VLP capsid could be observed by transmission electron microscopy. These particles measured approximately 30nm in diameter and appeared in icosahedra form. Our data demonstrate that EV71 virus-like particles (VLPs) can be successfully done in small space by using the suggested LOD system.

Keywords: Enterovirus 71, Virus-like particles, Baculovirus

First Isolation of Taylorella equigenitalis from Horses in Republic of Korea

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Contagious equine metritis (CEM) is a highly contagious venereal disease of the equid species caused by the bacterium Taylorella equigenitalis. CEM and T. equigenitalis have been reported from at least 30 countries of different continents including only Japan in Asia, but they have not been reported in Korea which has been considered to be free from CEM historically. In this study, 5 strains of T. equigenitalis were isolated firstly in Korea horses. The isolated bacterium was Gram-negative stained and small coccoid rod-shaped which was positive in the oxidase, catalase and phosphatase tests. Sequencing of the 16S rDNA gene and phylogenetic analysis confirmed that the 5 isolates were T. equigenitalis by showing the high sequence identity (99-100%) with other T. equigenitalis strains. This study describes the first isolation of T. equigenitalis in thoroughbred horses raised in South Korea and the characterization of the T. equigenitalis isolate.

Keywords: Taylorella equigenitalis, contagious equine metritis, horses

Fluid Mixing Control on Lab-on-a-Disc Using Coriolis Force

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Lab-on-a-Disc (LOD) has the advantage of being able to control the fluid, such as blood and reagent, for various instrument in the laboratory using only centrifugal force which integrated into the Compact Disc (CD). Since the control of the fluid is only regulated by the centrifugal force, total experimental process becomes simpler and faster in LOD. In terms of fluid control on LOD, mixing process is important part. Most of the mixing methods have some problems because it requires sufficient volume. In this study, we purposed to make new mixing system which designed by only one micro-channel. This system is a multi-lamination structure, which means that fluid is mixed by split, flip, and recombination processes. These mixing processes are explained by 'Coriolis pseudo force'. This force is deflection of moving objects in a rotating reference frame. We performed experiments to mix inks of two colors. Both came from the loading-chamber, and LOD was designed to make inks go through to the result-chamber by passing the micro-channel. As a result, combined solution inks are shown in the result-chamber. The outcome of this experiment shows that micro-mixing is possible on LOD only using channel which integrated to lead Coriolis pseudo force of the liquid flow. It is expected that the automation of mixing various reagents for microorganism experiments and controlling of small amount of liquid can be successfully done in small space by using the suggested LOD system.

Keywords: Lab-on-a-Disc (LOD), Mixing, Coriolis force, Multi-lamination, Micro-channel

Existence of Diverse dsRNA Mycoviruses in Trichoderma spp. Causing Green Mold Disease of Shiitake Lentinula edodes

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A total of 315 fungal isolates causing green mold disease were collected from contaminated artificial logs and sawdust bags used for cultivating shiitake mushroom Lentinula edodes in Korea and were analyzed for the presence of dsRNA, dsRNA, which was purified using dsRNA-specific chromatography and verified by dsRNA-specific RNAaseII digestion, was detected in 32 isolates. The molecular taxonomy of dsRNA-infected isolates based on the ITS region of the rDNA indicated that all isolates belonged to the Trichoderma spp. The number and size of dsRNAs varied among isolates and the band patterns could be categorized into 15 groups. The most common dsRNA group, group VI, occurred in 10 isolates encompassing three species of Trichoderma. Partial cDNA clones were obtained from two selected dsRNA groups and the sequence comparison of the cloned fragments of these dsRNAs revealed a high degree of similarity to sequences of a hypothetical protein and polypeptide genes of other mycoviruses, indicating the existence of mycoviruses in Trichoderma spp. Northern blot analysis using cloned cDNA showed specific hybridizing patterns in the dsRNA bands for isolates from which the clones were obtained, suggesting that many different mycoviruses, which have not been identified yet, exist in Trichoderma spp.

Keywords: Trichoderma, mycovirus, Lentinula edodes, Shiitake
**J-9**

Opuntia ficus-indica var. Saboten Mediates the Antidiabetic Activity in Pancreatic β cell

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The prickly pear cactus (Opuntia ficus-indica) has a global distribution and have been used for medicinal benefits such as arthritis, diabetes, gastritis, and hyperglycemia. However, very little information is currently available for their mechanism. The prickly pear variety Opuntia ficus-indica var. Saboten (OFS) is widely cultivated in Cheju Island, southwestern region of Korea, and used as a functional food. Present study investigated the effects of OFS on pancreatic β-cell function using pancreatic islet β cells (HIT cell). Alpha-glucosidase inhibition, glucose uptake, insulin secretion, insulin sensitivity, and pancreatic β cell proliferation were determined. The inhibitory effect of ethanol extract of OFS stem on α-glucosidase enzyme was measured in a cell free system. Glucose uptake was determined using fluorescent glucose analogue, 2-NBDG. Insulin secretion was measured by ELISA assay. Cell proliferation was measured by MTT assay. Ethanol extracts of OFS dose-dependently inhibited α-glucosidase activity as well as glucose uptake. insulinitropic effect of OFS extract was observed at high glucose media in pancreatic β-islet cells. Furthermore, pancreatic β cell regeneration was also observed. These results suggest that OFS mediates the antidiabetic activity mainly via α-glucosidase inhibition, glucose uptake, and improved insulin sensitivity.

Keywords: Prickly pear cactus, Pancreatic β cell, Insulinotropic effect

**J-10**

A New Record of Xylogone sphaerospora from Crop Field Soil in Korea

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Soil fungal diversity study was undertaken to assess the diversity of fungi in the crop field soil from Kangwon-do, Korea in 2015. Total five hundreds samples were collected and various fungi were isolated to study the fungal diversity from Kangwon-do, Korea. Fungi was isolated through the serial dilution technique, then purified and differentiated by morphological and microscopic characteristics. Genomic DNA of the isolates was extracted by using QIAGEN® Plasmid Mini Kit (QIAGEN Sciences, USA) and the identification of fungi was carried out by sequence analysis of internal transcribed spacer (ITS) region of the 18S ribosomal DNA (18S rDNA). According to the results, among the identified fungal species, Xylogone sphaerospora was found new in Korea which was not officially reported.

Keywords: Diversity, Morphology, Xylogone sphaerospora

**J-11**

Surface Display of Cyclodextrin Glycosyltransferase (CGTase) in Pichia pastoris using Glycosylphosphatidylinositol (GPI)-Anchored Protein as an Anchoring Motif

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Cyclodextrin glycosyltransferase (CGTase) is a member of the α-amylase family known as glycosyl-hydrolases. CGTase catalyzes the transglycosylation reaction which transfers maltooligosaccharide to an acceptor substrate such as ascorbic acid. Through the reaction of CGTase, ascorbic acid 2-glucoside (AA2G) can be synthesized. AA2G is a stabilized form of ascorbic acid with glucose. In this study, CGTase was displayed on the surface of Pichia pastoris SMD1168 using glycosylphosphatidylinositol (GPI)-anchored protein of Saccharomyces cerevisiae. In order to synthesize AA2G from ascorbic acid, the surface displayed P. pastoris was used as whole cell catalyst. For the production of AA2G, maltodextrin and ascorbic acid were mixed with CGTase-displaying P. pastoris and incubated at 37° for 48 hours followed by incubation at 37° for further 3 hours with glucose. The reaction mixture was analyzed by HPLC. As a result, 10 g/L of AA2G was produced. Furthermore, it could be successfully recycled at least 3 times. These results suggested that CGTase-displaying P. pastoris could be worth using in industrial application as whole cell catalyst.

Keywords: CGTase, GPI-anchored protein, Pichia pastoris, Surface display, Ascorbic acid 2-glucoside

**J-12**

Opuntia ficus-indica var. Saboten Enhances Glucose Uptake and Balances Adipogenesis and Lipolysis Properties

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The prickly pear variety Opuntia ficus-indica var. Saboten (OFS) is widely cultivated in Cheju Island, southwestern region of Korea, and used as a functional food. Present study investigated the effects of OFS on adipogenesis, lipolysis, glucose uptake, and glucose transporter (GLUT4) expression using preadipocyte 3T3-L1 cells. Adipogenesis was determined by preadipocyte differentiation and triglyceride accumulation assessed by Oil Red O staining. Lipolysis was determined as the rate of glycerol release. Insulin stimulated glucose uptake and GLUT4 expression were measured using fluorescent glucose analogue, 2-NBDG. and ELISA, respectively. Quantitative real-time RT-PCR was performed to investigate the effects of OFS on mRNA expression of peroxisome proliferator-activated receptor γ (PPARY), a regulator of adipocyte differentiation. Ethanol extracts of OFS dose-dependently enhanced adipocyte differentiation and cellular triglyceride levels indicating the enhancement of the differentiation of preadipocytes into adipocytes. Insulin stimulated glucose uptake and GLUT4 expression were also dose-dependently increased by OFS treatment. Furthermore, OFS treatment also increased the mRNA levels of PPARY. These effects of OFS on adipocytes suggest that OFS is potentially beneficial for type 2 diabetes by due to its enhanced glucose uptake and balanced adipogenesis and lipolysis properties.

Keywords: Opuntia ficus-indica, Adipogenesis, Lipolysis, Glucose uptake, Glucose transporter
The Korea National Microbiological Research Resource Center is the core center of the twelve microorganism banks designated by the Ministry of Education, Science and Technology. The KNMRRRC supports microorganism banks with necessary guidelines, standards, training for efficient operation of the banks. It also provides with an effective forum to solve common issues of the related banks. The ultimate goal of the KNMRRRC is the followings: 1construction of standardized and integrated management system, 2construction of Core center and other organs network, 3 Quality Control(QC) of microbial resources in the member banks, 4conservation of Resources in the member banks and the interrupted banks, Sedation for professionals in the member banks, 6Public Relations for raising people's awareness of the importance of microbiological resources.

Keywords: Bacteria, Virus, Fungi, cDNA library and clone

J-14

Investigation of Distribution Characteristics of Indoor Airborne Bacteria in Greenhouse for Oyster Mushroom Cultivation

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This study was carried out to investigate of the levels of airborne bacteria in green house according to mushroom cultivation stage. We collected samples from oyster mushroom farm located in Jinju, Gyeongnam Province. The temperature and humidity in indoor of the greenhouse was 18.25±0.25°C and 86±2.34%, respectively. The Listeria sp. and Bacillus cereus were identified from indoor air of the greenhouse. Mean concentration of Listeria sp. in indoor of fermentation stage was 0.2×10 cfu/hr in spring and 0.8×10 cfu/hr in summer respectively, Mean concentration of Bacillus cereus in indoor of fermentation stage was 0.1×10 cfu/hr in spring and 0.2×10 cfu/hr in summer respectively, Mean concentration of Listeria sp. in indoor of growth stage was 0.3×10 cfu/hr in spring and 0.7×10 cfu/hr in summer respectively, Mean concentration of Bacillus cereus in indoor of growth stage was 0.6×10 cfu/hr in spring and 10×10 cfu/hr in summer respectively.

Keywords: Airborne bacteria, Greenhouse, Pleurotus ostreatus

J-15

Serological Surveillance of AHS, EIA and EVA in Korea in 2014

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AHS is an infectious but noncontagious viral disease affecting all species of equid. AHS is endemic in sub-Saharan Africa, although occasional outbreaks have occurred in northern Africa, the Middle East, and in Europe. AHS occurs world-wide and is characterized by recurrent febrile episodes, thrombocytopenia, anaemia, rapid loss of weight and oedema of the lower parts of the body. EVA is a contagious viral disease of equids caused by an RNA virus classified in the family Arteriviridae, and is found in horse populations o many countries worldwide. Korea is considered to be free from AHS, EIA, and EVA, and therefore surveillance is required to be able to quickly identify any new incursions of these diseases into the horse population. In this study, we tested a total of 1,530 horse sera, which were collected in 2014 through the support of the KRA. All sera were tested by Ingerim AHISV compact plus (INGENASA), EIA virus antibody test kit and EAV antibody test kit (VMRD). Sera that were positive for EVA by ELISA tests were confirmed by the VN test, which is the gold standard test recommended by the OIE. In this study, all samples were negative for AHS and EIA by ELISA tests and 16 samples were positive by ELISA for EVA. The positive samples were tested by VN, and 10 out of 16 horse sera were determined to be positive, which were all confirmed to have a history of vaccination against EVA. The serological surveillance supports the view that Korea is free from AHS, EIA, and EVA.

Keywords: African Horse Sickness, Equine Infectious Anaemia, Equine Viral Arteritis, ELISA test, virus neutralisation test

J-16

The Effects of Laminarin, a Polysaccharide from Seaweed, on Cecal and Fecal Microbiota of High Fat-fed Mice

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Seaweeds are one of the major food ingredients in Asian countries, especially South Korea and Japan. In this study, the effects of laminarin on weight gain, cecal and fecal microbiota of high fat-fed mice were investigated. Mice experiment was conducted to investigate effects of laminarin and designed in three groups: i) mice were fed with common non-high fat diet as a control (CTL); ii) 45% Kcal high fat-diet (HFD); and iii) 45% Kcal high fat-diet supplemented with 1% laminarin (HFL). Feeding was conducted for 4 weeks and additional two weeks without laminarin to investigate post effects of laminarin. Cecal and fecal microbiota were analyzed through sequencing of V4 region of the 16S rRNA gene by Illumina’s MiSeq. While most of the taxonomiy distribution were similar among samples, the phylum Verrucomicrobia species were more abundant in cecum and feces of HFD groups. On the other hand, operational taxonomic units (OTUs) distribution analysis indicated significant difference between CTL group from HFD and HFL groups, suggesting high-fat diet significantly changes gut microbiota. Non-metric multidimensional scaling (NMDS) showed that fecal microbiota of HFL was different from HFD during the laminarin feeding period. In contrast, HFL fecal microbiota shifted toward that of HFD during the two weeks of post-feeding period. In conclusion, our results suggested that laminarin significantly shifts gut microbiota of diet-induced obese mice.

Keywords: Laminarin, MiSeq, 16S rRNA gene, Cecal microbiota, Fecal microbiota
The purpose of this study was to evaluate effect of the cultivar and milling degree on quality characteristics of barley Makgeolli. Dry lees contents of 92.3 g, 69.4 g and 63.8 g were contained in Saessal-bori Groups (Si-4, Si-12 and Si-18), respectively, while 62.3 g, 42.2 g and 32.2 g in Hainchalsal-bori Groups (Hcs-6, Hcs-14 and Hcs-20). There were significant differences between milling degrees and cultivars (P<0.05). There were decreases in pH, total acidity and amino acidity according to the increase of the milling degree. There were no differences in total sugar content and alcohol content in Makgeolli according to the milling degree of barley, but there were differences between cultivars, and barley Makgeolli had 10.7~11.8 °Brix, 14.07~15.07%, which were significantly lower than 12.0~12.2 °Brix, 17.27~17.77% in rice Makgeolli (P<0.05). Lactic acid bacteria was respectively 7.21, 6.99 and 6.67 logCFU/mL in Si-4, Si-12 and Si-18, and 6.14, 5.39 and 5.65 logCFU/mL in Hcs-6, Hcs-14 and Hcs-20, which showed significant decreases according to the increase of the milling degree (P<0.05).

Keywords: barley, cultivar, milling degree, Makgeolli

Effect of Different Cultivars and Milling Degrees on Quality Characteristics of Barley Makgeolli
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First Report of Aspergillus awamori as a Fungal Pathogen of Garlic (Allium sativum)
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1College of Life Sciences and Biotechnology, Korea University, 2College of Life Sciences and Biotechnology, Korea University

Garlic is one of the most important produces for cuisines worldwide, especially in Korea. We isolated several black Aspergilli from black powdery spores on garlic (Allium sativum) bulbs. Among these isolates, we identified a representative isolate GL-125 comparing with a reference isolate Aspergillus awamori NRRL 4948 through morphological identification using different media as well as molecular identification using ITS, β-tubulin, and calmodulin genes. As a result, isolate GL-125 showed similar morphological characteristics with the reference isolate NRRL 4948 and those described in the literature. At some point (reverse color of CYA plate and spore roughness), isolate GL-125 showed a few differences with reference isolate and those described in the literature. However, molecular identification showed isolate GL-125 being constantly grouped with A. awamori. Therefore, we identified isolate GL-125 as A. awamori. In addition, we conducted pathogenicity test for isolate GL-125 against garlic and onion using two inoculation methods: (i) agar block inoculation onto the holes made by a cork borer and (ii) pin-prick inoculation onto the wounds with the spore suspension. Isolate GL-125 caused diseases on both garlic and onion either agar block or pin-prick inoculation. To the best of our knowledge, this is the first report of A. awamori as the fungal pathogen of garlic.

Keywords: Aspergillus awamori, Allium sativum, Fungal identification

A Newly Isolated Bacteriophage PBES 02 Infecting Cronobacter sakazaki
Hyung Ju Lee1, Wan Il Kim1, Young Chan Kwon1, and Hoejoon Myung2*
1Hana High School, 2Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

A novel bacteriophage infecting Cronobacter sakazaki was isolated and characterized. It has a spherical head of 90 nm in diameter and a tail of 140 nm in length, belonging to myoviridae as observed under transmission electron microscope. The major virion protein appeared 30 KDa in size. Its burst size was 75. Infectivity remained intact after exposure to temperatures from 4 to 55°C for one hour. It was also stable after exposure to pHs from 6 to 10 for one hour. It has a double stranded DNA genome of 149,732 bases. Its GC ratio was 50.7%. Sequence analysis revealed it had 299 open reading frames (ORFs) and 14 tRNA genes. 39 ORFs were annotated including 24 related to regulatory and replication, 10 related to structural proteins, and 5 related to DNA packaging. The genome of PBES02 was closely related to other C. sakazaki phages, CR3 and CR8.

Keywords: bacteriophage, Cronobacter sakazaki, genomic analysis

Loop-mediated Isothermal Amplification (LAMP) Assays for Rapidly Detection of Pea Enation Mosaic Virus Associated with Leguminous Crop
Jin-Young Lee1, Jin Ho Kim2, Ji-Yeon Kim3, Byeong-Hee Kim3, Siwon Lee3, and Jae-Young Rho3*
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Pea enation mosaic virus (PEMV) is a plant pathogenic virus that has spherical and enveloped morphology at about 28nm in diameter. PEMV can infect legume plants, broad bean, sweet pea, and alfalfa by seeds, aphid, and sap-transmission. Currently, diagnostic methods for the detection of PEMV is reverse transcription-polymerase chain reaction (RT-PCR) and nested PCR. However, these methods require over ten hours, which is inconvenient for field use. Therefore, loop-mediated isothermal amplification (LAMP) assay as a simple, rapid, and high detection of PEMV is reverse transcription-polymerase chain reaction (RT-PCR) and nested PCR. However, these methods require over ten hours, which is inconvenient for field use. Therefore, loop-mediated isothermal amplification (LAMP) assay as a simple, rapid, and high

Keywords: LAMP, PEMV, Pea enation mosaic virus

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Hyung Ju Lee1, Wan Il Kim1, Young Chan Kwon1, and Hoejoon Myung2*
1Hana High School, 2Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

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Keywords: bacteriophage, Cronobacter sakazaki, genomic analysis
Monokaryotic strains of _Lentinula edodes_ were isolated and characterized in terms of fruiting rate and yield through mating and cultivation of the mated mycelia in sawdust media. To accomplish this, monokaryotic strains of _L. edodes_ were isolated from basidiospores of the commercial dikaryotic strains, Chamaram (Cham) and Sanjo701 (SJ701). A total of 703 matings (538 self-matings and 165 outcrosses) were performed, which generated 133 self-mates and 84 outcross mates. The mating rate was 25% and 50% for self-mating and outcross, respectively. The bipolarity of the outcross indicated the multi-allelic nature of the mating type genes. The mating was only dependent on the A mating type locus, while the B locus showed no effect, implying that the B locus is multi-allelic. Next, 145 selected dikaryotic mates were cultivated in sawdust medium. The self-mated dikaryotic progenies showed 51.3% and 60.5% fruiting rates for Cham and SJ701, respectively, while the fruiting rate of the outcross mates was 63.2%. The dikaryotic mates generated 69.5% fruiting rates for Cham and SJ701, respectively, while the fruiting rate of the outcross mates was 63.2%. The dikaryotic mates generated by mating with one of the monokaryotic strains, including A20, B2, E1, and E3, showed good fruiting performance and tended to yield high fruiting body production, while many of the monokaryotic strains failed to form fruiting bodies. Overall, these findings suggest that certain monokaryotic strains have traits enabling better mating and fruiting. Keywords:
Antagonistic Action of Microbes Against Fusarium spp.

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Microbial Safety Team, National Academy of Agricultural Science

Fusarium diseases frequently have occurred in wheat, barley, corn, or rice throughout the world causing crop loss and mycotoxin contamination of grains. The contamination with Fusarium mycotoxin can be threatening to human and livestock health due to possible accumulation of harmful mycotoxins such as trichotecens in cereals. In order to control mycotoxin contamination of cereals, antagonistic microbes were isolated from cereal-grown soils. The 384 microbial isolates were screened by dual culture assay to check up antifungal activity against eight mycotoxigenic Fusarium species. As a result, 16 microbes were revealed to inhibit mycelial growth of all the Fusarium species tested. At the same time, conidia germination test was conducted using culture-filtrates of 298 microbes against five Fusarium species. A total of 19 culture-filtrates inhibited conidia germination for 27 days and five of them were selected further for in vivo assay on maize. The assay showed that three microbes successfully restricted fungal growth on maize ears. The most effective one was identified as Bacillus amyloliquefaciens based on 16s rRNA sequence homology. We believe that those antagonistic microbes have potential to be used as control agents against mycotoxigenic Fusarium species.

Keywords: Fusarium, Trichotecenes, Mycotoxin, Bacillus amyloliquefaciens, Antagonistic microbes
J-29

The Relationship Between Commensal Microbes and Gut Homeostasis on Lifespan of Drosophila

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Department of Biological Sciences, Inha University

Intestinal microbes are well known to affect host fitness. However, the effects of intestinal microbes on host lifespan are not well characterized. In this study, we investigated the effects of intestinal microbes on host lifespan using Drosophila melanogaster. The lifespan of flies was increased under axenic condition, and it was decreased by the oral ingestion of the extracts of guts from flies. To investigate the specific species of gut microbes affecting host lifespan, flies was subjected to oral ingestion with the single species of gut microbes. Interestingly, major microbes of young flies gut decreased the lifespan, whereas major microbes of old flies gut increased or did not affect the lifespan in axenic flies. We hypothesized dysbiosis of the microbiota leads to systemic influences on aging flies with increased intestinal permeability. We confirmed intestinal dysfuction increased the permeability of microbes in the gut of flies. However, the lifespan of fly exhibiting intestinal barrier dysfunction was not affected by the presence of intestinal microbes. Taken together, our study highlights the importance of intestinal microbes as one of the determinant of host lifespan.

Keywords: Gut microbes, Gut Homeostasis, Aging, Drosophila melanogaster

J-30

Effect of Various Protectants on Survivability During Freeze-drying Process of Neisseria gonorrhoeae and Streptococcus pneumoniae Isolates

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Although long-term storage of bacterial cells is an essential step for all researches in microbiology, preservation of some bacteria such as Streptococcus pneumoniae and Neisseria gonorrhoeae is difficult due to their sensitivity to temperature. Since freeze-drying is known as one of successful methods for long-term storage of bacterial cells, various hypoprotectants have used in laboratories. In this study, the optimal protectant for freeze-drying was investigated. Each three isolates of S. pneumoniae and N. gonorrhoeae were grown on 5% sheep blood agar and chocolate agar plates, respectively. Bacterial cultures were suspended five kinds of protective medium (10% skim milk, 20% skim milk, 10% skim milk+5% inositol, 10% skim milk+5% sucrose, 10% skim milk+5% trehalose). 100ul protective medium was filled into ampoules in 37ºC for two weeks. Survivability of isolates was determined as the optimum lyoprotectant for both S. pneumoniae and N. gonorrhoeae.

Keywords: Matrine, Neem, Insecticidal activity, Bacillus thuringiensis on Spodoptera exigua

J-31

Multiplex PCR Method for Specific Detection of Ralstonia solanacearum Race 3 Biovar 2 Strains

Eun-Sang Song, Jeong-Gu Kim, Heejung Cho, Seunghwan Kim, and Byoung-Moo Lee*
National Academy of Agricultural Science, Rural Development Administration

We describe the development of a multiplex PCR method for a precise and specific detection of the strains of race 3 biovar 2 of Ralstonia solanacearum, which is the causal organism of brown rot of potato. Two primer sets were designed to sequence within R. solanacearum SL2029 strain-specific genes that were identified in comparison to the six sequenced R. solanacearum strains. The sizes of PCR amplicons when using both primer sets (890F/890R and 920F/920R) were 366 bp and 700 bp, respectively. The PCR products were generated only from 16 isolates of R. solanacearum race 3 biovar 2 among 95 isolates of other races of Ralstonia species and Pseudomonas and Xanthomonas species. We believe that this PCR-based assay can be used as a simple and rapid tool for the detection of R. solanacearum race 3 biovar 2 strains.

Keywords: Brown rot, PCR, Potato, Ralstonia solanacearum

J-32

Combination Effects of Organic Materials and Bacillus thuringiensis on Spodoptera exigua

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The beet armyworm, Spodoptera exigua is one of the most difficult moth to control. For eco-friendly beet armyworm managements, we investigated insecticidal activity with organic materials, Bacillus thuringiensis and both. Matrine caused 100% mortality against 3rd instar larvae of beet armyworm with 0.016% solution and medial lethal time(LT50) of its was 2-42days. A mixture of matrine and BT showed different mortality based on BT concentration. In the case of neem, when the mixture of 0.1% neem and BT was applied to larvae of S. exigua, their mortality was 50%. Weight of larvae in a mixture of neem+BT were significantly different compare with control and only neem treatment. Control of beet armyworm larvae with BT and neem or matrine showed complementary effect.

Keywords: Matrine, Neem, Insecticidal activity, Bacillus thuringiensis, Spodoptera exigua
**Marine Fungal Resource Bank**

Jae Young Park, Myung Soo Park, Ji Eun Eom, and Young Woon Lim* Seoul National University

The Marine Fungal Resource Bank (MFRB), overseen by Dr. Young Woon Lim at Seoul National University, was designated as a marine bioresource bank of Korea by the Ministry of Oceans and Fisheries. The main goal of the MFRB is to establish a culture collection of marine fungi for educational, scientific, and industrial purposes. MFRB will undertake the following tasks: 1) Survey marine environments across Korea to catalogue marine fungal diversity, 2) Establish a robust system of polyphasic species identification, 3) Evaluate the usefulness of the discovered fungi, 4) Create a secure preservation and loan system, 5) Provide web-based access to the database. With a global focus on utilizing natural resources, marine fungal resources provide excellent opportunities for educating the public on marine ecosystems, vitalizing marine research, and discovering novel substances for use as medicine and energy. This work was supported by the Marine BioResource Bank Program of the Ministry of Ocean & Fisheries.

Keywords: Marine fungi, Depository body, Biological resources, Genetic resources, Preservation

**First Report of Brown Rot Caused by Cryptococcus pseudolongus on Fruit Body of Lentinula edodes (shiitake) in Korea**

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In 2014 autumn, browning phenomenon appeared on shiitake fruit body's caps. This phenomenon which is considered to be new disease was observed in mushroom farms located at Gwangju and Muju in Korea. We named the disease tentatively as brown rot. Disease incidence was nearly 20% in two mushroom farms where sawdust media were used for cultivation. This work was supported by the Marine BioResource Bank Program of the Ministry of Ocean & Fisheries.

Key words: MARINE FUNGI, DEPOSITORY BODY, BIOLIGICAL RESOURCES, GENETIC RESOURCES, PRESERVATION

**The Role of DesB on Virulence Traits in Pseudomonas aeruginosa**

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1Department of Food and Nutrition, Seokmyung Women, 2Department of Oral Microbiology, Wonsung University

The present study is aimed at investigating whether DesB(an aerobic desaturase) contributes to the pathogenic activities in host cells, such as exotoxin production, hemolysis, cell invasion and intracellular replication. For exotoxin production assay, HeLa cells were exposed to cell-free supernatant of wild type P. aeruginosa (WT) or its derived desB mutant, and exotoxins were indirectly quantified by cell viability assay. For invasion and intracellular replication assays, WT or desB mutant was inoculated in HeLa cells, and the efficiencies of invasion and intracellular replication of WT and desB mutant were compared. Hemolysis assay was performed by spotting overnight cultures of WT and mutants on blood-containing agar plate. In order to determine underlying mechanism of DesB on virulence at the molecular level, the transcriptional profiles of WT and desB mutant were compared by microarray analysis and qRT-PCR. DesB mutant had different efficiency in exotoxin production, invasion and intracellular replication in cells compared to WT. Furthermore, decrease in hemolysis was observed in desB mutant, but pch expression had no difference between WT and desB mutant, indicating that reduced hemolysis in desB mutant is not attributed to pch. The results demonstrate that P. aeruginosa DesB have effect on pathogenesis-related behaviors in host cells, including exotoxin production, hemolysis, cell invasion and intracellular replication.

Keywords: Pseudomonas aeruginosa, DesaturaseB, Pathogenicity

**The Effect of Chemical Disinfectants on Bacillus spp. from Activated Sludge in Slaughterhouse**

Mi An, Yongho Jung, Jeongsoo Kang, Hae Chul Park, Jaeyoung Song, and Kwang Jick Lee*
Animal and Plant Quarantine Agency

There have been notable disease outbreaks such as HPAI (high pathogenic avian influenza) and FMD (foot and mouth disease) in Korea. Secondary spreads of these diseases are achieved through human related activities such as a vehicle. Based on the epidemiological studies, slaughterhouse is blamed for spreading the virus. Therefore it is important that appropriated disinfection process for trucks could prevent diseases from stretching out farm to farm. Disinfection with chemical disinfectants is not allowed in slaughterhouse yet. It is assumed that chemical disinfectants could affect on sludge used for slaughterhouse waste-water purification. This study was aimed to reveal how much the chemical disinfectant effect on microorganisms from activated sludge. The seeds of the sludge used in slaughterhouse were purchased. The bacteria were isolated and then identified as Bacillus spp. General procedures for disinfectant efficacy test were followed by the guideline of disinfectant efficacy evaluation in QIA. Salmonella Typhimurium was inactivated by disinfectant diluted high concentration however there was no effect on Bacillus spp. at the same disinfectant concentration. Therefore chemical disinfectants were not affected on activated sludge owing to inactivation of disinfectants by organic matter in slaughterhouse. When the disinfection process has to be set up, organic materials should be cleaned up by water and then apply the disinfectants.

Keywords: disinfectant, slaughterhouse waste water, sludge
**J-37**  
**Evaluation of the Anti-inflammatory Effect of Ginsenosides from Black Ginseng Extract Against *Propionibacterium acnes***  
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Department of Systems Biotechnology, Chung-Ang University  

This study aimed to investigate the effects of anti-inflammatory activity of black ginseng extract against pathogenic microorganisms, such as *Propionibacterium acnes* KCTC 3314, *Staphylococcus aureus* KCTC 3811, and *Staphylococcus epidermidis* KCTC 1917, on human skin. The beneficial functions of the ginseng are mainly based on the bioactive component such as ginsenosides and polyphenol. Ginsenoside-enriched fractions of each ginseng extract were obtained from column chromatography with C18 silica gel and diaion HP-20 resin. In the non-saponin analysis, total sugar contents were 770.0±84.1% in fresh ginseng extract, 1072.7±237.8% in red ginseng, and 671.5±115.9% in black ginseng. Polyphenolic compound and acidic saccharide contents of black ginseng extract were 37.7±0.7% and 614.0±61.1% respectively. The antibacterial activity with disc diffusion assay were carried out with the crude extracts and column-related fractions using diaion HP-20. As a results, the diaion HP-20-eluted fraction 8 to 10 showed the antibacterial activity against *P. acnes*. It suggested that ginsenosides of black ginseng extract can be effected on skin care against acnes.  

Keywords: Acne, Black ginseng, *Propionibacterium acnes*

**J-38**  
**Biological Control of *Fusarium oxysporum* of Lettuce by *Trichoderma* sp.**  
Myoungjun Jang1, Changho Kim1, Yongkoo Cho1, Seongmin Kim1, Dongil Shin1, Hyunju Lee1, Youngsu Lee1, and Taeseok Oh*  
1Department of Plant Resources, Kongju National University, 2Environmental Agriculture Research Division, Gyeonggi-do Agricultural Research & Extension Services  

We investigated effect of *Trichoderma* sp. for biological control in *Fusarium oxysporum* of lettuce. Researching the ability to produce spores of *Trichoderma* sp., the harvested substrate of mushroom was excellent. In the spring culture at the field, mobility rate of *Fusarium oxysporum* is lowest, and control value is highest in J13 incubated at the harvested substrate of mushroom. In the autumn culture at the field, mobility rate of *Fusarium oxysporum* is lower than the control, and the control value is higher than J13 incubated at the harvested substrate of mushroom.  

Keywords: *Trichoderma* sp., *Fusarium oxysporum*, biological control, control value, mobility rate

**J-39**  
**Unreported Fungal Species of *Didymosphaeriaceae* from Soil in Korea**  
Mahesh Adhikari1,2,3, Sang Woo Kim1,2, Dil Raj Yadav1,2,3, Yong Hyun Um2,3, Hye Seung Kim2,3, and Youn Su Lee2,4*  
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Soil is a diversified micro-habitat for the development of microbial populations. Soil fungi are important integral components in the soil ecosystem and play a crucial role as a source of food for larger organisms, pathogens and maintain beneficial symbiotic relationships with plants or other organisms. Soil fungal diversity study was carried out to check the species diversity in the crop field soil of Chuncheonbuk-do, Korea in 2015. In total five hundred soil samples were collected and isolated through the serial dilution technique. Isolated fungi were then purified and differentiated according to their morphological and microscopic characteristics. Genomic DNA of the isolates was extracted by using QIAGEN® Plasmid Mini Kit(QIAGEN Sciences, USA) and the identification of fungi was carried out by sequence analysis of internal transcribed spacer (ITS) region of the 18S ribosomal DNA (18S rDNA). Morphologically 220 isolates were found distinct. Among them species of *Didymosphaeriaceae* family were encountered namely *Paraphaeosphaeria sporulosa*, new and unreported in Korea. This species was identified based on the molecular and morphological analysis and have not been reported officially in the past from Korea.  

Keywords: *Didymosphaeriaceae*, Diversity, Soil Fungi

**J-40**  
**A Serological Surveillance of West Nile and Japanese Encephalitis Virus in Horses from South Korea in 2014**  
Jihye Lee, Sunghee Kim, Jeeyong Park, Yongjoo Kim, Byounghan Kim, and Heyeong Jeong*  
Animal and Plant Quarantine Agency  

West Nile virus (WNV) and Japanese Encephalitis virus (JEV) are members of the genus Flavivirus in the family *Flaviviridae*. These are *arthropod-borne virus* that affects the nervous system of humans, horses, and birds, causing mild to severe illness and sometimes death. In this study, we conducted a serological surveillance of these viruses in South Korea to look for evidence of infection with arboviruses associated equine encephalomyelitis in horses. Total of 1,531 serum sample of horse, including riding horses, racing horses and horses in horse breeding farms and riding schools, were collected from 2014 across South Korea through the support of the Korean Racing Authority (KRA). WNV is has not been in Korea and all sample tested for WNV antibodies were shown to be negative. For JEV, because South Korea is endemic and horses in South Korea are vaccinated against JEV, various titers of antibodies for JEV were detected by VNT in 75.3% (1,153/1,531) of the sera in 2014. Also, although no WNV has been reported in Korea, WNV cases in wild birds and humans have been reported in neighboring countries, which demonstrates the possible risk of introduction of WNV into Korea. Therefore, continued surveillance will be needed to quickly identify this disease of public concern.  

Keywords: WNV, JEV, horses, arboviruses
Optimum Condition of Demucilaging Process for *Opuntia ficus-indica* var. Saboten (Pad) with Enzyme Treatment

Byung Wook Yang¹, Seon Jeong Maeng¹, Seung Il Ahn¹, Jae Hoo Choi¹, Heejung Jung¹, Yejin Cho², Byung Yong Kim¹, and Young Tae Hahn²*

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*Opuntia ficus-indica* is a member of the Cactaceae family that is widely grown in all the semi-arid countries throughout the world. *Opuntia ficus-indica* var. *Saboten* (OFS), commonly known as prickly pear cactus, is commercially cultivated as a dietary food and medicinal plant in Jeju Island, Korea. This study was conducted in order to establish the hot water extraction (HWE) conditions with the fresh stem of OFS for development of a functional food, which is controlling blood glucose level. And also, the contents of soluble dietary fiber (SDF) of the dried powder prepared by the extract were optimized using the response surface methodology (RSM), which included 20 experimental points with 3 replicates for two independent variables (extraction temperature and time). A central composite design was used to monitor the effect of extraction temperature (60-90°C), X1 and extraction time (1-10h, X2) on dependent variables, such as dietary fiber (Y1), soluble content (Y2), and dietary fiber yield (Y3). The determination coefficient (R²) value for the extraction yield of the fresh stem was 0.95 (p<0.01). The optimum extraction yield was predicted at an extraction temperature of 89.02°C, 598 min of extraction time and determined the value of 8.61% (wt/wt) in HWE yield, respectively. Estimated maximum values at predicted optimum conditions were in best agreement with experimental values.

Keywords: hot water extraction, *Opuntia ficus-indica*, response surface methodology, soluble dietary fiber

Optimum Conditions for Hot Water Extraction from *Opuntia ficus-indica* var. Saboten Using Response Surface Methodology

Byung Wook Yang¹, Seon Jeong Maeng¹, Seung Il Ahn¹, Jae Hoo Choi¹, Heejung Jung¹, Yejin Cho², Byung Yong Kim¹, and Young Tae Hahn²*

¹Department of System Biotechnology, Chung-Ang University, ²Department of Food Science and Biotechnology, Kyung Hee University

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Keywords: hot water extraction, *Opuntia ficus-indica*, response surface methodology, soluble dietary fiber

Development of Real-time RT-PCR to Differentiate Rift Valley Fever Virus from Clone 13 Vaccine

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RVFV is a mosquito-borne viral disease affecting both livestock and humans RVFV. It was first identified in Kenya in 1931 and reported endemic in Africa but has recently spread to Arabian Peninsula. Possibility of RVFV introduction was increasing as climate change and globalization of trade in animals and animal products. Thus, we developed a new rRT-PCR assay that safely and cost-effectively differentiates infected from vaccinated animals (DIVA) and is apply to RVF-free countries. The L, M and S segments of Smithburn strain and the Clone 13 strain were synthesized and used in rRT-PCR. The primer and probe sets were designed and synthesized. The performance of the new rRT-PCR was compared with the OIE reference method and quadruplex rRT-PCR. Diagnostic specificity was evaluated using other vector-borne viruses. For differentiate clone 13 vaccine, the new rRT-PCR assay targeting the S segment (N1s and N gene) was tested. The new rRT-PCR assay is able to differentiate the Smithburn strain from the Clone 13 vaccine strain. No cross reactivity with other vector-borne viruses, which is especially important in the South Korea was observed. These results indicated that the new rRT-PCR can be used as a safe, cost-effective and DIVA diagnosis in RVF-free countries including the South Korea.

Keywords: Rift valley fever virus, Real time RT-PCR, Clone 13 vaccine

Investigation of Methane Emission Mechanisms from Rice Based on Comparative Paddy Soil Metagenomics

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Rice fields are known as one of the anthropogenic sources of atmospheric methane. Quantitative balance of methanogens and methanotrophs in rice paddy soil, thus play an important role of controlling amount of methane emission from rice. Recent studies have shown the use of swine manure enhances the amount of methane emission from rice. To investigate the mechanism by which swine manure boosts methane emission, we conducted comparative metagenomics between inorganic fertilized paddy (NPK) and swine manure fertilized paddy (PIG). Taxonomy analysis using 16S ribosome RNA sequences database showed that more abundant methanogen archaea and methanotrophic bacteria were found in PIG compared with NPK. BLAST results showed more abundant carbohydrate metabolism functional genes in PIG. On the other hand, differential abundance analysis using RSEM showed that some of the key genes related to methane production were found more abundant in PIG with higher abundance of methanotrophs likely due to more abundant substrates (i.e., methane).

Keywords: Rice, Methane emission, Metagenomics, Methanogens, Methanotrophs
J-45

Center for Fungal Genetic Resources (CFGR): Housing Plant Pathogenic Fungi for Educational and Research Purposes
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Fungi are eukaryotic organisms, growing in a wide range of habitats. Fungi are significantly important in a variety of ways. They play an essential role in the decomposition of organic matter. They have been used as a source of food, and agents for fermentation of food products and for the production of various antibiotics and enzymes that are used in a field of research, industry, medicine, etc. In contrary, impact of many fungi on animals and plants is economically and socially detrimental. For example, *Mycosphaerella oryzae* causes the most destructive disease, “rice blast”. Annual yield loss of rice by rice blast is equivalent to rice that could feed about 60 million people. The Center for Fungal Genetic Resources (CFGR) was established to collect, maintain and distribute genetic resources mainly from plant pathogenic fungi, which are important for both educational and research purposes. This will contribute to development of new strategies for management of crop diseases and of new components for improvement of our lives. CFGR possesses important fungal species; a total of 42,000 isolates from 54 species of fungi including 20,902 T-DNA transformants of rice blast fungus and anthracnose fungus. In addition to the biological materials, CFGR has developed user-friendly databases to maintain genetic information of fungal stocks and help to solve questions about fungal pathogenicity, population genetics, development, and evolution.

Keywords: Plant pathogen, Fungi, Mutant, Plant quarantine, Pathogenicity genes

J-46

Korean Metagenome Bank for Exploiting Microbial Diversity
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Microorganisms have played important roles in biotechnology and bioindustry for long times. The recent use of molecular ecological methods and environmental DNA (eDNA) has changed our knowledge of microbial diversity dramatically and provided rapid access to genes of yet-uncultured microorganisms. Application of molecular ecological studies has shown that the majority (99%) of microorganisms present in the nature are under uncultivation. Many attempts to improve the recovery of microorganisms have been used as a source of food, agents for fermentation of food products and for the production of various antibiotics and enzymes that are used in a field of research, industry, medicine, etc. In contrary, impact of many fungi on animals and plants is economically and socially detrimental. For example, *Mycosphaerella oryzae* causes the most destructive disease, “rice blast”. Annual yield loss of rice by rice blast is equivalent to rice that could feed about 60 million people. The Center for Fungal Genetic Resources (CFGR) was established to collect, maintain and distribute genetic resources mainly from plant pathogenic fungi, which are important for both educational and research purposes. This will contribute to development of new strategies for management of crop diseases and of new components for improvement of our lives. CFGR possesses important fungal species; a total of 42,000 isolates from 54 species of fungi including 20,902 T-DNA transformants of rice blast fungus and anthracnose fungus. In addition to the biological materials, CFGR has developed user-friendly databases to maintain genetic information of fungal stocks and help to solve questions about fungal pathogenicity, population genetics, development, and evolution.

Keywords: Plant pathogen, Fungi, Mutant, Plant quarantine, Pathogenicity genes

J-47

Microbial Carbohydrate Resource Bank (MCRB)
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Microbial carbohydrates have a variety of characteristics and original functionalities comparing with those produced by animals and plants. Microbial carbohydrates are generally natural, non-toxic, biodegradable and biocompatible polymers. Most carbohydrates also ascribed to the GRAS(Generally Regarded As Safe) and their structural diversities lead to a variety of physical or chemical functions. Recently, many novel applications have been developed using microbial polysaccharides such as drug delivery systems using hydrogels, nanoparticles, microspheres in the pharmaceutical and biomedical industries, solubility study and characterization in the fields of fine chemicals and biochemical engineering. Microbial polysaccharides can also be utilized as stabilizer, emulsifier, thickener, gelling agent in food industries. The Microbial Carbohydrate Resource Bank (MCRB) in Korea was recently established to investigate and collect various functional polysaccharides and microorganisms in order to widely utilize the microbial resources. MCRB also takes a role as research resource bank offering a storage facility for various microbial carbohydrates, their chemical derivatives and microorganisms. MCRB will contribute to the advancement of polysaccharide-based industrial fields as well as academic area by providing the adequate microbial carbohydrate resources to various researchers for their needs according to the regulation of MCRB.

Keywords: Microbial Carbohydrate Resource, Carbohydrate, Polysaccharides, Microbe, Biomedical material

J-48

Bacteriophage Bank
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Bacteriophages are viruses growing on bacterial hosts. They are antagonistic to bacteria and first reported by Frederick Twort and Felix d’Herelle in 1915 and 1917, respectively. They are found in sea, air, land and even foods. It is assumed that 1030 to 1032 phages exist on earth and they play a role in maintenance of biological balance. Recently, new applications for phages are increasingly reported. As they are a part of useful biological resources, there are increasing demands for securing these resources. In response to these demands, the bacteriophage bank was established in 2010. The bank collects phages from environments as well as from working groups worldwide. Currently, 600 different phages are stocked. The host bacteria include E. coli, Salmonella enteritidis, Pseudomonas aeruginosa, Listeria monocytogenes, Acinetobacter, Campylobacter jejuni, Enterococcus faecium, Enterococcus faecalis, Cronobacter sakazaki, Serratia marcescens and Staphylococcus aureus. The number of stock is growing continuously. The bank also serves as a distributor for the collected phages. (www.phagebank.or.kr)

Keywords: Bacteriophage, 600 different phages, useful biological resources
Korea Bank for Pathogenic Viruses

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Korea Bank for Pathogenic Viruses (KBPV) has been established in 2005 as a repository agent for the collection, management and distribution of the various pathogenic viruses that are essential to use for researches in biomedical sciences. The Institution operates in collaboration with The Institute for Viral Disease at Department of Microbiology, College of Medicine, Korea University, founded in 1973. The bank has unique viral collections such as Hantaan, Seoul, Muju, Soochong, and imjin the various pathogenic viruses that are essential to use for researches in biomedical sciences. The Institution operates in collaboration with The Institute for Viral Disease at Department of Microbiology, College of Medicine, Korea University, founded in 1973. The bank has unique viral collections such as Hantaan, Seoul, Muju, Soochong, and imjin the etiologic agents of hemorrhagic fever with renal syndrome. To date, total of more than 43,000 materials (~100,000 vials) from human and animal sources have been collected and maintained. We have provided a highly collaborative environment for researchers in various fields by providing valuable viral resources including consulting service. We also provide the educational program related to pathogenic viruses including biosafety training. Requestors of such agents are required to register with KBPV and to supply details of their laboratory facilities and safety management. More details about KBPV can be found at http://kbpv.knu.ac.kr

Keywords: Pathogenic Viruses, biosafety, viral disease, genetic information, antibody

Lichen as a Novel Bioresources in Korea

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Lichens are symbiotic organisms composed of a fungus (mycobiont) and an alga (photobiont). They produce characteristic secondary metabolites, lichen substances, which seldom occur in other organisms. Lichen and their metabolites have many biological activities. In spite of the wide spectrum of biological activities shown by the lichens, they have long been neglected by mycologists and overlooked by agrochemical industry because of its slow growth in nature and difficulties in the artificial cultivation of organisms. Use of lichen-forming fungi can overcome the disadvantage of natural lichen extracts for industrialization of their metabolites because of their much faster growth and larger production of the metabolites in culture than the natural thalli. Korean Lichen and Allied Bioresources Center focuses on isolation, maintenance and distribution of lichen bioresources to research groups in universities, national institutes and industrial sectors. It also screens their biological activities, and investigates cultural conditions for large production of lichen substances. Chemical library of some lichen extracts is also available from the center.

Keywords: lichen, lichen-forming fungi, photobiont
It has been known that about 700 species of oral bacteria inhabit the human oral cavity. Of them, 350 species have been cultured. The oral bacteria are the major causative agents of systemic diseases such as cardiovascular diseases as well as oral diseases, periodontitis and dental caries. However, the causative bacterial species for oral diseases have not been known because the dental diseases are occurred by the multiple infections. In addition, the prevalence of the oral bacterial species is different by the geographic location of the host and individual. It is very important to obtain the oral bacteria from Koreans for pathogenesis studies related to oral infectious diseases. The purpose of Korean Collection for Oral Microbiology is to obtain the oral clinical strains and their genetic resources, such as 16S rDNA, species-specific PCR or qRT-PCR primers, and genome sequences, for offering them to the researchers.

Keywords: oral bacteria, oral diseases, pathogenesis, resource center

Today, the increasing clinical abuse of antimicrobials in people and animals, led to a high rate of occurrence of resistant microbes. In addition, drug resistance is easily transferred from one resistant species to another related one in many ways, thereby complicating the issue. Therefore, treatment for disease caused by antimicrobial resistant microbes has emerged as a critical issue worldwide, and development of new drugs that inhibit resistant microbes became an urgent issue of research. As the issue should be dealt across clinical research, regulation, and pharmaceutical development, communication and cooperation between researchers among those areas are necessary. Since Culture Collection of Antimicrobial Resistant Microbes was established in 1999, CCARM has played a role as a connector among various research fields by providing the antimicrobial resistant microbes with known mechanism and information. CCARM collects, keeps, and preserves the resistant microbes in a systematic manner for constant supply of certified microbes and share the information with researchers in various fields. CCARM has a collection of over 20,000 strains of bacteria and yeast from 87 genera and provides various information including international meeting, newest information related to resistance via homepage and newsletter.

Keywords: Antimicrobial Resistant, Research Resource Center, bacteria

Korea Environmental Microorganisms Bank
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Korea Environmental Microorganisms Bank (KEMB) has been established as a microbial and genetic resource center for environmental industries. The KEMB plays an essential role as follows: 1the collection and conservation of native environmental microorganisms and genetic resources, 2the construction of systematic management system for effective conservation and application of microbiological resources for environmental industries, 3the provision fundamental data for ecosystem research and microbial classification, and 4the development of biological treatment system for bioremediation of environmental pollutant and ecosystem restoration. There are about 14,000 strains of bacteria collected from environments, at this time. These collections are classified in accordance with scientific and functional characteristics, respectively. It is considered to promote academic and industrial activities by supplying basic materials for research and industrial applications, which accomplish the ecological recovery through constructing eco-friendly bioremediation system by supplying basic microbial resources.

Keywords: Microorganism, Environmental Restoration, Recovery of Ecosystem

Biocontrol of Lettuce Sclerotinia Rot Using Trichoderma virens and Trichoderma harzianum
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Sclerotinia sclerotiorum attacks over 408 plant species in 75 families in worldwide, and approximately 56 plant species are recorded as hosts in Korea. S. sclerotiorum causes severe damage on many economically valuable crops including vegetables. In order to select the effective antagonists for biological control against sclerotinia rot, 200 Trichoderma isolates were isolated from sclerotia of Sclerotinia sclerotiorum and rhizosphere of lettuce plants from several main lettuce culture locations in Korea. Thirteen isolates effectively parasitized sclerotia of S. sclerotiorum between 15°C and 30°C and the isolates successfully parasitized sclerotia of S. sclerotiorum. When spore suspension (5×10^5 conidia/ml) of the Trichoderma virens TR1047 and Trichoderma harzianum TR1048 were drenched onto soil surface around lettuce plants with inoculation of S. sclerotiorum. Incidence of Sclerotinia rot in the isolate treated plots was 14.4% and 6.7%, while that in the control plot 97.8%.

Keywords: Biocontrol, Lettuce, Sclerotinia Rot, Trichoderma
Antifungal Activity of Fungal Pathogens of Ginseng by Bacillus amyloliquefaciens

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Agricultural Microbiology Division, National Academy of Agricultural Science

Fungal diseases of ginseng (Panax ginseng) are very difficult to control using agrochemicals or other cultural methods because the pathogens are resistant to the applied chemicals and soil environment. People's anxiety of environmental pollution and medicine plant safety due to agrochemicals has led to demanding alternatives to chemical control of plant diseases. Subsequently biological control using antagonistic organisms has been applied as one of alternative methods for environment-friendly control and integrated management of plant diseases. Strain M35, isolated from leaf of cucumber was identified as a strain of B. amyloliquefaciens by 16S rRNA gene sequencing and physiological and biochemical analyses. Antagonistic strain M35 had strong antifungal activity against pathogens of damping-off, sclerotinia rot, root rot, anthracnose, alternaria blight and grey mold of ginseng.

Keywords: Antifungal Activity, Bacillus amyloliquefaciens, Fungal Pathogens, Ginseng

Salicylic Acid Reduces OmpF Expression to Become Salmonella Typhimurium More Resistant against Cephalosporins

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Salmonella typhimurium is one of the most important bacterial pathogen that cause diarrhoea. It is well-known that S. typhimurium is more resistant to various antibiotics after treatment with salicylic acid (Sal). Here, we studied the molecular mechanism of Sal against the increased cephalosporin resistance in these bacteria. The minimum inhibitory concentrations (MIC) of cephalosporins such as cephalothin, cefetazole and cephotaxime in bacteria cultured in 5 mM Sal increased 2-8 folds. Even in bacteria treated with CCCP, an inhibitor of bacterial efflux pumps, MICs of such antibiotics increased 2-8 folds in the presence of Sal. This was caused by the inhibition of cephalosporin entry by Sal, as demonstrated by increase in ethidium bromide (EtBr) accumulation rates in CCCP-treated bacteria and decrease upon treatment with Sal in a dose-dependent manner. We conclude that the inhibition of cephalosporin entry of the bacteria by Sal occurs via OmpF as OmpF proteins significantly decreased in the presence of Sal whereas other OmpA and OmpC increased. Consistent with this result, ompF transcripts decreased 20-fold and its antiseres regulator, mcrF increased 12-fold in the presence of Sal whereas transcripts of porins (ompA and ompC) and efflux pumps (acrA, acrD and tolC) were not altered significantly.

Keywords: Salicylic Acid, OmpF, Salmonella Typhimurium, Cephalosporin

Control Effect of Tomato Bacterial Wilt Caused by Ralstonia solanacearum Using Water Extract of Spent Mushroom Media of Hericium erinaceus

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Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Wanju 55365

Bacterial wilt caused by Ralstonia solanacearum is one of the most devastating soil-borne diseases of plants worldwide and affects many important crop species. R. solanacearum has been investigated both biochemically and genetically and recognized as a model system for the analysis of pathogenicity. Chemical control has become less effective due to the development of pathogen resistance beside the potentially undesirable effects of the fungicides on human, plants and other beneficial organisms. Control could be considered as an alternative of chemical control. Derived extract of spent mushroom (Hericium erinaceus) compost was screened for antibacterial activity against phytopathogenic bacteria. The Derived extract of spent mushroom (Hericium erinaceus) compost showed antibacterial activity against R. solanacearum in vitro. Also, this extract suppressed the disease development of Bacterial wilt on tomato in pot test.

Keywords: Control, Hericium erinaceus, Bacterial wilt, Spent Mushroom media, Tomato

Occurrence of Aspergillus/Penicillium Species in Korean Cereals

Sosoo Kim, Jae-Seon Park, Hyoenheui Ham, Soo Hyung Lee, Sung Kee Hong, Jae-Gee Ryu, and Theresa Lee*
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To monitor occurrence of aflatoxin, ochratoxin, or citrinin producing fungi in Korean cereals, grains were collected nationwide from rice in storage, barley or wheat fields. The grain samples were surface-sterilized and placed on potato dextrose agar plates for 5 days at 25°C. Among fungal colonies grown out from the grains, those with Aspergillus or Penicillium-like morphology were isolated and identified based on β-tubulin sequence homology. In 2015, seventeen Aspergillus strains were isolated and identified as A. flavus (5), A. oryzae (1), A. triticum (3), A. amstelodami (2), A. heteroarthrosporum (1), A. pseudoglaucus (1), A. nigri (1), A. cristatus (1), and Aspergillus spp. (2). Penicillium strains were P. auratigriseus (1), P. chrysogenum (1), P. cyclopium (1), P. glabrum (2), P. polonicum (1), P. viridicatum (1), and Penicillium spp. (6). The number of isolates and species range appeared lower and less diverse than previous years. PCRs to amplify norB-cypD region for aflatoxin production or PKS region for ochratoxin/citrinin production were performed with selected species respectively. The results showed none of the expected bands, indicating that the samples of 2015 tested might be safe from contamination with aflatoxin, ochratoxin, or citrinin.

Keywords: cereals, Aspergillus, Penicillium

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Salmonella typhimurium is one of the most important bacterial pathogen that cause diarrhoea. It is well-known that S. typhimurium is more resistant to various antibiotics after treatment with salicylic acid (Sal). Here, we studied the molecular mechanism of Sal against the increased cephalosporin resistance in these bacteria. The minimum inhibitory concentrations (MIC) of cephalosporins such as cephalothin, cefetazole and cephotaxime in bacteria cultured in 5 mM Sal increased 2-8 folds. Even in bacteria treated with CCCP, an inhibitor of bacterial efflux pumps, MICs of such antibiotics increased 2-8 folds in the presence of Sal. This was caused by the inhibition of cephalosporin entry by Sal, as demonstrated by increase in ethidium bromide (EtBr) accumulation rates in CCCP-treated bacteria and decrease upon treatment with Sal in a dose-dependent manner. We conclude that the inhibition of cephalosporin entry of the bacteria by Sal occurs via OmpF as OmpF proteins significantly decreased in the presence of Sal whereas other OmpA and OmpC increased. Consistent with this result, ompF transcripts decreased 20-fold and its antiseres regulator, mcrF increased 12-fold in the presence of Sal whereas transcripts of porins (ompA and ompC) and efflux pumps (acrA, acrD and tolC) were not altered significantly.

Keywords: Salicylic Acid, OmpF, Salmonella Typhimurium, Cephalosporin

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Keywords: Control, Hericium erinaceus, Bacterial wilt, Spent Mushroom media, Tomato

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Keywords: cereals, Aspergillus, Penicillium
**J-61**

Quantitative Analysis of Activated Coagulation Factor XI as an Impurity in Human Plasma-derived IVIG Products

Hokyung Oh, Kikyung Jung, Sang-Mi Park, Yong Seok Kang, Ji-Hye Kim, Garam Min, Kiwon Han, Sung Hwan Han, Hyun Song, Jiyoung Lee, Seon Hwa Seong, So Young Han, and Chiyoung Ahn

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The intravenous immunoglobulin (IVIG), a human plasma-derived medicinal product, is mainly used to treat primary immunodeficiency syndromes and a variety of autoimmune, infectious, transplantation-related, and chronic diseases. A small number of thromboembolic events (TEEs) occurring after the administration of IVIG preparations and infusions have been reported. These TEEs have been attributed to an increased residual content of activated procoagulant components - mainly activated coagulation factor XI (FXa) - in IVIG. Currently no TEEs associated with the use of domestically manufactured IVIG have been reported in Korea. To study the safety of the IVIG produced by the domestic industry, we investigated procoagulant activity in 40 IVIG lots from 2 domestic manufacturers using FXa chromogenic assay and thrombin generation assay. This study describes a rigorous, systematic investigation of FXa as an impurity in IVIG from 2 Korean blood fractionation companies. As a result, FXa was below the detection limit of the chromogenic assay and below the quantification limit of the TGA. This result gives us an useful information that 2 Korean manufacturers’ IVIG manufacturing process has the capability to remove thrombogenic factor. Keywords: Thrombosis, IVIG, FXa, Procoagulant

**J-62**

Different Pathogenicity of Entomopathogenic Fungi Cultured in Different Media to Control Aphid

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During crop cultivation, crop was infested several insect pests and/or plant diseases. Chemical control has been major control method, but development of resistance by pest or disease is demanding alternative control methods. Entomopathogenic fungi are important control agent to control sucking type insect pests such as aphid, and whitefly as alternative control agent. Narrow host range, low or slow and fluctuation in mortality are disadvantages using mycopesticides using fungi. Fungal pathogenicity can be changed by culture media. We studied comparison of pathogenicity of entomopathogenic fungi cultured in different media against two aphid species. Pathogenicity of entomopathogenic fungi differed from media. For example, an isolate showed higher virulence with spores cultured in liquid media than spores cultured in solid media. Some isolate increased mortality when cultured in different culture condition. Keywords: entomopathogenic fungi, culture media, pathogenicity

**J-63**

Development of Peptide Nucleic Acid Multi-probe- real-time PCR Method Targeting hsp65 Gene for Identification Between Mycobacterium abscessus Strains

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The Mycobacterium abscessus group can be divided into at least three distinct subspecies: M. abscessus subsp. abscessus (M. abscessus), M. abscessus subsp. massiliense (M. massiliense) and M. abscessus subsp. bolletii (M. bolletii). Furthermore, the separation of three genotypes within M. massiliense (Type I, II-1 and II-2) was also recently proposed. Here, we introduced a novel peptide nucleic acid (PNA) multi-probe real time PCR method targeting hsp65 gene (hsp65 PNA RT-PCR) for distinguishing four types within M. abscessus groups (M. abscessus and 3 types of M. massiliense) using 3 PNA probes designed by multiple alignment of mycobacterial hsp65 sequences. A developed hsp65 PNA RT-PCR was applied to 27 reference strains and 228 clinical isolates belonging to the M. abscessus groups. In reference strains, with 100% sensitivity and specificity, it enabled the separation of four M. abscessus subspecies or genotypes. In comparison with results obtained by the hsp65 direct sequencing protocol, it enabled the identification of 227 isolates of 228 (99.6%) as 99 M. abscessus and 128 M. massiliense strains. Furthermore, it could also differentiate all 128 M. massiliense isolates into three genotypes, Type I (71 isolates), Type II-1 (49 isolates) and Type II-2 (8 isolates). Our data suggest that this novel hsp65 PNA RT-PCR method could be a promising approach for identifying subspecies or genotypes among clinical isolates of the M. abscessus group routinely in a clinical setting. Keywords: Mycobacterium abscessus, Mycobacterium massiliense, peptide nucleic acid (PNA), real time PCR, hsp65 genotype

**J-64**

Detection of EBV-encoded Small RNA from Diffuse Large B-cell Lymphoma by rt-PCR

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Keywords: EBV, EBER, RT-PCR, DLBCL

Epstein-Barr virus (EBV) has a pathogenic role in several lymphomas including diffuse large B-cell lymphoma (DLBCL). In this study, we detected the EBV-encoded small RNA (EBER) in clinical samples from DLBCL patients by RT-PCR and compared the sensitivity of the RT-PCR product was detected by RT-PCR for EBER. When using the RT-PCR method, targeting EBV-encoded small RNA (EBER). The 150 base pair long PCR product was detected by RT-PCR for EBER. When using the RT-PCR method, targeting EBV-encoded small RNA (EBER). The 150 base pair long PCR product was detected by RT-PCR for EBER. When using the RT-PCR method, targeting EBV-encoded small RNA (EBER). The 150 base pair long PCR product was detected by RT-PCR for EBER. When using the RT-PCR method, targeting EBV-encoded small RNA (EBER). The 150 base pair long PCR product was detected by RT-PCR for EBER. When using the RT-PCR method, targeting EBV-encoded small RNA (EBER).
**J-65**

An Analysis and Study of Domestic and Foreign Cases by Institutional Biosafety Committee

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A researcher to execute the test of genetic recombination at the Class I or II LMO research institute in Korea shall declare the LMO research institute facility for safety management of LMO (Living Modified Organisms) to the Ministry of Science, ICT, and Future Planning in accordance with the “Transboundary Movement, etc. of Living Modified Organisms Act” (hereinafter referred to as LMO Act), taken effect since January 1st, 2008.

The formation and management of institutional biosafety committee shall be implemented by the facility declared as the one of Class II or above.

An institutional biosafety committee (IBC) is to secure the safety against hazard by a test of genetic recombination within a research institute, and it prevents biological risk and reviews and examines biosafety of researches including a test of genetic recombination.

This presentation is to introduce the analysis of operation system of foreign biosafety committee and administration of institutional biosafety committee administrated by an institute based on the contents studied by the Ministry of Science, ICT, and Future Planning from 2014 to September 2015.

Keywords: IBC, Living Modified Organisms, LMO, Recombinant DNA experiments

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**J-66**

Antibacterial Activity of Essential Oils against Acidovorax avenae subsp. citrulii, causing Bacterial Fruit blotch (BFB) of Cucurbit Plants

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Bacterial fruit blotch (BFB) of watermelon caused by Acidovorax avenae subsp. citrulii (AAC) is one of the most severe diseases of cucurbit plants worldwide. The aim of this work was to examine and evaluate the antibacterial activity of thirty-two plant essential oils against AAC. Using the half-divided petri plate assays, sweet basil and peppermint volatile oils were observed to be the most effective against AAC. Following tests confirmed that peppermint oil was significantly active against AAC. GC-MS analysis revealed that chemical compositions of the peppermint oil were 1,8-cineole, menthol, neomenthol, isomenthol, menthone, isomenthone, menthofuran, menthyl acetate, pipertone, (-)-pinene, (-)-limonene, and (+)-limonene. Among them menthol, neomenthol, isomenthol, and 1,8-cineole were significantly active against AAC. Using disc diffusion assays, peppermint and cinnamon oils were observed to be the most effective against AAC. The major compositions of cinnamon oil were analyzed using GC-MS analysis. Among them benzaldehyde and cinnamaldehyde exhibited significant antibacterial activity against AAC. Minimum inhibitory concentration and minimum bactericidal concentration values of essential oil components showing an important antibacterial activity were measured using broth dilution assays. The in vitro results suggest that these plant oils, considered as potential antibacterial agents, should be preferred for the control of BFB.

Keywords: Antibacterial activity, Essential oils, Bacterial fruit blotch (BFB) experiments

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**J-67**

Intestinal Microbes Affect the Lifespan Extension of Dietary Restriction in Drosophila melanogaster

Ah Yoon, Shin-Hae Lee, Hye-Yeon Lee, and Kyung-Jun Min*

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Dietary restriction (DR), undernutrition without malnutrition, is the most well-known intervention to retard aging and to extend the lifespan in diverse organisms. Recently, commensal bacteria reside in the digestive tracts were reported to affect the host fitness and lifespan. However, the relationship of intestinal microbes and the longevity effect of DR are not illustrated yet. To test our hypothesis that the longevity effect of DR is related with intestinal microbes, we used Drosophila melanogaster, which is an optimal model organism to study host-microbes interaction. We generated the axenic fly through the dechorionation of eggs and measured the lifespan under the various concentration of yeast extract, the protein source of fly. In addition, we examined the fecundity, feeding amount, and body weight of conventional or axenic flies exposed with various DR concentration. Furthermore, the change of microbial flora of conventional or axenic flies by DR was examined via the CFU assay. Our results showing the relationship of intestinal microbes and longevity effect of DR will be provide fundamental knowledge to understand the underlying mechanisms of lifespan extension by DR.

Keywords: Dietary restriction, Intestinal microbe, Longevity, Host-microbes interaction, Drosophila melanogaster

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**J-68**

DNA Delivery and Immunogenicity of VLP Forming Baculoviral Vaccine against Influenza pdmH1N1

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An outbreak of influenza H1N1 in 2009 has recorded the first influenza pandemic of the 21st century (pH1N1), it has transmitted over a million and took 18,449 lives. Despite the advantages of DNA vaccines, overcoming their lower efficacy relative to that of conventional vaccines remains a challenge. Here, we constructed a human endogenous retrovirus (HERV) envelope-coated, non-replicable, baculovirus-based virus like particle (VLP) forming DNA vaccine against pandemic influenza A/California/04/2009 (pH1N1). Previous we reported the efficacy of influenza HA vaccine using a baculoviral DNA vaccine (AcHERV HA). However, AcHERV HA vaccine only elicits immune response against HA antigen, was lower than that of the commercial killed vaccine. For that reason, we constructed a baculovirus is able to form VLP in host cell to overcome the limitation of previous vaccine.

In BALB/c mice, AcHERV VLP immunization showed higher humoral immune response and cross-reactivity against heterologous H1 strain (PR8) was observed, while killed vaccine immunization was absent from results against heterologous H1 strain (PR8). In addition, AcHERV VLP elicits strong neutralizing antibody production, high level of IFN-γ secretion in splenocyte and lower virus shedding in lung after lethal dose of influenza virus challenge. In conclusion, VLP forming baculovirus DNA vaccine (AcHERV VLP) could be a potential vaccine candidate to achieve an efficacy comparable to that of killed virus vaccines.

Keywords: Influenza, Recombinant baculovirus, Virus-like particle, Immune response, DNA vaccine
J-69

Enhanced Immune Response for Foot-and-Mouth Disease Virus Vaccine with Baculovirus-Based Granulocyte Macrophage Colony Stimulating Factor-Flagellin Fusion Adjuvant

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Foot-and-mouth disease (FMD) is an economically important disease of cloven-hoofed animals that is primarily controlled by vaccination of susceptible animals. Granulocyte-macrophage colony-stimulating factor (GMCSF) is produced by a variety of cell types including T cells, macrophages, endothelial cells and fibroblasts upon receiving immune stimuli. Salmonella typhimurium Flagellin 2 (STF2) activates Toll-like receptor 5-mediated innate immune signaling pathways and induces inflammatory responses through the activation of antigen-presenting cells (APCs). In this study, we investigated the effects of STF2 linked GMCSF-Flagellin 2 fusion (Ac-ieI-PERVB-GMCSF-STF2) on the immune response of commercial FMD vaccine (KBNP, O Manisa, A Malaysia97, Asia 1 serum strain). We constructed a recombinant baculovirus based GMCSF-STF2 fusion encoding adjuvant (Ac-ieI-PERV-GMCSF-STF2). BALB/c Mice were immunized with two times with 2x107 FFU of Ac-ieI-PERV-GMCSF-STF2 at 2-week intervals. Immunized mice sera were collected and the immunological effects of the Ac-ieI-PERV-GMCSF-STF2 were determined by ELISA, T-cell proliferation assay, and IFN-γ. The data revealed that GMCSF-STF2 fusion as an adjuvant of FMD vaccine could stimulate both humoral and cell-mediated immune response. Interestingly, GMCSF-STF2 fusion showed much better adjuvant effects than that of FMD vaccine only. In conclusions, STF2 and GM-CSF could be used as a potential adjuvant for FMD vaccine.

Keywords: baculo virus, GMCSF, STF2, adjuvants, FMD Vaccine

J-70

Baculovirus Based DNA Vaccine against Porcine Reproductive and Respiratory Syndrome Virus Vaccine

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Porcine reproductive and respiratory syndrome virus (PPRSV) causes an economically devastating of porcine industry, by reproductive failure in pregnant sows and by respiratory tract illness in piglets. PPRS vaccine belongs to Arteriviridae family. The GP5 and M proteins are the key immunogenic proteins of PPRS. In this study, we constructed a baculovirus based Recombinant DNA vaccine (AcPERV-CS/C6). SPF 6-weeks BALB/c mice and SPF 5-weeks miniature pigs were immunized intramuscular AcPERV-CS/C6 and commercial PPRS killed vaccine. All of the groups were immunized 3 weeks interval, two times injection. Serum samples were collected at 3 and 5 weeks after post-immunization. There results showed that AcPERV-CS/C6 can significantly enhance PPRS-specific antibody, PPRS-specific neutralizing antibody, IFN-γ level rather than those of commercial PPRS killed vaccine. Therefore, this study suggests that AcPERV-CS/C6 DNA Vaccine can be a potential efficient prophylactic vaccine candidate.

Keywords: PPRS, Baculovirus, DNA vaccine, Immunization, Immune response

J-71

Bacillus subtilis H801 Producing Cellulase and Xylanase, Isolated from Spent Mushroom (Pleurotus ostreatus) Substrates

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Spent mushroom substrate (SMS) is a by-product remaining after a crop of mushrooms. About 11 bacterial species were isolated from fresh SMS on TSA medium. Among them, one isolate was produced cellulase and xylanase as hydrolyase. The strain grew at temperatures of 20-60°C and pH 5.0-7.0. Chemotaxonomic data (G+C content: 44%, major fatty acids: anteiso-C14:0, C15:0, and iso-C16:0) supported the affiliation of the isolate to the genus Bacillus. Analysis of comparative 16S rDNA sequence showed that the isolate formed a distinct phylogenetic tree within the genus Bacillus and was most closely related to Bacillus subtilis with 98% of 16S rDNA sequence similarity. Based on phenotype, chemotaxonomic characteristics and phylogenetic inference, this isolate was assigned to the genus Bacillus subtilis.

Keywords: Bacillus subtilis, Cellulase, Pleurotus ostreatus, Spent mushroom substrates, Xylanase

J-72

Evaluation of Commercial Probiotics for Animal from Korea Using a Barcoded Pyrosequencing

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Labeling on commercial probiotic products for animal feeding often do not represent the true microbiological content. The aim of this work was to verify the commercial probiotic products for animal feeding. Probiotic samples were collected twenty-four commercial probiotics from sixteen brands. Bacterial communities were analyzed using a barcoded pyrosequencing approach. The bacterial 16S rDNA genes of probiotic samples were PCR-amplified by using a universal bacterial primer set (27F/518R) with a unique identifying barcode for each sample, allowing us to analyze all of the amplified samples in a single pyrosequencing run. Pyrosequencing data were processed and classified using RDP pipeline (http://pyro.cme.msu.edu) and potential pathogenic bacteria were found by Blasts. Microorganisms labeled on the products were mainly constituted of Bacillus, Clostridium (Bacteria) and Saccharomycyes (Yeast). The pyrosequencing results indicated that some probiotics were similar between bacteria claimed on the label and classification results; however, discrepancies were also observed in other probiotics. Almost probiotics showed presence of not declared bacteria. About 2% of the total sequences were closely related to known pathogenic bacteria sequence, including Pseudomonas aeruginosa, Burkholderia cepacia and Escherichia coli. Additionally, detection of virulence genes of the potential pathogenic bacteria by PCR will be performed and discussed.

Keywords: barcoded pyrosequencing, probiotics, pathogenic bacteria
Pedobacter humicola sp. nov., Isolated from Soil of Hwaseong in South Korea
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An aerobic, Gram staining negative, oxidase-negative, catalase-positive, non-motile, non-spor-forming, rod-shaped, light pink-pigmented bacterium designated strain R135T was isolated from soil in Hwaseong, South Korea. Flexinbin-type pigments were absent. A phylogenetic analysis based on its 16S rRNA gene sequence revealed that strain R135T formed a lineage within the family Sphingobacteriaceae of the phylum Bacteroidetes that was distinct from the most closely related to various species of the genus Pedobacter (98.13% sequence similarity), Pedobacter jeongganensis BH45T (97.71% sequence similarity), Pedobacter rusean CG-GP80T (97.24% sequence similarity), Pedobacter yonginense HMD1002T (97.14% sequence similarity), Pedobacter sanakohulus DS-27T (96.95% sequence similarity), Pedobacter yonginensis KACC 16221T (96.31% sequence similarity), and Pedobacter soli 15-5T (96.11% sequence similarity). The major isoprenoid quinone was Menaquione-7 (MK-7) and major polar lipids were phosphatidylethanolamine (PE). The major cellular fatty acids were summed feature 3 (C16:1ω7c and/or C16:1ω6c; 37.0%), iso-C15:0 (19.2%), iso-C17:0 3-OH (8.1%), and C16:0 (6.0%). The DNA G+C content of strain R135T was 40.4 mol%. On the basis of phenotypic, genotypic and phylogenetic analysis, strain R135T represents a novel species of genus Pedobacter, for which the name Pedobacter humicola sp. nov. is proposed. Keywords: Pedobacter humicola sp. nov., Bacteroidetes, taxonomy, soil

Variovorax soli sp. nov., Isolated from Forestry Soil Using Modified Uncultured Method
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Department of Life Science, Kyonggi University

Growth of strains UC10T and UC38T occurred at pH 5–9 in a temperature range between 4°C and 40°C and in the presence of 0–5% (w/v) NaCl on R2A agar plates. Colonies on the agar plates were tiny, yellow, and convex after 5-day incubation at 28°C. Comparative analysis of the nearly full-length 16S rRNA gene sequences were closely related to Variovorax guangxiensis GXGD002T with similarities of 98.57-98.64%; 98.01-98.04% with V. soli GIH 9-3T; 97.75-97.82% with V. dokdonensis DS-43T; 97.32-97.72% with V. ginsengisoli GSol 3165T; 97.68-97.95% with V. paradoxae IAM 12373T; 97.43-97.64% with V. defluvii 2C1-bT; 97.28-97.41% with V. horiconiumcule BAM-48T. The predominant ubiquinone is Q-8. The primary polar lipids were diphostatidylglycerol, phosphatidylethanolamime, and phosphatidylglycerol. Genomic DNA similarity between strain UC10T and UC38T shared 89.20-94.3% DNA-DNA relatedness; and isolated strains showed levels of relatedness range from 29.15-38.92% with its reference strains. Based on these results, strain UC38T was designated a novel member of the genus Variovorax with the proposed name, Variovorax soli. Keywords: Variovorax soli sp. nov., taxonomy, forestry soil

Development of Nested PCR Assay for Detection of Mycosphaerella nawae in Korea
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Mycosphaerella nawae, causal agent of circular leaf spot which leading to severe yield losses on persimmon orchard. To diagnose circular leaf spot on persimmon, we designed specific primer for nested polymerase chain reaction (PCR) based on draft genome sequence of M. nawae KNU003 isolate. Among the obtained genomic DNA sequences, we selected specific region of M. nawae using the Blast search in NCBI comparing with other fungal pathogen. Several primer sets were tested against M. nawae genomic DNA, other fungal pathogens causing disease on persimmon, various persimmon cultivar leaves and allied species of M. nawae. Finally, primer sets were selected as Ghf-F1/Ghf-R1 (600 bp) for the first PCR, and Ghf-F5/Ghf-R5 (200bp) for the nested PCR base on hydroxyl acid related gene sequences. As the result, target DNA fragments were amplified in M. nawae genomic DNA while no targeted DNA fragments were amplified in other fungal pathogens and persimmon leaf DNA samples. Although two allied species were amplified among 12 allied species in first PCR primer sets, however, those species have not been reported in Korea yet. Considering its specificity, developed PCR assay can be recommended as the method in the diagnosis of circular leaf spot on persimmon. Keywords: Mycosphaerella nawae, nested PCR assay, Specific primer

Diagnosis of Various Types of Symptoms on Apple in Korea
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Up to now, it has been reported that 41 pathogens are associated with apple. Among those pathogen, two pathogens are considered as main disease on fruit; Apple anthracnose caused by Colletotrichum gloeosporioides and white rot caused by Botryosphaeria dothidea. Due to global warming and cropping system changes in recent years, there is high probability that unforeseen diseases can be occurred in apple orchards. In this study, we collected over 500 apple fruits which appearing abnormal symptoms such as discolored, brown spot, black rot and so on in nationwide from 2013-2015. It was hard to diagnosis them due to the symptoms were small and appearing like a little spot. To diagnose abnormal symptoms, we observe the surface of symptoms using light microscope, isolate the pathogen from the each of symptoms and then total genomic DNA was extracted from culture and PCR was conducted to analyze molecular marker gene. As the results, the isolated pathogen were identified as Alternaria spp., C. gloeosporioides, B. dothidea and Fusarium sp. with its conidial morphology. Also PCR analysis results were corresponded with morphological identification results. According to our results, it is needed to change the fungicide spray program which focus on not only apple anthracnose and white rot also other disease caused by Alternaria spp. and Fusarium sp.

Keywords: Abnormal fruit disease, Diagnosis, Alternaria spp.
**Degradation of Biogenic Amine by *Lactobacillus arizonensis* in Food**

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Biogenic amines are existed in all fermented food products and may have detrimental effects on the human health. Biogenic amines are therefore considered as the principal risk factor in foods. In this study, the probiotic strains were screened from Kimchi. Among many isolates, one isolate was identified by 16S rRNA as a *Lactobacillus arizonensis* strain. Next, the reduction of biogenic amine contents in foods was monitored by determining the biogenic amine contents by high performance liquid chromatography. The remarkable biogenic amine reduction was observed after BCNU 9200 treatment. In conclusion, this isolate BCNU 9200 might be usable as a potential functional starter culture for reducing biogenic amines in fermentative food manufacturing process.

Keywords: Probiotic bacteria, Biogenic amine, starter culture

**Degradation of Biogenic Amines by *Staphylococcus equorum* Isolate from Jeotgal**

Bo Ram Lim, Hye Jung Choi and Woo Hong Joo*
Department of Biology and Chemistry, Changwon National University, Changwon 51140

Biogenic amines are found in many fermentative food products and may have detrimental effects on the human health. In this study, the probiotic strains were screened from jeotgal. One isolate was identified using 16S rRNA sequencing as belonging to *Staphylococcus equorum*. This isolate BCNU 9200 was then used to reduce the biogenic amine contents in foods. The cell growth and biogenic amines were monitored during the cultivation period by UV-vis spectrophotometry and HPLC, respectively. This bacterium exhibits remarkable biogenic amine-reducing activities. Therefore, this isolate BCNU 1405 might have potential for use as functional starter cultures for reducing biogenic amines in fermentative food manufacture.

Keywords: Lactic acid bacteria, Biogenic amines, jeotgal, *Staphylococcus equorum*

**Bacillus subtilis BCNU 1330 Strain and its Use in Biogenic Amine Reduction**

Bo Ram Lim, Hye Jung Choi and Woo Hong Joo*
Department of Biology and Chemistry, Changwon National University, Changwon 51140

Biogenic amines are considered as the principal risk factor in foods, because biogenic amines are existed in all fermented food products and may have detrimental effects on the human health. In this study, many probiotic strains were isolated from Korean Doenjang. One isolate, among many isolates, was identified by 16S rRNA as a *Bacillus subtilis* strain. The reduction of biogenic amine contents in foods was monitored by high performance liquid chromatography. *Bacillus subtilis* BCNU 1330 was determined to reduce biogenic amines remarkably. Therefore, *Bacillus subtilis* BCNU 1330 strain might be a bioresource with potential for use in fermentative food manufacturing process.

Keywords: Probiotic bacteria, *Bacillus subtilis* BCNU 1330, iogenic amine reduction

**Reduction of Biogenic Amine by *Ocenobacillus Sojae* Isolate from Jeotgal**

Bo Ram Lim, Hye Jung Choi and Woo Hong Joo*
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All fermented food products possess the biogenic amines. Screening of many probiotic strains from jeotgal was attempted in the present study as a way of overcoming this problem of biogenic amines production in foods, since the biogenic amines in all fermented food products may have detrimental effects on the human health and are therefore considered as the principal risk factor in foods. Among many isolates, one isolate was identified by 16S rRNA as *Ocenobacillus sojae*. This isolate BCNU 1404 was then attempted in the reduction process of biogenic amine contents. During all cell cultivation, biogenic amines contents were monitored by high performance liquid chromatography. BCNU 1404 had exerted remarkable effects on the biogenic amine reduction. Therefore, this isolate BCNU 1404 might have potential for use as a functional starter culture for overcoming the principal risk factor in food manufacturing.

Keywords: Probiotic bacteria, Biogenic amines, jeotgal, *Ocenobacillus sojae*
Tyrosinase Inhibitory and Antioxidant Activities of Cryptococcus albidosimilis BCNU3010 Isolated from Persimmon
Sung Min Ha, Hye Jung Choi and Woo Hong Joo*
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Mushroom tyrosinase is a copper containing oxidase that catalyzes both the hydroxylation of tyrosine into o-diphenols and the oxidation of o-diphenols into o-quinones, and then forms brown or black pigments. In the present study, we isolated and selected one potential strain which show tyrosinase inhibitory activities. A selected yeast strain was identified by 18S rRNA sequencing as belonging to Cryptococcus albidosimilis. Antioxidative activities of culture filtrates were then examined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, reducing power and superoxide anion radical scavenging measurement. Tyrosinase inhibitory activities were also determined by following the ordinary methods. Optimal cultivation conditions were determined to be PDB broth medium, pH 7, 28°C, and 2 day. Tyrosinase and DPPH and superoxide anion radical inhibition after optimal cultivation of Cryptococcus albidosimilis BCNU3010 were 42.1%, 55.82% and 80.24%, respectively. Therefore, BCNU3010 may be potential resources for development of new cosmetics and for biomedical applications.

Keywords: Tyrosinase inhibitor, antioxidant activities, Cryptococcus albidosimilis

Evolution of CTX Phages of Vibrio cholerae
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Since the El Tor biotype strains first emerged in 1962, the classical biotype strains have been diminished around the world. The classical biotype strains are not isolated from the cholera patients since the 1980’s and considered to be extinct. The classical biotype strains and El Tor biotype strains typically harbored biotype specific cholera toxin phages (CTX) on their genome and produced biotype specific cholera toxin (CT). CTX phage genome is composed of 10 genes, rstR, rstA, rstB, pshA, cpa, orfY, ace, zo1, ctxAB. Classical biotype strains harbored CTX1 and El Tor biotype strains contained CTX1-2, or CTX-1. Two CTX phages differ by the rstR, that have entirely different DNA sequence, rstR1 and rstR2. Other genes are different between two phages by a number of SNPs. The El Tor biotype strains usually produced the biotype-specific cholera toxin, therefore, called prototype El Tor strains or Wave 1 strains of seventh cholera pandemic. However, the El Tor biotype strains containing the cholera toxin of the classical biotype strains have been recognized for the early 2000’s. These strains have been called as altered strains, El Tor variant, and atypical El Tor strains. The earliest isolation of the atypical El Tor strains is back to 1991 in Indian subcontinent. We analyzed DNA sequences of various CTX phages in F. cholerae O1 serogroup strains, including CTX-1, CTX-2, CTX-3, CTX-4, and a CTX phage in US Gulf Coast strain.

Keywords: CTX

Wild Mushroom Diversity in Dongbaekdongsan in Jeju Island
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Dongbaekdongsan located in Gotjawal Forest, North-eastern face of Mt. Halla in Jeju Island is the habitat for many nationally and internationally endangered species. The region includes Dongbaekdongsan wetland that has been designated as the Ramsar Wetland in March 14, 2011 for continuous preservation and protection. Therefore, it has been recognized for the importance and value of scientific research. However, there has been no research on mushroom species. In an effort to understand biodiversity in Dongbaekdongsan, we performed a day survey of mushroom in July, 2015. Approximately, 100 specimens of wild mushrooms were collected and identified based on morphology. In spite of a day survey, surprisingly, a total of 26 genera and 44 species were found. Major two genera were Amanita and Russula. Amanita genus included A. ceciliae, A. farinose, A. pantherina, A. rubescens, A. spissacea, and A. sp. While, Russula genus included R. abbovedulata, R. aurata, R. compacta, R. rubescens, R. subnigricans, and R. sp. The results of our study showed that although Dongbaekdongsan is volcanic and rocky ground environment, it is a good habitat of diverse wild mushroom. This is first report of mushroom diversity in Gotjawal Forest, an enclave of the Southern Korea evergreen forests ecoregion.

Keywords: Dogbaekdongsan, Wild mushroom, mushroom diversity, Gotjawal
Prevalence, Biochemical Properties, and Toxin Characterization of *Clostridium perfringens* Isolated from Beef in Meat Retailer Differing in Processing Temperature

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*Clostridium perfringens* is a foodborne pathogen inducing food poisoning and enteritis necroticans in human and a good hygienic indicator in food industry. The prevalence of *Cl. perfringens* in meat retailers have been actively studied to date, however, the impact of processing temperature of meat retailer on the prevalence of this bacterium have not been studied yet. The aims of the study were to compare of the prevalence of *Clostridium perfringens* in beef from meat retailer differing in processing temperature and to investigate the biochemical properties and toxin gene profiles of the isolates. Beef samples were purchased from 13 meat retailers which process beef under 15°C and 17 meat retailers which process over 15°C. Two beef samples were collected per market. *Cl. perfringens* were tested following Korean Food Code. Biochemical diversity and toxin genes was investigated for the isolates. There were no significant differences between the prevalence of *Cl. perfringens* in meat retailers processing beef under 15°C (2/26, 7.69%) and over 15°C (1/34, 2.94%). In biochemical diversity analysis, one isolate was highly similar with clinical strains and another isolate was highly similar with standard strain (> 90%). Toxin gene profiles were different between the isolates. Our data suggest that the processing temperature of meat retailer has only partial contribution on reducing the prevalence of *Cl. perfringens* in beef.

Keywords: *Clostridium perfringens*, prevalence, meat retailer, biochemical analysis, toxin profiling
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<td><strong>Main Technology &amp; Products</strong></td>
<td></td>
</tr>
<tr>
<td>Non-Defrosting with Fineless Condenser Refrigerator &amp; Cryogenic freezer applying mixed refrigerants double copper pipe</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hanil Sci-Med Co., Ltd.</th>
<th>Booth No.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>(주)한일사이메드</td>
<td>CEO Jeon Hyeonggon</td>
</tr>
<tr>
<td>TEL 042-825-4260</td>
<td>FAX 042-825-4263</td>
</tr>
<tr>
<td>Homepage <a href="mailto:hanil@scimed.co.kr">hanil@scimed.co.kr</a></td>
<td>E-mail <a href="mailto:hanil@scimed.co.kr">hanil@scimed.co.kr</a></td>
</tr>
<tr>
<td>Address 5-4, 48beong-gil, Songnim-ro, Yuseong-gu, Daejeon 305-358, Korea.</td>
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<tr>
<td><strong>Main Technology &amp; Products</strong></td>
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</tr>
<tr>
<td>Centrifuge &amp; Continuous Centrifuge, Continuous Filtering System</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>JinSung Uni-Tech. Co., Ltd.</th>
<th>Booth No.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>(주)진성유니텍</td>
<td>CEO Sungbin Jun</td>
</tr>
<tr>
<td>TEL</td>
<td>FAX</td>
</tr>
<tr>
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<td>E-mail <a href="mailto:sbjun@jsunitech.com">sbjun@jsunitech.com</a></td>
</tr>
<tr>
<td>Address #443, A complex, 140 Tongil-ro, Deogyang-gu, Goyang-si, gyeonggi-do 412-090, Korea</td>
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<td><strong>Main Technology &amp; Products</strong></td>
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</tr>
<tr>
<td>Anaerobic System (Anaerobic Chamber, Jar Gassing System, Gas pack etc..) Plastic Ware PCR Kits Prepared Media</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>JinSung Uni-Tech. Co., Ltd.</th>
<th>Booth No.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>기산바이오(주)</td>
<td>CEO J.W. SUN</td>
</tr>
<tr>
<td>TEL 02-529-2282</td>
<td>FAX 02-529-2284</td>
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<tr>
<td>Homepage</td>
<td>E-mail <a href="mailto:kisan@kisanbio.com">kisan@kisanbio.com</a></td>
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<tr>
<td>Address 2F, Kisan B/D, 11, Yangjaecheon-ro 31-gil, SeoCho-Gu, Seoul #137-890</td>
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<td><strong>Main Technology &amp; Products</strong></td>
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</tr>
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<td>Main Product : Microbial Media, Ready Media, Microbial Identification kit We provide various media according to the standards of MFDS, QIA, NIER and KP.</td>
<td></td>
</tr>
</tbody>
</table>
Korean Collection for Type Cultures (KCTC)  
Booth No.09

한국생명공학연구원 미생물자원센터  
CEO  
Doo-Sang Park

TEL  
FAX  
Homepage  
E-mail  
sales@kribb.re.kr

Address  
Korean Collection for Type Cultures (KCTC),  
Korea Research Institute of Bioscience and Biotechnology (KRIIBB)  
125 Gwahak-ro, Yuseong-gu, Daejeon, Korea, 305-806

Main Technology & Products
- Microorganism: Type strain, Reference strain, Patent strain  
- Cell lines: Animal cell line, Plant cell line  
- Identification of Microbe  
- Package service: Culture extract  
- Genomic DNA, Proteome for Microorganism  
- Metagenome library

Korea National Research Resource Center  
Booth No.06

(재)연구소재중앙센터  
CEO  
Yeonhee Lee

TEL  
FAX  
Homepage  
E-mail  
knrrc@knrrc.or.kr

Address  
#324, Golden 50 Commemoration Hall, Seoul Women’s University, Hwarang-ro 621,  
Nowon-gu, Seoul 01797, Korea

Main Technology & Products
The Korea National Research Resources Center Project is supported by Ministry of Science, ICT and Future Planning (MSIP) and National Research Foundation of Korea (NRF) since 1995. As of September 2015, Korea National Research Resource Center (KNRRC) consists of the headquarters, 5 core centers (human-originated resources, animals, plants, microorganisms, and fusion-matters), 2 research resource centers (RRC) of special purposes, and 32 RRCs. RRCs distribute various resources and accept deposit for preservation and distribution. Also, RRCs provide consulting services and conduct researches.

Life Science Publishing Co.  
Booth No.01

(주)라이프사이언스  
CEO  
Kim Hyo Joong

TEL  
FAX  
Homepage  
E-mail  
life@lifescience.co.kr

Address  
A-303 Halla APT Sangga, 9 Ttuseom-ro 35-gil, Gwangjin-gu, Seoul 143-878, Korea

Main Technology & Products
Text Book (Life science, Biology)
Exhibition

National Culture Collection for Pathogens  Booth No.02

CEO  Seong, won keun
TEL  043-719-6871
Fax  043-719-6871
Address  200, Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, Korea

Main Technology & Products
NCCP as the sole national pathogen bank of Korea established pathogen network contributing to invigoration of health care R&D research by distribution of collected and standardized pathogens (bacteria, fungi, viruses, parasites).

National Culture Collection for Pathogens  Booth No.21

CEO  Song, Ju-Seop
TEL  043-719-6871
Fax  02-2652-1500
Address  211, Mokdongseo-ro, Yangcheon-gu, Seoul, 158-051 Rep, of Korea

Main Technology & Products
Life Science Book
Medical Book
Health Science Book

SaeHyun-tech  Booth No.12

CEO  Woon Mo, Hwang
TEL  82-31-439-4226
Fax  82-31-439-4228
Address  Saehyun B/D 4F, 60-13, Doli-ro Danwon-gu, Ansan-city, Kyunggi-do, 425-83, Korea

Main Technology & Products
Microbiologics® Quality Control Microorganism Products

SolGent  Booth No.11

CEO  Hyun Kun Myong, Sung Jun Lee
TEL  +82-42-864-5695
Fax  +82-42-864-5690
Address  3F, 32 Techno 6-ro, Yuseong-gu, Daejeon, 305-509, Korea

Main Technology & Products
DNA/RNA Amplification (PCR Enzyme, RT & RT-PCR, qPCR, Isothermal Amplification)
Cloning (Cloning Kit, DNA Ligase)
Service (Sequencing, Genotyping)
### Exhibition

<table>
<thead>
<tr>
<th>Takara Korea Biomedical Inc.</th>
<th>Booth No.08</th>
</tr>
</thead>
<tbody>
<tr>
<td>다카라코리아바이오메디칼(주)</td>
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<tr>
<td><strong>TEL</strong> 02-2081-2525</td>
<td>Dong Geun Lee</td>
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<tr>
<td><strong>FAX</strong> 02-2081-2500</td>
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<tr>
<td><strong>Homepage</strong> <a href="http://www.takara.co.kr">www.takara.co.kr</a></td>
<td>E-mail</td>
</tr>
<tr>
<td><strong>Address</strong> #601, New T Castle, 108, Gasan digital 2-ro, Geumchun-gu, 153-779, Seoul</td>
<td><a href="mailto:support@takara.co.kr">support@takara.co.kr</a></td>
</tr>
</tbody>
</table>

**Main Technology & Products**

Takara PCR, qPCR reagents and instrument, Clontech, Lonza, Exiqon, Mupid, Titertek-Berthold spectrometer, luminometer, washer
Author Index
### Author Index

<table>
<thead>
<tr>
<th>Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhikari, Mahesh</td>
<td>J-10, S8-4</td>
</tr>
<tr>
<td>Ahmad, Shabir</td>
<td>B-42, YS2-3</td>
</tr>
<tr>
<td>Ahn, Byung-Koo</td>
<td>B-66</td>
</tr>
<tr>
<td>Ahn, Chiyoung</td>
<td>J-61</td>
</tr>
<tr>
<td>Ahn, Donghyun</td>
<td>G-10</td>
</tr>
<tr>
<td>Ahn, Dong-Hyun</td>
<td>G-6</td>
</tr>
<tr>
<td>Ahn, Geum Ran</td>
<td>J-84</td>
</tr>
<tr>
<td>Ahn, Hae Jun</td>
<td>C-22</td>
</tr>
<tr>
<td>Ahn, Jin Hyun</td>
<td>E-29</td>
</tr>
<tr>
<td>Ahn, Jin-Hyun</td>
<td>F-40, F-41, G-14, G-2, YS3-3</td>
</tr>
<tr>
<td>Ahn, Jung Oh</td>
<td>D-9</td>
</tr>
<tr>
<td>Ahn, Kwangseog</td>
<td>PL-3</td>
</tr>
<tr>
<td>Ahn, Min Ju</td>
<td>I-9, I-25, I-26, I-27, I-33</td>
</tr>
<tr>
<td>Ahn, Seung Il</td>
<td>B-43, J-41, J-42</td>
</tr>
<tr>
<td>Ahn, Sharon</td>
<td>A-41</td>
</tr>
<tr>
<td>Ahn, Tae-Young</td>
<td>A-36, A-38, A-40</td>
</tr>
<tr>
<td>Ahn, Ye-Hyeon</td>
<td>S5-2</td>
</tr>
<tr>
<td>Ali, Asjad</td>
<td>S12-1</td>
</tr>
<tr>
<td>Ali, Irshad</td>
<td>G-1</td>
</tr>
<tr>
<td>An, Byoung Rak</td>
<td>F-17</td>
</tr>
<tr>
<td>An, Due Rae</td>
<td>C-15</td>
</tr>
<tr>
<td>An, Jieun</td>
<td>D-7</td>
</tr>
<tr>
<td>An, Mi</td>
<td>J-36</td>
</tr>
<tr>
<td>Anirban, Jyoti Md</td>
<td>F-30</td>
</tr>
<tr>
<td>Bae, Hyeun-Jong</td>
<td>H-8</td>
</tr>
<tr>
<td>Bae, Jin-Woo</td>
<td>B-23</td>
</tr>
<tr>
<td>Bae, Joon-Yong</td>
<td>S15-2</td>
</tr>
<tr>
<td>Bae, Minsuk</td>
<td>J-3</td>
</tr>
<tr>
<td>Bae, Nanyoung</td>
<td>G-10</td>
</tr>
<tr>
<td>Bae, Nan-Young</td>
<td>G-6</td>
</tr>
<tr>
<td>Bae, Sang-Min</td>
<td>A-27</td>
</tr>
<tr>
<td>Bae, Yong-Soo</td>
<td>F-72, G-18</td>
</tr>
<tr>
<td>Bae, Yu-Ra</td>
<td>B-49</td>
</tr>
<tr>
<td>Baek, Kyunghwa</td>
<td>B-67</td>
</tr>
<tr>
<td>Baek, Min-Jeong</td>
<td>E-26, E-27</td>
</tr>
<tr>
<td>Baek, Seong Yeol</td>
<td>D-11</td>
</tr>
<tr>
<td>Baek, Song Yeol</td>
<td>D-28</td>
</tr>
<tr>
<td>Baek, Song-Yi</td>
<td>B-32</td>
</tr>
<tr>
<td>Baek, Youlchang</td>
<td>B-3</td>
</tr>
<tr>
<td>Bahn, Yong-Sun</td>
<td>LS-1</td>
</tr>
<tr>
<td>Baik, Keunsk</td>
<td>A-43</td>
</tr>
<tr>
<td>Bak, Gyeryeong</td>
<td>B-12</td>
</tr>
<tr>
<td>Bak, Won Chull</td>
<td>I-11, I-13</td>
</tr>
<tr>
<td>Bak, Wonchull</td>
<td>C-9</td>
</tr>
<tr>
<td>Bai, Jyotiranjan</td>
<td>D-10, D-20</td>
</tr>
<tr>
<td>Bang, Iel Soo</td>
<td>F-81, F-82</td>
</tr>
<tr>
<td>Bang, John J.</td>
<td>S23-1</td>
</tr>
<tr>
<td>Bang, Woo Young</td>
<td>A-31</td>
</tr>
<tr>
<td>Bang, Wooyoung</td>
<td>H-10</td>
</tr>
<tr>
<td>Batjargal, Uziltuya</td>
<td>I-22, I-23</td>
</tr>
<tr>
<td>Beatty, Deanna</td>
<td>A-25</td>
</tr>
<tr>
<td>Beom, Ji Yoon</td>
<td>C-18</td>
</tr>
<tr>
<td>Berg, Leslie J.</td>
<td>S18-2</td>
</tr>
<tr>
<td>Bhak, Jong</td>
<td>F-35</td>
</tr>
<tr>
<td>Bong, Ki Moon</td>
<td>D-23</td>
</tr>
<tr>
<td>Canete, Rachelle</td>
<td>H-26</td>
</tr>
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<td>Carroll, Emily</td>
<td>I-31</td>
</tr>
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<td>Cha, Chang-Jun</td>
<td>S9-1</td>
</tr>
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<td>B-55</td>
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<td>H-42</td>
</tr>
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<td>Cha, In-Tae</td>
<td>E-2, I-6</td>
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<td>Cha, Ju-Hee</td>
<td>S9-1</td>
</tr>
<tr>
<td>Cha, Kiweon</td>
<td>F-59</td>
</tr>
<tr>
<td>Cha, KyoungEun</td>
<td>J-48</td>
</tr>
<tr>
<td>Cha, Seung Hae</td>
<td>F-63</td>
</tr>
<tr>
<td>Chae, Jong-Chan</td>
<td>A-28, B-47, S9-2</td>
</tr>
<tr>
<td>Chae, Su-Jin</td>
<td>F-46</td>
</tr>
<tr>
<td>Chae, Tong Un</td>
<td>D-2</td>
</tr>
<tr>
<td>Chang, Chi Young</td>
<td>S5-2</td>
</tr>
<tr>
<td>Chang, Dong-Ho</td>
<td>A-41, E-25</td>
</tr>
<tr>
<td>Chang, Jaesoo</td>
<td>B-21, B-22</td>
</tr>
<tr>
<td>Chang, Minhee</td>
<td>H-44</td>
</tr>
<tr>
<td>Chaudhary, Dhiraj Kumar</td>
<td>B-61</td>
</tr>
<tr>
<td>Cheng, Jinhua</td>
<td>E-23</td>
</tr>
<tr>
<td>Cheon, Seon Ah</td>
<td>H-12</td>
</tr>
<tr>
<td>Cheong, Woo-Chang</td>
<td>F-45, F-78</td>
</tr>
<tr>
<td>Cho, Ahnna</td>
<td>S2-4</td>
</tr>
<tr>
<td>Cho, Dong-Hyun</td>
<td>S16-3</td>
</tr>
<tr>
<td>Cho, Eun Hee</td>
<td>LS-2</td>
</tr>
<tr>
<td>Cho, Eun Jin</td>
<td>H-8</td>
</tr>
<tr>
<td>Cho, Eunjuong</td>
<td>I-5</td>
</tr>
<tr>
<td>Cho, Eun-Min</td>
<td>S23-2</td>
</tr>
<tr>
<td>Cho, Geonyeong</td>
<td>J-68, J-69, J-70, S18-4</td>
</tr>
<tr>
<td>Cho, Hae Jin</td>
<td>J-69</td>
</tr>
<tr>
<td>Cho, Han Ah</td>
<td>J-69</td>
</tr>
<tr>
<td>Cho, Hansam</td>
<td>E-21, E-23</td>
</tr>
<tr>
<td>Cho, Hee-Jung</td>
<td>J-31</td>
</tr>
<tr>
<td>Cho, Hye In</td>
<td>J-94</td>
</tr>
<tr>
<td>Cho, Hyun-Soo</td>
<td>G-12</td>
</tr>
<tr>
<td>Cho, Jae-Chang</td>
<td>B-2, B-4</td>
</tr>
<tr>
<td>Cho, Jae-Han</td>
<td>A-30</td>
</tr>
<tr>
<td>Cho, Jaeyeong</td>
<td>J-66</td>
</tr>
<tr>
<td>Cho, Jin Won</td>
<td>H-12</td>
</tr>
<tr>
<td>Cho, Kye Man</td>
<td>I-1, I-25, I-26, I-27, I-33</td>
</tr>
<tr>
<td>Cho, Kyung-Ah</td>
<td>B-29, B-30</td>
</tr>
<tr>
<td>Cho, Kyuseon</td>
<td>S1-3</td>
</tr>
<tr>
<td>Cho, Myung-Je</td>
<td>F-79</td>
</tr>
<tr>
<td>Cho, Nam-Hyuk</td>
<td>S18-2</td>
</tr>
<tr>
<td>Cho, Sang-Nae</td>
<td>E-1</td>
</tr>
<tr>
<td>Cho, Sang-Woo</td>
<td>I-20</td>
</tr>
<tr>
<td>Cho, Seongyeong</td>
<td>B-25</td>
</tr>
<tr>
<td>Cho, Seung-Hak</td>
<td>G-22</td>
</tr>
<tr>
<td>Cho, Seungsk</td>
<td>I-5, J-3</td>
</tr>
<tr>
<td>Cho, Soo-Young</td>
<td>I-8</td>
</tr>
<tr>
<td>Cho, Soo Jeong</td>
<td>J-14, J-71</td>
</tr>
<tr>
<td>Cho, Sukyung</td>
<td>J-66</td>
</tr>
<tr>
<td>Cho, Sungjin</td>
<td>A-5</td>
</tr>
<tr>
<td>Cho, Yeondong</td>
<td>J-68, J-69, J-70, S18-4</td>
</tr>
<tr>
<td>Cho, Yong Gon</td>
<td>F-79</td>
</tr>
<tr>
<td>Cho, Yong-Joon</td>
<td>E-1</td>
</tr>
<tr>
<td>Cho, Yongkoo</td>
<td>J-38</td>
</tr>
<tr>
<td>Cho, You-Hee</td>
<td>S10-1</td>
</tr>
<tr>
<td>Cho, Youngae</td>
<td>F-29</td>
</tr>
<tr>
<td>Choe, Byed</td>
<td>A-18, A-59</td>
</tr>
<tr>
<td>Choe, Ae Ran</td>
<td>C-20</td>
</tr>
<tr>
<td>Choe, Ahyoung</td>
<td>A-62</td>
</tr>
<tr>
<td>Choe, Bong-Kyu</td>
<td>F-11, F-54</td>
</tr>
<tr>
<td>Choe, Bongkyu</td>
<td>F-55</td>
</tr>
<tr>
<td>Choe, Chang Uk</td>
<td>I-37</td>
</tr>
<tr>
<td>Choe, Chul Hee</td>
<td>G-21</td>
</tr>
<tr>
<td>Choe, Chul-Hae</td>
<td>G-9, G-13</td>
</tr>
<tr>
<td>Choe, Dahye</td>
<td>D-8</td>
</tr>
<tr>
<td>Choe, Dassom</td>
<td>I-28</td>
</tr>
<tr>
<td>Choe, Eun-Ha</td>
<td>E-31</td>
</tr>
<tr>
<td>Choe, Eun-Ji</td>
<td>F-56</td>
</tr>
<tr>
<td>Choe, Eun-Kyoung</td>
<td>F-24, F-25</td>
</tr>
<tr>
<td>Choe, Eun-Sun</td>
<td>F-58, F-59</td>
</tr>
<tr>
<td>Choe, Geun</td>
<td>H-44</td>
</tr>
<tr>
<td>Choe, Grace Eun Ju</td>
<td>A-25</td>
</tr>
<tr>
<td>Choe, Hak-Jong</td>
<td>D-14</td>
</tr>
<tr>
<td>Choe, Hang-Seek</td>
<td>B-31</td>
</tr>
<tr>
<td>Choe, Han-Gyu</td>
<td>G-11, G-20, G-21</td>
</tr>
<tr>
<td>Choe, Hanul</td>
<td>J-68, S18-4</td>
</tr>
<tr>
<td>Choe, Hyey Young</td>
<td>J-49, S22-1</td>
</tr>
<tr>
<td>Choe, Hong-Seek</td>
<td>F-24, F-25</td>
</tr>
</tbody>
</table>

Choi, Hye Jung | I-36 |
Choi, Hyeong Tae | C-2 |
Choi, Hyun Bae | B-14 |
Choi, Hyung Yell | F-63 |
Choi, In Seong | H-8 |
Choi, Induck | J-17 |
Choi, In-Ged | E-8, E-21, E-26, E-27 |
Choi, Jae Hee | I-10, J-41, J-42 |
Choi, Jway Woon | S6-1 |
Choi, Jaelm | B-34 |
Choi, Jaeyong | E-20 |
Choi, Janggyu | B-12 |
Choi, Jeong-Soo | J-43 |
Choi, Ji Hyun | H-35 |
Choi, Jihye | I-3 |
Choi, Jin Myung | C-31 |
Choi, Jinhwe | B-42, YS2-3 |
Choi, Jin-Hwan | I-20 |
Choi, Jintack | B-55 |
Choi, Jong Deok | B-14 |
Choi, Jong-il | E-7 |
Choi, Jong-Soo | B-10, S14-3 |
Choi, Jung Sup | D-26 |
Choi, Jung Hye | S9-1 |
Choi, Jung Sub | D-14 |
Choi, Kyung Hee | J-35, YS3-1 |
Choi, Kyunghwa | J-65 |
Choi, Min-Jin | H-26 |
Choi, Min-Young | B-23, B-65, B-66 |
Choi, Moon-Tae | B-66 |
Choi, Myoung-Ju | F-25 |
Choi, Okhee | J-66 |
Choi, Sang Ho | I-4, YS1-4 |
Choi, Sanghwa | F-20 |
Choi, Seon | A-43 |
Choi, Seong Yeo | B-34, D-4, D-15, YS4-4 |
Choi, SeOUNg | G-11, G-20 |
Choi, Seung-Chul | B-66 |
Choi, Sol | D-2, H-1, H-3 |
Choi, Soo In | F-10 |
Choi, Soo Young | E-1 |
Choi, Soohee | F-84 |
Choi, Soo-jeong | H-34, I-18 |
Choi, Soo-Keun | S6-2 |
Choi, Sungmi | A-71 |
Choi, Sungyol | J-83 |
Choi, Won Il | B-66 |
Choi, Woosuk | S13-2 |
Choi, Woo Young | F-13, F-36, F-37 |
Choi, Wooyong | F-14 |
Choi, Yejin | J-41, J-42 |
Choi, Yasun | I-7 |
<table>
<thead>
<tr>
<th>Author Index</th>
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<tbody>
<tr>
<td>Choi, Yong Seon</td>
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<th>Author Index</th>
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<td>Elgabbar, Mohammed A, Abdo</td>
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</table>

<table>
<thead>
<tr>
<th>Author Index</th>
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<tbody>
<tr>
<td>Fenical, William</td>
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<td>Fong, Jonathan J.</td>
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<td>Hahn, Yoonsoo</td>
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<td>Halda, Josef</td>
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<td>Han, Dong Min</td>
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<tr>
<td>Jin, Hyun Mi</td>
</tr>
</tbody>
</table>

Author Index

Joo, Woohong: I-36
Joo, Youn-Lee: A-18, A-59
Joung, Wans-Kyu: S12-1
Joo, Yoon Jung: C-19
Jung, Moon-Beom: D-25, D-26
Jung, Yu Jong Ran: F-3, F-36, YS3-2
Jung, Hyo-Eun: F-11
Jung, Hyo-Kiyoung: F-55
Jung, Jee-Hun: E-16, S8-3
Jung, Joo-Jung: F-22, F-23
Jung, Joo-Kyu: F-9, YS3-4
Jung, Joo-Nyung: C-18
Jung, Joo-Yong: I-20
Jung, Jun-Kyu: S21-3
Jung, Jung-Hyun: E-7
Jung, Kikyung: J-61
Jung, Kwang-Hwan: F-70
Jung, Kwang-Soo: F-54
Jung, Kyeong Cheon: S18-1
Jung, Kyung: B-53, I-16
Jung, Kyu Seok: A-55, E-30, I-34
Jung, Myung-Hwan: F-48, H-11
Jung, Myung-Soon: A-11
Jung, Myung-Jung: F-47
Jung, Paul Eunil: A-48
Jung, Seun-Ho: J-47, S22-2
Jung, Subin: C-29
Jung, Suk-Yul: H-44, H-46
Jung, Sun-Mi: F-43, F-44, G-22
Jung, Sunyoung: F-47
Jung, Yong-Tae: F-63
Jung, Young-Jung: F-11
Jung, Yu-Jin: F-16, F-21, S21-2
Jyoti, Md. Anirban: F-86
<table>
<thead>
<tr>
<th>Author Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="http://www.fkms.kr">www.fkms.kr</a></td>
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<td>373</td>
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</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
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</thead>
<tbody>
<tr>
<td>Kim, Byoung-Chan</td>
<td>A-41, E-25</td>
</tr>
<tr>
<td>Kim, Byoung-Han</td>
<td>J-43</td>
</tr>
<tr>
<td>Kim, Byoung-Han</td>
<td>A-45, B-15, J-15</td>
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<td>J-6, J-40</td>
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<td>Kim, Byoung-Han</td>
<td>A-45, B-15, J-15</td>
</tr>
<tr>
<td>Kim, Byoung-Jun</td>
<td>F-49, J-63</td>
</tr>
<tr>
<td>Kim, Byoung-Chul</td>
<td>F-45</td>
</tr>
<tr>
<td>Kim, Byung-Chul</td>
<td>F-35</td>
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<tr>
<td>Kim, Byung Joo</td>
<td>I-9, I-25, I-26, I-27</td>
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<tr>
<td>Kim, Byung-Soo</td>
<td>A-10, B-8</td>
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<tr>
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<td>J-41, J-42</td>
</tr>
<tr>
<td>Kim, Cha Soon</td>
<td>F-8, F-26</td>
</tr>
<tr>
<td>Kim, Chang Sun</td>
<td>A-49, A-54</td>
</tr>
<tr>
<td>Kim, Chang Su</td>
<td>H-5, H-31</td>
</tr>
<tr>
<td>Kim, Changho</td>
<td>J-38</td>
</tr>
<tr>
<td>Kim, Chang-Jin</td>
<td>D-25, D-26</td>
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<tr>
<td>Kim, Changmu</td>
<td>A-2, H-10</td>
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<tr>
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<td>F-3, YS3-2</td>
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<tr>
<td>Kim, Chul Hwan</td>
<td>J-14, J-71</td>
</tr>
<tr>
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<td>S24-3</td>
</tr>
<tr>
<td>Kim, Dae Jung</td>
<td>H-35</td>
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<tr>
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<td>I-14</td>
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<td>F-38, F-39</td>
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<tr>
<td>Kim, Da Shin</td>
<td>A-3, S2-3</td>
</tr>
<tr>
<td>Kim, Da-Wei</td>
<td>S9-1</td>
</tr>
<tr>
<td>Kim, Da- Won</td>
<td>D-21, D-22</td>
</tr>
<tr>
<td>Kim, Daewoon</td>
<td>J-11</td>
</tr>
<tr>
<td>Kim, Dajeong</td>
<td>I-29</td>
</tr>
<tr>
<td>Kim, Daker</td>
<td>B-19, J-57, J-58</td>
</tr>
<tr>
<td>Kim, Dockyu</td>
<td>B-20</td>
</tr>
<tr>
<td>Kim, Dohak</td>
<td>A-38</td>
</tr>
<tr>
<td>Kim, Dong Han</td>
<td>F-63</td>
</tr>
<tr>
<td>Kim, Dong Wook</td>
<td>J-83</td>
</tr>
<tr>
<td>Kim, Dong Ho</td>
<td>E-7</td>
</tr>
<tr>
<td>Kim, Dongho</td>
<td>F-73, F-74</td>
</tr>
<tr>
<td>Kim, Donghwan</td>
<td>S15-2, J-85</td>
</tr>
<tr>
<td>Kim, Dong-Hyeon</td>
<td>I-28, YS2-4</td>
</tr>
<tr>
<td>Kim, Donghyun</td>
<td>F-42</td>
</tr>
<tr>
<td>Kim, Dongin</td>
<td>H-1</td>
</tr>
<tr>
<td>Kim, Dong-Uk</td>
<td>B-26</td>
</tr>
<tr>
<td>Kim, Duwoon</td>
<td>I-8, S14-3</td>
</tr>
<tr>
<td>Kim, Eui- Joong</td>
<td>S6-2</td>
</tr>
<tr>
<td>Kim, Eun Ji</td>
<td>C-30</td>
</tr>
<tr>
<td>Kim, Eun Jin</td>
<td>J-83</td>
</tr>
<tr>
<td>Kim, Eun Young</td>
<td>H-23</td>
</tr>
<tr>
<td>Kim, Eung Sook</td>
<td>H-27</td>
</tr>
<tr>
<td>Kim, Eunbin</td>
<td>A-11, B-20</td>
</tr>
<tr>
<td>Kim, Eun-Ha</td>
<td>F-56</td>
</tr>
<tr>
<td>Kim, Eunji</td>
<td>A-71</td>
</tr>
<tr>
<td>Kim, Eun-Jin</td>
<td>D-5</td>
</tr>
<tr>
<td>Kim, Eunju</td>
<td>I-7</td>
</tr>
<tr>
<td>Kim, Eun-Sun</td>
<td>A-8, E-8</td>
</tr>
<tr>
<td>Author Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Kim, Ga-Na</td>
<td>F-49, J-63</td>
</tr>
<tr>
<td>Kim, Gi Eun</td>
<td>B-7, B-35</td>
</tr>
<tr>
<td>Kim, Gieun</td>
<td>B-17, B-39</td>
</tr>
<tr>
<td>Kim, Gu Hwan</td>
<td>J-1</td>
</tr>
<tr>
<td>Kim, Gyu Hyoek</td>
<td>H-33</td>
</tr>
<tr>
<td>Kim, Hae-Ryoung</td>
<td>B-37</td>
</tr>
<tr>
<td>Kim, Hanbok</td>
<td>G-5</td>
</tr>
<tr>
<td>Kim, Haneul</td>
<td>A-67</td>
</tr>
<tr>
<td>Kim, Hansol</td>
<td>A-38</td>
</tr>
<tr>
<td>Kim, Han-Sol</td>
<td>F-63</td>
</tr>
<tr>
<td>Kim, Hanwo</td>
<td>B-20</td>
</tr>
<tr>
<td>Kim, Hae-Jun</td>
<td>F-24</td>
</tr>
<tr>
<td>Kim, Hae-Kwon</td>
<td>B-66</td>
</tr>
<tr>
<td>Kim, Heenam</td>
<td>S16-4</td>
</tr>
<tr>
<td>Kim, Hey-Min</td>
<td>YS2-1</td>
</tr>
<tr>
<td>Kim, Hong</td>
<td>F-60, F-61</td>
</tr>
<tr>
<td>Kim, Hong Chul</td>
<td>J-71</td>
</tr>
<tr>
<td>Kim, Hong Gi</td>
<td>A-57, A-61</td>
</tr>
<tr>
<td>Kim, Hong-Jin</td>
<td>H-17</td>
</tr>
<tr>
<td>Kim, Hong-Seok</td>
<td>I-28, J-85</td>
</tr>
<tr>
<td>Kim, Hwa Jung</td>
<td>S11-2</td>
</tr>
<tr>
<td>Kim, Hwa-Jung</td>
<td>F-69, G-9, G-11, G-13, G-20, G-21</td>
</tr>
<tr>
<td>Kim, Hyangmi</td>
<td>A-62</td>
</tr>
<tr>
<td>Kim, Hye Jin</td>
<td>F-83</td>
</tr>
<tr>
<td>Kim, Hye Kyung</td>
<td>J-9, J-12</td>
</tr>
<tr>
<td>Kim, Hye Soo</td>
<td>J-14, J-71, F-79</td>
</tr>
<tr>
<td>Kim, Hye-Jin</td>
<td>B-2, B-4, J-5</td>
</tr>
<tr>
<td>Kim, Hyejin</td>
<td>S15-2</td>
</tr>
<tr>
<td>Kim, Hyeong Jin</td>
<td>C-22</td>
</tr>
<tr>
<td>Kim, Hyeong Min</td>
<td>B-51</td>
</tr>
<tr>
<td>Kim, Hyeon-Jeong</td>
<td>I-12</td>
</tr>
<tr>
<td>Kim, Hyeshin</td>
<td>A-26</td>
</tr>
<tr>
<td>Kim, Hyoij</td>
<td>S21-2</td>
</tr>
<tr>
<td>Kim, Hyoung-Pyo</td>
<td>S5-1</td>
</tr>
<tr>
<td>Kim, Hyun</td>
<td>B-26</td>
</tr>
<tr>
<td>Kim, Hyun Ju</td>
<td>C-10, E-32</td>
</tr>
<tr>
<td>Kim, Hyun Jung</td>
<td>I-30</td>
</tr>
<tr>
<td>Kim, Hyun Seung</td>
<td>J-10, J-39, S4-4, S8-4</td>
</tr>
<tr>
<td>Kim, Hyun Uk</td>
<td>D-3</td>
</tr>
<tr>
<td>Kim, Hyun Woo</td>
<td>F-17, F-18, J-27, J-64</td>
</tr>
<tr>
<td>Kim, Hyung Sun</td>
<td>F-83</td>
</tr>
<tr>
<td>Kim, Hyung-Seok</td>
<td>S5-2</td>
</tr>
<tr>
<td>Kim, Hyunji</td>
<td>F-24</td>
</tr>
<tr>
<td>Kim, Hyun-Joo</td>
<td>J-43</td>
</tr>
<tr>
<td>Kim, Hyun-Sea</td>
<td>F-14</td>
</tr>
<tr>
<td>Kim, Hyun-Sook</td>
<td>B-41</td>
</tr>
<tr>
<td>Kim, Hyunuk</td>
<td>H-1, H-3</td>
</tr>
<tr>
<td>Kim, In Hwang</td>
<td>F-27, F-70</td>
</tr>
<tr>
<td>Kim, In-Hwang</td>
<td>F-71</td>
</tr>
<tr>
<td>Kim, In Kyoo</td>
<td>E-12</td>
</tr>
<tr>
<td>Kim, In Soo</td>
<td>B-21</td>
</tr>
<tr>
<td>Kim, Insu</td>
<td>B-22</td>
</tr>
<tr>
<td>Kim, Jae Bum</td>
<td>S16-1</td>
</tr>
<tr>
<td>Author Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Kim, Kangmin</td>
<td>B-47</td>
</tr>
<tr>
<td>Kim, Ki Daek</td>
<td>I-15, J-19</td>
</tr>
<tr>
<td>Kim, Ki Woo</td>
<td>S4-2</td>
</tr>
<tr>
<td>Kim, Ki Yoon</td>
<td>E-4, E-5, E-6, E-10</td>
</tr>
<tr>
<td>Kim, Ki-Bum</td>
<td>E-31</td>
</tr>
<tr>
<td>Kiju</td>
<td>F-29</td>
</tr>
<tr>
<td>Kothbongwoori</td>
<td>G-10</td>
</tr>
<tr>
<td>Kim, Kunho</td>
<td>B-52</td>
</tr>
<tr>
<td>Kim, Kun-Soo</td>
<td>F-27, F-70, F-71</td>
</tr>
<tr>
<td>Kim, Kwang Kyu</td>
<td>A-3</td>
</tr>
<tr>
<td>Kim, Kwang-Ho</td>
<td>A-69, B-64</td>
</tr>
<tr>
<td>Kim, Kwangseok</td>
<td>C-9</td>
</tr>
<tr>
<td>Kim, Kyong Su</td>
<td>E-3</td>
</tr>
<tr>
<td>Kim, Kyooong</td>
<td>B-18</td>
</tr>
<tr>
<td>Kim, Kyung Mo</td>
<td>S1-2</td>
</tr>
<tr>
<td>Kim, Kyung-Soo</td>
<td>A-8</td>
</tr>
<tr>
<td>Kim, Mal Nam</td>
<td>B-1, B-5</td>
</tr>
<tr>
<td>Kim, Man Su</td>
<td>B-9, B-14</td>
</tr>
<tr>
<td>Kim, Marie</td>
<td>A-29</td>
</tr>
<tr>
<td>Kim, Mi Hee</td>
<td>J-4</td>
</tr>
<tr>
<td>Kim, Mi Hyun</td>
<td>J-59</td>
</tr>
<tr>
<td>Kim, Mi Jin</td>
<td>F-85</td>
</tr>
<tr>
<td>Kim, Mi Jung</td>
<td>J-26</td>
</tr>
<tr>
<td>Kim, Mihyun</td>
<td>I-5</td>
</tr>
<tr>
<td>Kim, Min Ho</td>
<td>E-29</td>
</tr>
<tr>
<td>Kim, Min Jeong</td>
<td>S6-1</td>
</tr>
<tr>
<td>Kim, Min Soo</td>
<td>B-33, F-75, F-76</td>
</tr>
<tr>
<td>Kim, Mincheol</td>
<td>S2-4</td>
</tr>
<tr>
<td>Kim, Min-jeong</td>
<td>B-6</td>
</tr>
<tr>
<td>Kim, Min-Ji</td>
<td>B-68, G-6</td>
</tr>
<tr>
<td>Kim, Minji</td>
<td>G-10</td>
</tr>
<tr>
<td>Kim, Minjin</td>
<td>H-41</td>
</tr>
<tr>
<td>Kim, Minju</td>
<td>B-36</td>
</tr>
<tr>
<td>Kim, Min-Keun</td>
<td>S12-1</td>
</tr>
<tr>
<td>Kim, Min-Kyeong</td>
<td>A-7, A-44</td>
</tr>
<tr>
<td>Kim, Minseok</td>
<td>B-3, E-13</td>
</tr>
<tr>
<td>Kim, Min-Sik</td>
<td>C-20</td>
</tr>
<tr>
<td>Kim, Min-Soo</td>
<td>B-27</td>
</tr>
<tr>
<td>Kim, Minsoo</td>
<td>D-6</td>
</tr>
<tr>
<td>Kim, Min-Woo</td>
<td>F-75, F-76</td>
</tr>
<tr>
<td>Kim, Misun</td>
<td>A-42</td>
</tr>
<tr>
<td>Kim, Moonjong</td>
<td>E-3</td>
</tr>
<tr>
<td>Kim, Myoun Su</td>
<td>C-30</td>
</tr>
<tr>
<td>Kim, Myoung-Dong</td>
<td>D-27, H-47</td>
</tr>
<tr>
<td>Kim, Myoung Hee</td>
<td>I-4, S17-4, YS1-4</td>
</tr>
<tr>
<td>Kim, Myoung-Gyou</td>
<td>J-9, J-12</td>
</tr>
<tr>
<td>Kim, Myungkil</td>
<td>D-7</td>
</tr>
<tr>
<td>Kim, Myung-Kil</td>
<td>H-9</td>
</tr>
<tr>
<td>Kim, Nam Kyu</td>
<td>J-49, S22-1</td>
</tr>
<tr>
<td>Kim, Nan-Ok</td>
<td>F-43, F-44</td>
</tr>
<tr>
<td>Kim, Nan-Young</td>
<td>D-5</td>
</tr>
<tr>
<td>Kim, Ok-Sun</td>
<td>S2-4</td>
</tr>
<tr>
<td>Kim, Pil</td>
<td>H-27</td>
</tr>
<tr>
<td>Kim, Pyoung II</td>
<td>D-23</td>
</tr>
<tr>
<td>Kim, Sae Kyul</td>
<td>I-10, J-41, J-42</td>
</tr>
<tr>
<td>Kim, Sa-Hyun</td>
<td>F-17, F-18, J-27, J-64</td>
</tr>
<tr>
<td>Kim, Sang Cheol</td>
<td>A-66</td>
</tr>
<tr>
<td>Kim, Sang Woo</td>
<td>J-10, J-39, S4-4, S8-4</td>
</tr>
<tr>
<td>Kim, Sang-Joon</td>
<td>B-59</td>
</tr>
<tr>
<td>Kim, Sang-Yoon</td>
<td>D-12, H-28</td>
</tr>
<tr>
<td>Kim, Seun</td>
<td>C-9</td>
</tr>
<tr>
<td>Kim, Sehyun</td>
<td>J-70</td>
</tr>
<tr>
<td>Kim, Sei Hyun</td>
<td>J-68, S18-4</td>
</tr>
<tr>
<td>Kim, Seojeong</td>
<td>J-35, YS3-1</td>
</tr>
<tr>
<td>Kim, Seomi</td>
<td>F-56</td>
</tr>
<tr>
<td>Kim, Seong Hwan</td>
<td>J-84, YS1-6</td>
</tr>
<tr>
<td>Kim, Seonghun</td>
<td>H-24</td>
</tr>
<tr>
<td>Kim, Seonghwan</td>
<td>J-34</td>
</tr>
<tr>
<td>Kim, Seong-Hyun</td>
<td>B-23</td>
</tr>
<tr>
<td>Kim, Seongmin</td>
<td>J-38</td>
</tr>
<tr>
<td>Kim, Seonjoo</td>
<td>B-6</td>
</tr>
<tr>
<td>Kim, Seon-Won</td>
<td>H-26</td>
</tr>
<tr>
<td>Kim, Seung Burn</td>
<td>A-7, A-44, E-9</td>
</tr>
<tr>
<td>Kim, Seung Min</td>
<td>I-30</td>
</tr>
<tr>
<td>Kim, Seungcheol</td>
<td>F-1</td>
</tr>
<tr>
<td>Kim, Seunghwan</td>
<td>E-21, E-23, J-31</td>
</tr>
<tr>
<td>Kim, Si Wouk</td>
<td>A-65, D-13</td>
</tr>
<tr>
<td>Kim, Sinil</td>
<td>S6-3</td>
</tr>
<tr>
<td>Kim, So-Jeong</td>
<td>S1-3</td>
</tr>
<tr>
<td>Kim, Song Hak</td>
<td>I-8</td>
</tr>
<tr>
<td>Kim, Song-Gun</td>
<td>A-58</td>
</tr>
<tr>
<td>Kim, Soo-Jin</td>
<td>F-5</td>
</tr>
<tr>
<td>Kim, Soohyun</td>
<td>B-7</td>
</tr>
<tr>
<td>Kim, Soo-Kyong</td>
<td>D-19, F-51, F-57, YS2-5</td>
</tr>
<tr>
<td>Kim, Soonok</td>
<td>A-31, H-10</td>
</tr>
<tr>
<td>Kim, SooYeon</td>
<td>D-15</td>
</tr>
<tr>
<td>Kim, Sora</td>
<td>I-16</td>
</tr>
<tr>
<td>Kim, Sosoo</td>
<td>J-60</td>
</tr>
<tr>
<td>Kim, So-Yeon</td>
<td>F-27, F-71</td>
</tr>
<tr>
<td>Kim, Sookyung</td>
<td>I-7</td>
</tr>
<tr>
<td>Kim, Su Yeon</td>
<td>C-2, C-4, F-3, YS3-2</td>
</tr>
<tr>
<td>Kim, Suae</td>
<td>H-50</td>
</tr>
<tr>
<td>Kim, Sudeok</td>
<td>S14-2</td>
</tr>
<tr>
<td>Kim, Su-Jin</td>
<td>A-17, I-12</td>
</tr>
<tr>
<td>Kim, Sukyung</td>
<td>F-86</td>
</tr>
<tr>
<td>Kim, Sukyung</td>
<td>F-30</td>
</tr>
<tr>
<td>Kim, Sun Ick</td>
<td>A-57</td>
</tr>
<tr>
<td>Kim, Sung Chul</td>
<td>I-8</td>
</tr>
<tr>
<td>Kim, Sung Chul</td>
<td>PL-3</td>
</tr>
<tr>
<td>Kim, Sung-Hee</td>
<td>A-45, B-15, J-6, J-15</td>
</tr>
<tr>
<td>Kim, Sunhee</td>
<td>J-40</td>
</tr>
<tr>
<td>Kim, Sung-Hyun</td>
<td>S14-3</td>
</tr>
<tr>
<td>Kim, SungJe</td>
<td>J-11</td>
</tr>
<tr>
<td>Kim, Sung-Kyu</td>
<td>J-43</td>
</tr>
<tr>
<td>Kim, Sun-Tae</td>
<td>S8-1</td>
</tr>
<tr>
<td>Kim, Sun-Yeong</td>
<td>I-20</td>
</tr>
<tr>
<td>Kim, Suok-su</td>
<td>D-3</td>
</tr>
<tr>
<td>Kim, Su-Yeon</td>
<td>H-16</td>
</tr>
</tbody>
</table>

www.fkms.kr | 375
Author Index

Ko, Gwang Pyo ........................................... B-29, B-30, F-28
Ko, Han Gyu ................................. YS1-6, J-34
Ko, Han-Kyu ........................................... S12-2
Ko, Kwang Soo ........................................... S9-5
Ko, Pyung Yeol ........................................... J-84
Ko, Suk-Hyung ........................................... A-3, S2-3
Ko, Yo-Han .............................................. J-8
Ko, Yun-Ki .............................................. F-54
Ko, Yuri ................................................. H-44
Koh, Han Soo ........................................... S2-2
Koh, Su Im .............................................. F-77
Koh, Young JIn ......................................... J-52
Koh, Young-Sang ................................. G-1, G-8
Kondratyuk, Anna ..................................... A-51
Kondratyuk, Sargii .................................... A-19, A-51
Kondratyuk, T. O. ..................................... A-50
Kong, Hyun Gi ........................................... B-42, YS2-3
Kong, In-Soo .......................................... C-23
Kong, Seoung Wook ................................... D-9
Koo, Bon-sung .......................................... H-16
Koo, Eung Seo .......................................... B-9, B-14
Koo, Minseon .......................................... I-19
Kook, Joong-Ki .......................................... J-53, S22-3
Kook, Yoon-Hoh ....................................... F-49, J-63
Kown, Ki Moon .......................................... F-41
Kristyanto, Sylvia ....................................... B-63
Ku, Hye-Jin ............................................. E-24
Kuang, Zhizhou .......................................... S13-2
Kwang, Young-Nam ................................... A-49, A-54
Kwaik, Amin ............................................ E-15, A-60
Kwaik, Jinhwan ......................................... A-26
Kwaik, Myounghai ..................................... A-31
Kwon, Gayeung ......................................... C-28, G-4
Kwon, Hae Naem ......................................... I-4, YS1-4
Kwon, HeeUn .......................................... D-4
Kwon, Hy GJ ............................................ F-17, F-18, J-27, J-64
Kwon, Hyeok Il ......................................... F-56
Kwon, Hyo Il ........................................... F-22
Kwon, Hyo IL ........................................... F-23
Kwon, Hyuck Se ......................................... J-70
Kwon, Hyuk Woo ......................................... YS1-6
Kwon, Hyukwoo .......................................... J-34
Kwon, Joseph ............................................. B-10, 1-8, S14-3
Kwon, KaeYoung ....................................... S1-2
Kwon, Ki Mun ........................................... G-2
Kwon, Min .............................................. B-12
Kwon, Mun Ju ........................................... F-53
Kwon, Mun-Jee .......................................... H-17
Kwon, Nanhee ........................................... J-3
Kwon, Oh Sang .......................................... A-18

Kim, T. Doohun .......................................... C-15
Kim, Tae Gwan ......................................... H-43
Kim, Tae Yong .......................................... D-3
Kim, Tae-Gyun .......................................... S5-1
Kim, Taehoon .......................................... A-1
Kim, Tae-Ok ........................................... B-9
Kim, Tae-Su ........................................... A-44
Kim, Tae-Woon .......................................... D-14
Kim, Wan Il ........................................... J-20
Kim, Wan-Hoe .......................................... A-40
Kim, Won Jun ........................................... D-2
Kim, Won Mun .......................................... H-38, H-39, H-40
Kim, Won-Keun .......................................... A-20, A-24, F-19
Kim, Woo-Hyung ........................................ A-37
Kim, Yang Hyun .......................................... S24-2
Kim, Yangseon .......................................... H-10
Kim, Yangsup ........................................... F-41
Kim, Ye Ji ................................................. C-22
Kim, Yeon Hu ........................................... D-9
Kim, Yeon Ran ........................................... S16-1
Kim, Yeon-Hee .......................................... F-58, F-59
Kim, Yeon-Ran .......................................... YS2-1
Kim, Yeu-Chun .......................................... S18-3
Kim, Yi Sak .............................................. G-19
Kim, Yong Jin ........................................... J-55
Kim, Yong-Gyu .......................................... F-6, F-7
Kim, Yonghoon .......................................... A-36
Kim, Yong-Joo .......................................... A-45, B-15, J-15
Kim, Yong-Joo .......................................... J-40
Kim, Yong-Sun .......................................... F-24, F-25
Kim, Yoo Joong .......................................... F-22, F-23
Kim, Yoonji ............................................. S13-5
Kim, Yoon-Won .......................................... F-75, F-76
Kim, Youmi ............................................. H-48
Kim, Youn-Chul .......................................... G-15
Kim, Young Bong ......................................... J-68, J-69, J-70, S18-4
Kim, Young Deuk ......................................... F-75, F-76
Kim, Young Nam .......................................... A-36
Kim, Young Ran .......................................... S17-2
Kim, Young-Eui .......................................... F-40, F-41, YS3-3
Kim, Young-Eun .......................................... G-14
Kim, Young-Il .......................................... F-56
Kim, Young-Ji .......................................... F-85
Kim, Young-Jin .......................................... F-75, F-76
Kim, Youngjun .......................................... B-7
Kim, Younhee ............................................. E-18, H-27, H-32
Kim, Youn-Hee .......................................... YS3-5, F-62
Kim, Yu Ri ................................................. A-7, E-9, F-58
Kim, Yun-Ji ............................................. I-35
Kim, Yu Ri ................................................. F-59
Klein, Terry A ........................................... A-20, A-24
Klein, Terry A ........................................... F-19, F-37
Ko, Ara ................................................. H-49
Ko, Gwang Pyo .......................................... B-56, B-57
<table>
<thead>
<tr>
<th>Author</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwon, Ohsik</td>
<td>I-1</td>
</tr>
<tr>
<td>Kwon, Ohsuk</td>
<td>D-12, H-28</td>
</tr>
<tr>
<td>Kwon, Soo Jeong</td>
<td>C-1</td>
</tr>
<tr>
<td>Kwon, Yong Dae</td>
<td>S18-4</td>
</tr>
<tr>
<td>Kwon, Young Chan</td>
<td>J-20</td>
</tr>
<tr>
<td>Lamarche, Martin G.</td>
<td>S13-3</td>
</tr>
<tr>
<td>Lau, Gae W.</td>
<td>S13-2</td>
</tr>
<tr>
<td>Lee, Ae-Ra</td>
<td>F-45</td>
</tr>
<tr>
<td>Lee, Ara</td>
<td>F18-1</td>
</tr>
<tr>
<td>Lee, Aslian Hwanhwi</td>
<td>H-33</td>
</tr>
<tr>
<td>Lee, Beeyoung Gun</td>
<td>A-4</td>
</tr>
<tr>
<td>Lee, Beeyoung-Gun</td>
<td>A-34</td>
</tr>
<tr>
<td>Lee, Boleumi</td>
<td>A-12</td>
</tr>
<tr>
<td>Lee, Bonghyung</td>
<td>B-58</td>
</tr>
<tr>
<td>Lee, Bo-Ram</td>
<td>A-59</td>
</tr>
<tr>
<td>Lee, Bum Jin</td>
<td>S16-3</td>
</tr>
<tr>
<td>Lee, Byong Won</td>
<td>I-9, I-25, I-26, I-27</td>
</tr>
<tr>
<td>Lee, Byoung-Moo</td>
<td>E-21, E-23, J-31</td>
</tr>
<tr>
<td>Lee, Byung Ju</td>
<td>J-22</td>
</tr>
<tr>
<td>Lee, Chan Hee</td>
<td>E-12, E-29</td>
</tr>
<tr>
<td>Lee, Chang-Muk</td>
<td>H-16</td>
</tr>
<tr>
<td>Lee, Chang-Ro</td>
<td>A-39, C-29</td>
</tr>
<tr>
<td>Lee, Chang-won</td>
<td>C-7, C-19</td>
</tr>
<tr>
<td>Lee, Chan-Jung</td>
<td>J-14, J-71</td>
</tr>
<tr>
<td>Lee, Cheonghoon</td>
<td>B-29, B-30</td>
</tr>
<tr>
<td>Lee, Choongwo</td>
<td>I-9, I-26, I-27</td>
</tr>
<tr>
<td>Lee, Chowo</td>
<td>I-25</td>
</tr>
<tr>
<td>Lee, Chul Won</td>
<td>B-18</td>
</tr>
<tr>
<td>Lee, Daesang</td>
<td>A-20, A-24, F-19</td>
</tr>
<tr>
<td>Lee, Dae-yun</td>
<td>H-26</td>
</tr>
<tr>
<td>Lee, Deog-Yong</td>
<td>F-46, F-47, S3-2</td>
</tr>
<tr>
<td>Lee, Do-Hoon</td>
<td>S9-1</td>
</tr>
<tr>
<td>Lee, Dong Hwan</td>
<td>I-16</td>
</tr>
<tr>
<td>Lee, Dong Wook</td>
<td>A-53</td>
</tr>
<tr>
<td>Lee, Dong-Soek</td>
<td>E-18</td>
</tr>
<tr>
<td>Lee, Dong-Woo</td>
<td>C-10, C-31, E-32</td>
</tr>
<tr>
<td>Lee, Eun-Jung</td>
<td>I-35</td>
</tr>
<tr>
<td>Lee, Gang Burn</td>
<td>J-1</td>
</tr>
<tr>
<td>Lee, Gil Jae</td>
<td>B-56, B-57</td>
</tr>
<tr>
<td>Lee, Gwan-Hyeong</td>
<td>B-50</td>
</tr>
<tr>
<td>Lee, Geojun</td>
<td>B-12</td>
</tr>
<tr>
<td>Lee, Gyu-Cheol</td>
<td>B-6</td>
</tr>
<tr>
<td>Lee, Haichon</td>
<td>G-23</td>
</tr>
<tr>
<td>Lee, Hakmi</td>
<td>J-54</td>
</tr>
<tr>
<td>Lee, Ha-Na</td>
<td>C-6</td>
</tr>
<tr>
<td>Lee, Yoon-Dong</td>
<td>J-76</td>
</tr>
<tr>
<td>Lee, Hae Yul</td>
<td>I-9, I-25, I-26, I-27, I-33</td>
</tr>
<tr>
<td>Lee, Hae-Jung</td>
<td>J-69, J-70</td>
</tr>
<tr>
<td>Lee, Hae-Min</td>
<td>S14-3</td>
</tr>
<tr>
<td>Lee, Hae-Soo</td>
<td>S9-4</td>
</tr>
<tr>
<td>Lee, Haung-Shick</td>
<td>E-18, H-27, H-32</td>
</tr>
<tr>
<td>Lee, Hwa Jeong</td>
<td>F-82</td>
</tr>
<tr>
<td>Lee, Hyang Bum</td>
<td>A-56, A-63, A-64, B-16</td>
</tr>
<tr>
<td>Lee, Hye Won</td>
<td>B-16</td>
</tr>
<tr>
<td>Lee, Hye-Hyun</td>
<td>S21-3</td>
</tr>
<tr>
<td>Lee, Hye-Mi</td>
<td>C-4, F-2, F-38, F-39, F-69</td>
</tr>
<tr>
<td>Lee, Hyeon-Seo</td>
<td>H-26</td>
</tr>
<tr>
<td>Lee, Hye-Yeon</td>
<td>J-29, J-67</td>
</tr>
<tr>
<td>Lee, Hye Jung</td>
<td>J-72</td>
</tr>
<tr>
<td>Lee, Hye-Ji</td>
<td>F-16</td>
</tr>
<tr>
<td>Lee, Hyojin</td>
<td>A-9</td>
</tr>
<tr>
<td>Lee, Hyosun</td>
<td>B-26</td>
</tr>
<tr>
<td>Lee, Hyun</td>
<td>A-48, S22-1</td>
</tr>
<tr>
<td>Lee, Hyun Ho</td>
<td>J-1</td>
</tr>
<tr>
<td>Lee, Hyun Jung</td>
<td>A-31</td>
</tr>
<tr>
<td>Lee, Hyun Sook</td>
<td>C-20</td>
</tr>
<tr>
<td>Lee, Hyun Hwan</td>
<td>J-11</td>
</tr>
<tr>
<td>Lee, Hyung Ju</td>
<td>J-20</td>
</tr>
<tr>
<td>Lee, Hyunjoo</td>
<td>J-38</td>
</tr>
<tr>
<td>Lee, In Kyung</td>
<td>F-8, F-26</td>
</tr>
<tr>
<td>Lee, In-Kyung</td>
<td>D-21, D-22, D-24</td>
</tr>
<tr>
<td>Lee, Jae Eun</td>
<td>C-11</td>
</tr>
<tr>
<td>Lee, Jae Kwan</td>
<td>S24-4</td>
</tr>
<tr>
<td>Lee, Jae Myun</td>
<td>F-83</td>
</tr>
<tr>
<td>Lee, Jae-Chan</td>
<td>A-16, A-17</td>
</tr>
<tr>
<td>Lee, Jae-Hwa</td>
<td>H-34, I-18</td>
</tr>
<tr>
<td>Lee, Jaehwan</td>
<td>B-35</td>
</tr>
<tr>
<td>Lee, Jaewang</td>
<td>J-64</td>
</tr>
<tr>
<td>Lee, Jae-Woo</td>
<td>S12-3</td>
</tr>
<tr>
<td>Lee, Je Chul</td>
<td>F-22, F-23</td>
</tr>
<tr>
<td>Lee, Jeong-Hyo</td>
<td>H-6, H-13, H-14, H-25</td>
</tr>
<tr>
<td>Lee, Jeongmin</td>
<td>C-4</td>
</tr>
<tr>
<td>Lee, Ji Young</td>
<td>C-18</td>
</tr>
<tr>
<td>Lee, Jiheea</td>
<td>A-6, A-42</td>
</tr>
<tr>
<td>Lee, Ji-Hye</td>
<td>A-45, J-6, J-15</td>
</tr>
<tr>
<td>Lee, JiHyee</td>
<td>J-40</td>
</tr>
<tr>
<td>Lee, JiHyun</td>
<td>A-11, C-4, F-2, F-38, F-39</td>
</tr>
<tr>
<td>Lee, Jilm</td>
<td>F-1</td>
</tr>
<tr>
<td>Lee, Jinho</td>
<td>H-48, H-49</td>
</tr>
<tr>
<td>Lee, Jin-Hyung</td>
<td>F-6, F-7, H-37</td>
</tr>
<tr>
<td>Lee, Jinjae</td>
<td>I-24</td>
</tr>
<tr>
<td>Lee, Jintae</td>
<td>F-6, F-7, H-37</td>
</tr>
<tr>
<td>Lee, Jin-Young</td>
<td>J-18</td>
</tr>
<tr>
<td>Lee, Jiyeon</td>
<td>H-50</td>
</tr>
<tr>
<td>Lee, Jiyong</td>
<td>I-21</td>
</tr>
<tr>
<td>Lee, Jiyung</td>
<td>I-22, I-23</td>
</tr>
<tr>
<td>Lee, Jong Min</td>
<td>C-23</td>
</tr>
<tr>
<td>Lee, Jong-Bok</td>
<td>J-70</td>
</tr>
<tr>
<td>Lee, Jong-Hee</td>
<td>D-14</td>
</tr>
<tr>
<td>Lee, Jongmin</td>
<td>F-62</td>
</tr>
</tbody>
</table>
Author Index

Lee, Jong-Soo .................................. A-27, S17-3, S24-3
Lee, Jong suk .................................... F-1
Lee, Joong-Bok ................................... J-69
Lee, Joon-Hae ................................... D-19, F-51, F-57, YS2-5
Lee, Ju-Hoon ................................... E-24, S16-2
Lee, Jun Hak ................................... D-9
Lee, Junghyuk ................................... A-58
Lee, Joon-Yoo ................................... J-5
Lee, Jung Hun ................................... F-33
Lee, Jung-Ah ................................... J-5
Lee, Jung-hun ................................... I-22
Lee, Jung-hwan ................................ F-12, F-15
Lee, Jung-Hyun ................................ S1-2
Lee, Jung-Ok ................................... S21-3
Lee, Jung-Shin ................................ J-2, S19-4
Lee, Jung-Sook ................................ A-3, S2-3
Lee, Junhyoung ................................ A-26
Lee, Kalam ...................................... D-16
Lee, Kang-Hyo .................................. A-30
Lee, Kang-In .................................. G-11, G-20, G-21, S11-2
Lee, Keon Jin .................................. D-14
Lee, Keun Chul ................................ A-3, S2-3
Lee, Keun Hwa ................................ S15-4
Lee, Keun-Woo ................................ F-70
Lee, Kui-Jae ................................... B-47
Lee, Kwang Jick ................................. J-36
Lee, Kwanyeong ................................ B-18
Lee, Kwen-Woo ................................ F-27
Lee, Kyeong Min ............................... J-4, J-30
Lee, Kyeongiu .................................. F-28
Lee, Kyongs .................................... B-51
Lee, Kyoung-Tae ............................... D-6
Lee, Kyu-Ho .................................... E-19, YS1-1, F-70
Lee, Kyung Yong ............................... J-23
Lee, Kyung-Jo .................................. E-19, YS1-1
Lee, Kyungmin .................................. B-18
Lee, Kyuyeon .................................. F-32, J-28, YS1-2, YS4-3
Lee, Mi-Hwa ................................... A-44, A-66, H-50
Lee, Min Ho ................................... F-17, F-18, J-27, J-64
Lee, Min Jeong ................................ C-22
Lee, Min Yeon ................................ J-23
Lee, Minam ..................................... B-55
Lee, Min jung ................................. F-30, I-24
Lee, Minyoung .................................. J-54
Lee, Moo-Seung ............................... S13-1
Lee, Moran ...................................... A-55, E-30, I-34
Lee, Myoung Kyu ......................... F-32, YS2-1, S16-3
Lee, Sang Hae .................................. F-33
Lee, Sang Jun ................................ C-10, E-32
Lee, Sang Soeb ................................ A-32, J-13, J-55
Lee, Sang Won ................................ J-5
Lee, Sang Yup ................................ D-1, D-2, D-3, E-14, H-1, H-2, H-20, H-21, H-22, H-23, H-3, H-4, YS4-2, YS4-1
Lee, Sang-Hoon ................................ S1-2
Lee, Sanghoon ................................ S1-3
Lee, Sang-Hwa ................................ J-75
Lee, Sanghyun ................................ PL-3
Lee, Sang-II ................................... D-14
Lee, Sangjun .................................. I-21
Lee, Sangseob ................................ A-5
Lee, Sang-Seob B-11, B-40, B-41, B-52, D-16, G-3
Lee, Sangyeb ................................ E-15, J-32
Lee, Sang-Yeong .............................. I-20
Lee, Se-Hoon ................................ C-4
Lee, Seok-Joo ................................ F-11
Lee, SeokJoo ................................ F-55
Lee, Seon-Woo ................................ B-42, S2-1, YS2-3
Lee, Seoung-Ae ................................ F-60, F-61
Lee, Seung Hwan ............................... H-23
Lee, Seung Yeup .............................. B-42, YS2-3
Lee, Seung-Goo ............................... H-28
Lee, Seung-Ho ................................ A-20, A-24, F-19
Lee, SeungJin ................................ F-31, F-84
Lee, Seung-Yeol .............................. A-72, J-75, J-76
Lee, Shee Eun ................................ F-50, F-9, S11-1, YS3-4
Lee, Shin Ae .................................. A-70
Lee, Shin-Hae ................................ J-29, J-67
Lee, Song Hee ................................ J-8
Lee, Soobok ................................... I-3
Lee, Soohyung ................................ J-25, J-60
Lee, SookK-Young ........................... A-20, A-24, F-19
Lee, Sook-Kyoung .............................. I-28
Lee, Suk Kyeong ............................... S21-2
Lee, Sun Hwa ................................ F-26, F-8
Lee, Sung Haeng ............................... C-31
Lee, Sung-Joon ................................ S16-3
Lee, Sung-Keun ................................ S19-3
Lee, Sung-Suk ................................ H-9
Lee, Su-Yeon ................................... D-7, H-9
Lee, Taeh Hee .................................. G-7
Lee, Theresa .................................... J-25, J-60
Lee, Won-Ja ................................... F-13, F-14, F-36, F-37
Lee, WonJa .................................... H-42
Lee, Won-Kil .................................. F-79
Lee, Woo-Kon .................................. F-79
Lee, Wooseong ................................ I-36
Lee, Ye-jil ...................................... F-13
Lee, Ye-Ji ...................................... F-36, F-37
Lee, Yena ....................................... F-75
Lee, Yeong Soon ............................. C-4, F-3, YS3-2
Lee, Yeonhee ................................ J-54
Lee, Ye-Rim ................................... A-14
Lee, Yi ......................................... E-4, E-5, E-6, E-10
### Author Index

<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee, Yin-Won</td>
<td>E-17, E-33</td>
</tr>
<tr>
<td>Lee, Yong Jae</td>
<td>H-36, YS4-5</td>
</tr>
<tr>
<td>Lee, Yong-Hwan</td>
<td>E-20, J-45</td>
</tr>
<tr>
<td>Lee, Yong-Jk</td>
<td>C-31</td>
</tr>
<tr>
<td>Lee, Yong-Jun</td>
<td>F-75, F-76</td>
</tr>
<tr>
<td>Lee, Yoo Kyung</td>
<td>E-2</td>
</tr>
<tr>
<td>Lee, Yoo-Chul</td>
<td>F-79</td>
</tr>
<tr>
<td>Lee, Yoon-Hae</td>
<td>J-43</td>
</tr>
<tr>
<td>Lee, Yoonji</td>
<td>E-33</td>
</tr>
<tr>
<td>Lee, Yoon-Ju</td>
<td>D-22</td>
</tr>
<tr>
<td>Lee, Youn Su</td>
<td>J-10, J-39, S4-4, S8-4</td>
</tr>
<tr>
<td>Lee, Young-Han</td>
<td>B-66</td>
</tr>
<tr>
<td>Lee, Youngju</td>
<td>F-62</td>
</tr>
<tr>
<td>Lee, Young-Nam</td>
<td>F-75</td>
</tr>
<tr>
<td>Lee, Youngsa</td>
<td>J-38</td>
</tr>
<tr>
<td>Lee, Yu Young</td>
<td>I-9, I-25, I-26, I-27</td>
</tr>
<tr>
<td>Lee, Yujin</td>
<td>B-6</td>
</tr>
<tr>
<td>Lee, Yunkyoung</td>
<td>C-25</td>
</tr>
<tr>
<td>Leem, Kang-Hyun</td>
<td>J-9, J-12</td>
</tr>
<tr>
<td>Leng, Gang</td>
<td>S19-2</td>
</tr>
<tr>
<td>Li, Xi-Hui</td>
<td>D-19, F-51, YS2-5</td>
</tr>
<tr>
<td>Li, Yi-hui</td>
<td>F-57</td>
</tr>
<tr>
<td>Lim, Bo Ra</td>
<td>H-15, H-18, H-19, H-29, H-30</td>
</tr>
<tr>
<td>Lim, Bo Ram</td>
<td>H-18, H-19, J-77, J-78, J-79, J-80</td>
</tr>
<tr>
<td>Lim, Bu Geon</td>
<td>J-1</td>
</tr>
<tr>
<td>Lim, Heeji</td>
<td>J-5</td>
</tr>
<tr>
<td>Lim, HeeSeon</td>
<td>E-2, I-6</td>
</tr>
<tr>
<td>Lim, Hyosang</td>
<td>B-24</td>
</tr>
<tr>
<td>Lim, Hyoun Soo</td>
<td>S2-4</td>
</tr>
<tr>
<td>Lim, Jee-min</td>
<td>D-25</td>
</tr>
<tr>
<td>Lim, Jeong-A</td>
<td>A-55, B-53, E-30, I-16</td>
</tr>
<tr>
<td>Lim, Jong-Hwan</td>
<td>C-14</td>
</tr>
<tr>
<td>Lim, Joo Yeen</td>
<td>C-3</td>
</tr>
<tr>
<td>Lim, Ju Young</td>
<td>S17-2</td>
</tr>
<tr>
<td>Lim, Mi Sun</td>
<td>B-1</td>
</tr>
<tr>
<td>Lim, Miri</td>
<td>A-6</td>
</tr>
<tr>
<td>Lim, Sangyong</td>
<td>E-7, F-73, F-74</td>
</tr>
<tr>
<td>Lim, Seung Yeol</td>
<td>C-22</td>
</tr>
<tr>
<td>Lim, Shunmei</td>
<td>F-74</td>
</tr>
<tr>
<td>Lim, Sooyeon</td>
<td>A-41, E-25</td>
</tr>
<tr>
<td>Lim, Suk-Kyung</td>
<td>S9-4</td>
</tr>
<tr>
<td>Lim, Yang-Sook</td>
<td>A-72, J-75</td>
</tr>
<tr>
<td>Lim, Yong-Taik</td>
<td>S24-2</td>
</tr>
<tr>
<td>Lim, Yoo-jeong</td>
<td>B-31</td>
</tr>
<tr>
<td>Lim, Young Woon</td>
<td>A-48, J-33, J-49, S22-1</td>
</tr>
<tr>
<td>Lim, Young-Hye</td>
<td>C-28, G-4, G-16</td>
</tr>
<tr>
<td>Lim, Yum-Ji</td>
<td>F-12, F-15</td>
</tr>
<tr>
<td>Liu, Dong</td>
<td>A-47</td>
</tr>
<tr>
<td>Lokos, Laszlo</td>
<td>A-19</td>
</tr>
<tr>
<td>Luu, Van Trinh</td>
<td>H-17</td>
</tr>
<tr>
<td>Lyoo, Hye-Rhyoung</td>
<td>J-43</td>
</tr>
<tr>
<td>Ma, Xiaoning</td>
<td>B-38</td>
</tr>
<tr>
<td>Madhavara, Lavanya</td>
<td>A-65</td>
</tr>
<tr>
<td>Maeng, Seon Jeong</td>
<td>J-41, J-42</td>
</tr>
<tr>
<td>Maeng, Seonjeong</td>
<td>J-37</td>
</tr>
<tr>
<td>Malbunkaew, Sawarat</td>
<td>D-12</td>
</tr>
<tr>
<td>Mannaa, Mohamed</td>
<td>I-15</td>
</tr>
<tr>
<td>Manzoor, Zahid</td>
<td>G-8</td>
</tr>
<tr>
<td>Min, Byoungnam</td>
<td>E-26, E-27</td>
</tr>
<tr>
<td>Min, Garam</td>
<td>J-61</td>
</tr>
<tr>
<td>Min, Gyeongin</td>
<td>A-60, E-15</td>
</tr>
<tr>
<td>Min, Kwan Sik</td>
<td>H-35</td>
</tr>
<tr>
<td>Min, Kyung-Jin</td>
<td>J-29, J-67, S24-4</td>
</tr>
<tr>
<td>Min, Youngju</td>
<td>A-2</td>
</tr>
<tr>
<td>Mitchell, Robert</td>
<td>B-34, D-4, D-15</td>
</tr>
<tr>
<td>Mitchell, Robert J.</td>
<td>F-10, F-52, YS4-4, YS1-5</td>
</tr>
<tr>
<td>Mitchell, Robert James</td>
<td>A-33</td>
</tr>
<tr>
<td>Mohankandhasamy, Ramasamy</td>
<td>H-37</td>
</tr>
<tr>
<td>Mohankumar, Murugan</td>
<td>H-37</td>
</tr>
<tr>
<td>Moon, Cheol</td>
<td>F-17, F-18, J-27, J-64</td>
</tr>
<tr>
<td>Moon, Dong-Chan</td>
<td>S9-4</td>
</tr>
<tr>
<td>Moon, Gi-Seong</td>
<td>I-2</td>
</tr>
<tr>
<td>Moon, Hye Yoon</td>
<td>H-12, H-17</td>
</tr>
<tr>
<td>Moon, Saet-Byeol</td>
<td>B-11</td>
</tr>
<tr>
<td>Moon, Soo Young</td>
<td>C-23</td>
</tr>
<tr>
<td>Moon, Sungyoon</td>
<td>F-62</td>
</tr>
<tr>
<td>Mueller, Jaclyn A.</td>
<td>S1-1</td>
</tr>
<tr>
<td>Muhairic, Salama Al</td>
<td>S15-3</td>
</tr>
<tr>
<td>Mun, Ji-Young</td>
<td>D-11, D-28</td>
</tr>
<tr>
<td>Myoung, Jinjong</td>
<td>F-64, F-65, F-66, F-67, F-68, I-32</td>
</tr>
<tr>
<td>Myung, Heejeon</td>
<td>H-41, J-20, J-48</td>
</tr>
<tr>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Na, DoKyun</td>
<td>D-1, H-22</td>
</tr>
<tr>
<td>Na, Hae-young</td>
<td>F-43, F-44</td>
</tr>
<tr>
<td>Na, Hongjun</td>
<td>E-24</td>
</tr>
<tr>
<td>Na, Seok Hyeon</td>
<td>F-23</td>
</tr>
<tr>
<td>Na, Seok Hyun</td>
<td>F-22</td>
</tr>
<tr>
<td>Nahar, Shamsun</td>
<td>B-45</td>
</tr>
<tr>
<td>Nair, G. Balakrish</td>
<td>PL-2</td>
</tr>
<tr>
<td>Nam, Hyejin</td>
<td>E-17</td>
</tr>
<tr>
<td>Nam, Kung-Woo</td>
<td>F-86</td>
</tr>
<tr>
<td>Nam, Sang-Jip</td>
<td>A-25</td>
</tr>
<tr>
<td>Nam, Seon Young</td>
<td>F-26, F-8</td>
</tr>
<tr>
<td>Nam, Young-Do</td>
<td>H-50</td>
</tr>
<tr>
<td>Nam, Youn-Kool</td>
<td>E-8</td>
</tr>
<tr>
<td>Nguyen, Son G.</td>
<td>J-16, J-44</td>
</tr>
<tr>
<td>Nguyen, Thi Thoong Thoong</td>
<td>A-63, A-64</td>
</tr>
<tr>
<td>Nguyen, Thoong Thoong</td>
<td>A-56</td>
</tr>
<tr>
<td>Nguyen, Trieu Dieu Linh</td>
<td>C-2</td>
</tr>
<tr>
<td>Author Index</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Nguyen, Tuan Manh</td>
<td>J-74</td>
</tr>
<tr>
<td>Nguyen, Van Minh</td>
<td>D-22, D-24</td>
</tr>
<tr>
<td>Nguyen, Vu H.</td>
<td>S11-1</td>
</tr>
<tr>
<td>Nicholas, Asiimwe</td>
<td>F-22</td>
</tr>
<tr>
<td>No, Jin Sun</td>
<td>A-20, A-24, F-19</td>
</tr>
<tr>
<td>Noh, Gyuyou</td>
<td>C-23</td>
</tr>
<tr>
<td>Noh, Won</td>
<td>H-23</td>
</tr>
<tr>
<td>Nunez, Gabriel</td>
<td>F-42</td>
</tr>
<tr>
<td>Oh, Doo-Byoung</td>
<td>D-12, H-28</td>
</tr>
<tr>
<td>Oh, Hokyung</td>
<td>J-61</td>
</tr>
<tr>
<td>Oh, Hye Ji</td>
<td>H-12</td>
</tr>
<tr>
<td>Oh, Jeong-II</td>
<td>C-5, C-6</td>
</tr>
<tr>
<td>Oh, Ji Hye</td>
<td>S1-2</td>
</tr>
<tr>
<td>Oh, Ji Yeon</td>
<td>J-19</td>
</tr>
<tr>
<td>Oh, Jong-Won</td>
<td>I-35</td>
</tr>
<tr>
<td>Oh, Jungmin</td>
<td>D-19, F-51, F-57, YS2-5</td>
</tr>
<tr>
<td>Oh, Kyung Seo</td>
<td>I-8</td>
</tr>
<tr>
<td>Oh, Kyung-Hwan</td>
<td>F-5, F-53</td>
</tr>
<tr>
<td>Oh, Man Hwan</td>
<td>F-23</td>
</tr>
<tr>
<td>Oh, Min Ji</td>
<td>A-8, E-8</td>
</tr>
<tr>
<td>Oh, Sanghun</td>
<td>A-26</td>
</tr>
<tr>
<td>Oh, Sang-Keun</td>
<td>S8-2</td>
</tr>
<tr>
<td>Oh, Se Eun</td>
<td>G-2</td>
</tr>
<tr>
<td>Oh, Sea Kwan</td>
<td>J-17</td>
</tr>
<tr>
<td>Oh, Se-Jin</td>
<td>H-11</td>
</tr>
<tr>
<td>Oh, Soon Ok</td>
<td>A-34, A-47</td>
</tr>
<tr>
<td>Oh, Soon-Ock</td>
<td>A-19, A-49, A-54</td>
</tr>
<tr>
<td>Oh, Su-bin</td>
<td>I-23</td>
</tr>
<tr>
<td>Oh, Sung-Man</td>
<td>F-12, F-15</td>
</tr>
<tr>
<td>Oh, Tae Seok</td>
<td>J-38</td>
</tr>
<tr>
<td>Oh, Young Hoon</td>
<td>H-23</td>
</tr>
<tr>
<td>Oh, Young Kyoon</td>
<td>B-3</td>
</tr>
<tr>
<td>Oh, Young Taek</td>
<td>F-34, YS1-3</td>
</tr>
<tr>
<td>Oh, Youn-Lee</td>
<td>A-8, E-8, E-21</td>
</tr>
<tr>
<td>Oh, Yunjung</td>
<td>D-25</td>
</tr>
<tr>
<td>Park, Daep</td>
<td>J-3</td>
</tr>
<tr>
<td>Park, Daesu</td>
<td>F-35</td>
</tr>
<tr>
<td>Park, Dami</td>
<td>J-25</td>
</tr>
<tr>
<td>Park, Dong Suk</td>
<td>B-60</td>
</tr>
<tr>
<td>Park, Dong Woon</td>
<td>H-25</td>
</tr>
<tr>
<td>Park, Dong-Jin</td>
<td>D-25, D-26</td>
</tr>
<tr>
<td>Park, Eun Jung</td>
<td>S5-2</td>
</tr>
<tr>
<td>Park, Eung-Roh</td>
<td>A-18, A-59</td>
</tr>
<tr>
<td>Park, Eun-Jin</td>
<td>C-5</td>
</tr>
<tr>
<td>Park, Geun Woo</td>
<td>S14-1</td>
</tr>
<tr>
<td>Park, Gwan</td>
<td>I-17, I-21</td>
</tr>
<tr>
<td>Park, Gyungsoo</td>
<td>E-31</td>
</tr>
<tr>
<td>Park, Hae Chul</td>
<td>J-36</td>
</tr>
<tr>
<td>Park, Hae Woong</td>
<td>D-14</td>
</tr>
<tr>
<td>Park, Haju</td>
<td>B-20</td>
</tr>
<tr>
<td>Park, Hee-Moon</td>
<td>C-1, C-3</td>
</tr>
<tr>
<td>Park, Honggi</td>
<td>B-24, B-55</td>
</tr>
<tr>
<td>Park, Hongje</td>
<td>E-27</td>
</tr>
<tr>
<td>Park, Hong-Tae</td>
<td>S3-3</td>
</tr>
<tr>
<td>Park, Hye Min</td>
<td>H-23</td>
</tr>
<tr>
<td>Park, Hye-Gwon</td>
<td>D-1</td>
</tr>
<tr>
<td>Park, Hye-Jae</td>
<td>S13-4</td>
</tr>
<tr>
<td>Park, Hyeok</td>
<td>B-31, B-48</td>
</tr>
<tr>
<td>Park, Hye-Soo</td>
<td>G-11, G-20, G-21</td>
</tr>
<tr>
<td>Park, Hye-Won</td>
<td>A-15</td>
</tr>
<tr>
<td>Park, Hye-young</td>
<td>J-17</td>
</tr>
<tr>
<td>Park, Hyun-Eui</td>
<td>S3-3</td>
</tr>
<tr>
<td>Park, Hyun-Jin</td>
<td>I-18</td>
</tr>
<tr>
<td>Park, Hyunjo</td>
<td>F-32, J-28, YS1-2, YS4-3</td>
</tr>
<tr>
<td>Park, Hyun-woog</td>
<td>A-29</td>
</tr>
<tr>
<td>Park, In-Chool</td>
<td>B-46, D-23</td>
</tr>
<tr>
<td>Park, Inchoel</td>
<td>B-50</td>
</tr>
<tr>
<td>Park, Inhee</td>
<td>F-21</td>
</tr>
<tr>
<td>Park, Jae Young</td>
<td>J-33, J-49, S22-1</td>
</tr>
<tr>
<td>Park, Jae-Bong</td>
<td>F-24</td>
</tr>
<tr>
<td>Park, Jae-Eun</td>
<td>F-58</td>
</tr>
<tr>
<td>Park, Jae-Seon</td>
<td>A-39</td>
</tr>
<tr>
<td>Park, Jaman</td>
<td>G-5</td>
</tr>
<tr>
<td>Park, Jee-Young</td>
<td>A-45, B-15, J-6, J-15</td>
</tr>
<tr>
<td>Park, Jeeyong</td>
<td>J-40</td>
</tr>
<tr>
<td>Park, Jeong-Ho</td>
<td>F-24</td>
</tr>
<tr>
<td>Park, Jeong-Kyu</td>
<td>F-69, G-9, G-13</td>
</tr>
<tr>
<td>Park, Ji Seon</td>
<td>E-12</td>
</tr>
<tr>
<td>Park, Ji Yong</td>
<td>I-9, I-25, I-27</td>
</tr>
<tr>
<td>Park, Jeun</td>
<td>C-28</td>
</tr>
<tr>
<td>Park, Jiheon</td>
<td>I-11</td>
</tr>
<tr>
<td>Park, Ji hye</td>
<td>G-10</td>
</tr>
<tr>
<td>Park, Ji-Hye</td>
<td>G-6</td>
</tr>
<tr>
<td>Park, Jin Hwan</td>
<td>I-4, YS1-4</td>
</tr>
<tr>
<td>Park, Jin-Woo</td>
<td>A-55, E-30, I-34</td>
</tr>
<tr>
<td>Park, Jin-woo</td>
<td>B-53, I-16</td>
</tr>
<tr>
<td>Park, Jin-young</td>
<td>I-17</td>
</tr>
<tr>
<td>Park, Jisun</td>
<td>A-7</td>
</tr>
<tr>
<td>Park, Jiyoung</td>
<td>B-65</td>
</tr>
<tr>
<td>Park, Jong-Han</td>
<td>B-54</td>
</tr>
<tr>
<td>Park, Joo-Hae</td>
<td>F-45</td>
</tr>
<tr>
<td>Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Park, Joon-Song</td>
<td>H-27</td>
</tr>
<tr>
<td>Park, Ju Young</td>
<td>A-61</td>
</tr>
<tr>
<td>Park, Jun Seok</td>
<td>D-3</td>
</tr>
<tr>
<td>Park, Junbae</td>
<td>G-12</td>
</tr>
<tr>
<td>Park, Jung Chul</td>
<td>H-27</td>
</tr>
<tr>
<td>Park, Jung In</td>
<td>A-11</td>
</tr>
<tr>
<td>Park, Jung-Shin</td>
<td>A-34, A-35</td>
</tr>
<tr>
<td>Park, Jun-Sun</td>
<td>F-3, S3-2</td>
</tr>
<tr>
<td>Park, Ju-Ri</td>
<td>A-37</td>
</tr>
<tr>
<td>Park, Keun-Tae</td>
<td>G-4</td>
</tr>
<tr>
<td>Park, Ki Hoon</td>
<td>J-68, S18-4</td>
</tr>
<tr>
<td>Park, Ki Ho</td>
<td>E-10</td>
</tr>
<tr>
<td>Park, Kwang Seung</td>
<td>B-9</td>
</tr>
<tr>
<td>Park, Kwon-Sam</td>
<td>F-33</td>
</tr>
<tr>
<td>Park, Kye Ryeong</td>
<td>C-11</td>
</tr>
<tr>
<td>Park, Kyu Ri</td>
<td>I-19</td>
</tr>
<tr>
<td>Park, Kyungmin</td>
<td>S15-2</td>
</tr>
<tr>
<td>Park, Man-Seong</td>
<td>S15-2</td>
</tr>
<tr>
<td>Park, Min</td>
<td>F-17, F-18, J-27, J-64</td>
</tr>
<tr>
<td>Park, Min Jung</td>
<td>J-1</td>
</tr>
<tr>
<td>Park, Minsa</td>
<td>E-11</td>
</tr>
<tr>
<td>Park, Myung Soo</td>
<td>A-48, J-33</td>
</tr>
<tr>
<td>Park, Na-Young</td>
<td>F-27, F-70</td>
</tr>
<tr>
<td>Park, Sang-Jin</td>
<td>F-75</td>
</tr>
<tr>
<td>Park, Sangkyu</td>
<td>D-2</td>
</tr>
<tr>
<td>Park, Sang-Myi</td>
<td>D-2</td>
</tr>
<tr>
<td>Park, Sang-Sang</td>
<td>E-20, E-22</td>
</tr>
<tr>
<td>Park, Sehee</td>
<td>C-27</td>
</tr>
<tr>
<td>Park, Seho</td>
<td>F-29</td>
</tr>
<tr>
<td>Park, Seok Hyun</td>
<td>F-29</td>
</tr>
<tr>
<td>Park, Seong Hoe</td>
<td>E-20, E-22</td>
</tr>
<tr>
<td>Park, Seung Pyo</td>
<td>J-53</td>
</tr>
<tr>
<td>Park, Seung Chun</td>
<td>C-27</td>
</tr>
<tr>
<td>Park, Seung-Hwan</td>
<td>F-2, F-38, F-39</td>
</tr>
<tr>
<td>Park, Shin-Young</td>
<td>F-56</td>
</tr>
<tr>
<td>Park, Si Jae</td>
<td>G-17</td>
</tr>
<tr>
<td>Park, Sijae</td>
<td>H-1, H-23</td>
</tr>
<tr>
<td>Park, So Young</td>
<td>G-17</td>
</tr>
<tr>
<td>Park, Sook-Young</td>
<td>E-20, E-22</td>
</tr>
<tr>
<td>Park, Soon-Nang</td>
<td>J-53</td>
</tr>
<tr>
<td>Park, Soyeon</td>
<td>F-29</td>
</tr>
<tr>
<td>Park, So-Young</td>
<td>F-2, F-38, F-39</td>
</tr>
<tr>
<td>Park, Soyoungh</td>
<td>A-59</td>
</tr>
<tr>
<td>Park, Su Jeong</td>
<td>S5-3</td>
</tr>
<tr>
<td>Park, Su-Hyung</td>
<td>F-56</td>
</tr>
<tr>
<td>Park, Su-Jin</td>
<td>F-56</td>
</tr>
<tr>
<td>Park, Sukho</td>
<td>J-59</td>
</tr>
<tr>
<td>Park, Sung Min</td>
<td>A-65</td>
</tr>
<tr>
<td>Park, Sung-Jun</td>
<td>B-29, B-30</td>
</tr>
<tr>
<td>Park, Sungman</td>
<td>B-29, B-30</td>
</tr>
<tr>
<td>Park, Sunhee</td>
<td>G-10</td>
</tr>
<tr>
<td>Park, Sun-Hae</td>
<td>G-6</td>
</tr>
<tr>
<td>Park, Sun-Whan</td>
<td>F-13</td>
</tr>
<tr>
<td>Park, Sun-Whan</td>
<td>F-13</td>
</tr>
<tr>
<td>Park, Sun-Yang</td>
<td>S17-1</td>
</tr>
<tr>
<td>Park, Won Il</td>
<td>F-36</td>
</tr>
<tr>
<td>Park, Woo-Ju</td>
<td>B-31</td>
</tr>
<tr>
<td>Park, Yong-Bae</td>
<td>D-6</td>
</tr>
<tr>
<td>Park, Yongwoo</td>
<td>A-1</td>
</tr>
<tr>
<td>Park, Yoon Jeong</td>
<td>S16-1</td>
</tr>
<tr>
<td>Park, Yoon Mee</td>
<td>B-81</td>
</tr>
<tr>
<td>Park, Young Kyoung</td>
<td>J-30</td>
</tr>
<tr>
<td>Park, Youngae</td>
<td>C-9, I-13</td>
</tr>
<tr>
<td>Park, Youngdo</td>
<td>C-25</td>
</tr>
<tr>
<td>Park, Young-Ha</td>
<td>C-27, S2-1</td>
</tr>
<tr>
<td>Park, Young-jun</td>
<td>S-4</td>
</tr>
<tr>
<td>Park, Young-Min</td>
<td>I-20</td>
</tr>
<tr>
<td>Park, Youn-Je</td>
<td>H-5</td>
</tr>
<tr>
<td>Park, Yu-Jin</td>
<td>F-72</td>
</tr>
<tr>
<td>Paul, Lauren</td>
<td>A-25</td>
</tr>
<tr>
<td>Pengkit, Anchalee</td>
<td>E-31</td>
</tr>
<tr>
<td>Pontes, Mauricio H.</td>
<td>S1-7</td>
</tr>
<tr>
<td>Poo, Haryoung</td>
<td>S2-4</td>
</tr>
<tr>
<td>Puth, Sao</td>
<td>F-50</td>
</tr>
<tr>
<td>Qi, Longbin</td>
<td>D-18</td>
</tr>
<tr>
<td>Rahman, Md. Mzur</td>
<td>S9-1</td>
</tr>
<tr>
<td>Ramana, Ch Venkata</td>
<td>S23-3</td>
</tr>
<tr>
<td>Rhee, Dong-Kwon</td>
<td>C-8</td>
</tr>
<tr>
<td>Rhee, Jin-Kyu</td>
<td>B-10</td>
</tr>
<tr>
<td>Rhee, Joon Haeng</td>
<td>F-9, F-50, S11-1, S17-2, S3-4</td>
</tr>
<tr>
<td>Rhee, Moon-Soo</td>
<td>A-41</td>
</tr>
<tr>
<td>Rhee, Sung-Keun</td>
<td>S1-3</td>
</tr>
<tr>
<td>Rhee, Si-Eun</td>
<td>F-58, F-59</td>
</tr>
<tr>
<td>Rho, Jae-young</td>
<td>D-5, J-18</td>
</tr>
<tr>
<td>Rim, Minji</td>
<td>F-72, G-18</td>
</tr>
<tr>
<td>Ro, Eun Young</td>
<td>I-20</td>
</tr>
<tr>
<td>Ro, Hyeon-Su</td>
<td>E-11, E-13, J-21, S6-3</td>
</tr>
<tr>
<td>Ro, Yu-Mi</td>
<td>B-50</td>
</tr>
<tr>
<td>Robitaille, Sophie</td>
<td>S13-3</td>
</tr>
<tr>
<td>Roh, An-Sung</td>
<td>B-66</td>
</tr>
<tr>
<td>Roh, Eun Jung</td>
<td>I-16</td>
</tr>
<tr>
<td>Roh, Eunjing</td>
<td>A-55, B-53, E-30, I-34</td>
</tr>
<tr>
<td>Roh, Yong Yeol</td>
<td>F-36</td>
</tr>
<tr>
<td>Roh, Minho</td>
<td>D-2, D-3, H-4, H-22, YS4-2</td>
</tr>
<tr>
<td>Roh, Seong Woon</td>
<td>B-10</td>
</tr>
<tr>
<td>Roh, Younghee</td>
<td>J-65</td>
</tr>
<tr>
<td>Rooney, Alejandro P.</td>
<td>A-21</td>
</tr>
<tr>
<td>Ryoo, Rhiin</td>
<td>I-11, I-13</td>
</tr>
<tr>
<td>Ryou, Chongsuk</td>
<td>C-4, F-2, F-38, F-39</td>
</tr>
<tr>
<td>Ryu, Bum Han</td>
<td>C-15</td>
</tr>
<tr>
<td>Ryu, Eun-Ju</td>
<td>F-11, F-54</td>
</tr>
</tbody>
</table>
### Author Index

<table>
<thead>
<tr>
<th>Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryu, Ho-Yoon</td>
<td>D-5</td>
</tr>
<tr>
<td>Ryu, Jae San</td>
<td>S12-1</td>
</tr>
<tr>
<td>Ryu, Jae-Gee</td>
<td>I-16, J-25, J-60</td>
</tr>
<tr>
<td>Ryu, Jaewon</td>
<td>D-13</td>
</tr>
<tr>
<td>Ryu, Jong-su</td>
<td>B-12</td>
</tr>
<tr>
<td>Ryu, Ki Hyun</td>
<td>J-51</td>
</tr>
<tr>
<td>Ryu, Phil Youl</td>
<td>J-59</td>
</tr>
<tr>
<td>Ryu, Sanggyeol</td>
<td>E-24, I-29</td>
</tr>
<tr>
<td>Ryu, Seon</td>
<td>F-85</td>
</tr>
<tr>
<td>Ryu, Seungbo</td>
<td>F-78</td>
</tr>
<tr>
<td>Ryu, Sun-Hwa</td>
<td>D-7, H-9</td>
</tr>
<tr>
<td>Ryu, Wang-Shick</td>
<td>S21-1, YS3-6</td>
</tr>
<tr>
<td>Shin, Eunkyoung</td>
<td>F-5, F-53</td>
</tr>
<tr>
<td>Shin, Hea Luang</td>
<td>C-18</td>
</tr>
<tr>
<td>Shin, Hea Soon</td>
<td>F-20, F-77</td>
</tr>
<tr>
<td>Shin, Heuyn-Kil</td>
<td>J-28, YS1-2</td>
</tr>
<tr>
<td>Shin, Hyeun-Kil</td>
<td>F-32, YS4-3</td>
</tr>
<tr>
<td>Shin, Hyun Mu</td>
<td>S18-2</td>
</tr>
<tr>
<td>Shin, Jae-Ho</td>
<td>E-31</td>
</tr>
<tr>
<td>Shin, Min-Kyoung</td>
<td>F-80, S3-3</td>
</tr>
<tr>
<td>Shin, Ok Sarah</td>
<td>E-12, G-15</td>
</tr>
<tr>
<td>Shin, Pyung-Gyun</td>
<td>A-8, E-8</td>
</tr>
<tr>
<td>Shin, Saengchul</td>
<td>J-65</td>
</tr>
<tr>
<td>Shin, Seung-yeol</td>
<td>A-13</td>
</tr>
<tr>
<td>Shin, Saungyeol</td>
<td>A-9</td>
</tr>
<tr>
<td>Shin, Su-Kyoung</td>
<td>A-71, S23-2</td>
</tr>
<tr>
<td>Shin, Sung Jae</td>
<td>E-1</td>
</tr>
<tr>
<td>Si, Young-Jae</td>
<td>F-56</td>
</tr>
<tr>
<td>Siddique, Mahbubul Pratik</td>
<td>C-23</td>
</tr>
<tr>
<td>Sim, Eun Yeong</td>
<td>I-9, I-25, I-26, I-27</td>
</tr>
<tr>
<td>Sim, Hong Nam</td>
<td>F-63</td>
</tr>
<tr>
<td>Sim, Insuk</td>
<td>G-4</td>
</tr>
<tr>
<td>Sim, Jaehyun</td>
<td>F-54</td>
</tr>
<tr>
<td>Sim, Joon-So</td>
<td>H-16</td>
</tr>
<tr>
<td>Sim, Yong Sik</td>
<td>B-14</td>
</tr>
<tr>
<td>So, Mi Jin</td>
<td>J-49, S22-1</td>
</tr>
<tr>
<td>Sohn, Jung-Hoon</td>
<td>S6-4</td>
</tr>
<tr>
<td>Son, Bokyung</td>
<td>I-29</td>
</tr>
<tr>
<td>Son, Ho Sun</td>
<td>J-5</td>
</tr>
<tr>
<td>Son, Hokyung</td>
<td>E-17, E-33</td>
</tr>
<tr>
<td>Son, Jong Seong</td>
<td>J-1</td>
</tr>
<tr>
<td>Son, Minjae</td>
<td>A-60</td>
</tr>
<tr>
<td>Son, Soo-Ji</td>
<td>E-31</td>
</tr>
<tr>
<td>Son, Sujin</td>
<td>J-32</td>
</tr>
<tr>
<td>Son, Yeo-Jin</td>
<td>G-21</td>
</tr>
<tr>
<td>Son, Yeonyeong</td>
<td>H-7</td>
</tr>
<tr>
<td>Son, Young-Jin</td>
<td>C-4</td>
</tr>
<tr>
<td>Song, Bong Gu</td>
<td>F-36</td>
</tr>
<tr>
<td>Song, Bong Keun</td>
<td>H-23</td>
</tr>
<tr>
<td>Song, Chan Woo</td>
<td>YS1-4</td>
</tr>
<tr>
<td>Song, Chang-Hwa</td>
<td>F-12, F-15, F-69, G-9</td>
</tr>
<tr>
<td>Song, Chan Woo</td>
<td>D-1, E-14, H-20, H-21</td>
</tr>
<tr>
<td>Song, Daesub</td>
<td>S15-3</td>
</tr>
<tr>
<td>Song, Dong Hyun</td>
<td>A-20, A-24, F-19</td>
</tr>
<tr>
<td>Song, Eun-Sung</td>
<td>E-21, E-23, J-31</td>
</tr>
<tr>
<td>Song, Ha-Yeon</td>
<td>D-8, S12-2</td>
</tr>
<tr>
<td>Song, Hoijin</td>
<td>J-61</td>
</tr>
<tr>
<td>Song, Hong-Gyu</td>
<td>B-36, B-37</td>
</tr>
<tr>
<td>Song, Ho-Yeon</td>
<td>F-30, F-86</td>
</tr>
<tr>
<td>Song, Hyunjung</td>
<td>E-20</td>
</tr>
<tr>
<td>Song, Jae Ho</td>
<td>C-1</td>
</tr>
<tr>
<td>Song, Jaekyeong</td>
<td>A-70, B-23, B-65, B-66</td>
</tr>
<tr>
<td>Song, Jae Young</td>
<td>J-36, J-70, J-65</td>
</tr>
<tr>
<td>Song, Jeong Young</td>
<td>A-57, A-61</td>
</tr>
<tr>
<td>Song, Ji-kyoung</td>
<td>B-21, B-22</td>
</tr>
<tr>
<td>Song, Jin-Won</td>
<td>A-20, A-24, F-19</td>
</tr>
<tr>
<td>Song, Jong-Arn</td>
<td>S14-4</td>
</tr>
<tr>
<td>Song, Jun-Su</td>
<td>A-13</td>
</tr>
<tr>
<td>Author Index</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------</td>
<td>------</td>
</tr>
<tr>
<td>Song, Ki-Joon</td>
<td>J-50</td>
</tr>
<tr>
<td>Song, Kiwon</td>
<td>S19-2</td>
</tr>
<tr>
<td>Song, Moon Jung</td>
<td>F-45, F-78, S21-4</td>
</tr>
<tr>
<td>Song, Seokheon</td>
<td>A-11</td>
</tr>
<tr>
<td>Song, Taeksun</td>
<td>E-1</td>
</tr>
<tr>
<td>Song, Won-Geun</td>
<td>S14-4</td>
</tr>
<tr>
<td>Steward, Grieg F.</td>
<td>S1-1</td>
</tr>
<tr>
<td>Suh, Joo-won</td>
<td>D-18, E-23</td>
</tr>
<tr>
<td>Suh, JooWon</td>
<td>F-31</td>
</tr>
<tr>
<td>Sun, Li</td>
<td>S2-2, S2-3, S9-1</td>
</tr>
<tr>
<td>Sun, Ren</td>
<td>PL-4</td>
</tr>
<tr>
<td>Sundaraman, Aravind</td>
<td>A-32</td>
</tr>
<tr>
<td>Sung, Gi-Ho</td>
<td>A-30</td>
</tr>
<tr>
<td>Sung, Hoon-Ja</td>
<td>S9-1</td>
</tr>
<tr>
<td>Sung, Jung-Suk</td>
<td>B-32</td>
</tr>
<tr>
<td>Sung, Moon-Hee</td>
<td>S24-1</td>
</tr>
<tr>
<td>Sung, Pil Soo</td>
<td>S3-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takagi, Hiroshi</td>
<td>C-26</td>
</tr>
<tr>
<td>Tamanna, Zerin</td>
<td>F-30</td>
</tr>
<tr>
<td>Tan, Wenzhi</td>
<td>F-50</td>
</tr>
<tr>
<td>Tesf, Vernon L</td>
<td>S13-1</td>
</tr>
<tr>
<td>Thak, Eun Jung</td>
<td>H-12</td>
</tr>
<tr>
<td>Thawng, Cung Nawl</td>
<td>S9-1</td>
</tr>
<tr>
<td>Thines, Marco</td>
<td>S12-4</td>
</tr>
<tr>
<td>Truong, Quang Lam</td>
<td>F-29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Um, Ji-Hye</td>
<td>F-3, YS3-2</td>
</tr>
<tr>
<td>Um, Yong Hyun</td>
<td>J-10, J-39, S4-4, S8-4</td>
</tr>
<tr>
<td>Urino, Tatsuya</td>
<td>J-16, J-44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaidya, Bipin</td>
<td>I-B</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang, Eun-Jin</td>
<td>C-16, C-17</td>
</tr>
<tr>
<td>Waqas, Muhammad</td>
<td>C-4</td>
</tr>
<tr>
<td>Waqas, Muhammad</td>
<td>F-2, F-38, F-39</td>
</tr>
<tr>
<td>Wen, Yancheng</td>
<td>F-27, F-70</td>
</tr>
<tr>
<td>Whang, Jake</td>
<td>S11-2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whang, Kyung-Sook</td>
<td>A-13, A-16, A-17</td>
</tr>
<tr>
<td>Whang, Kyungsook</td>
<td>A-9</td>
</tr>
<tr>
<td>Widyasari, Kristin</td>
<td>B-62</td>
</tr>
<tr>
<td>Won, Ho Keun</td>
<td>H-11</td>
</tr>
<tr>
<td>Won, Mi Kyoungh</td>
<td>J-70</td>
</tr>
<tr>
<td>Won, Youn Hee</td>
<td>E-29</td>
</tr>
<tr>
<td>Woo, E-Eun</td>
<td>D-22, D-24</td>
</tr>
<tr>
<td>Woo, Hyun Jun</td>
<td>F-17, F-18, J-27, J-64</td>
</tr>
<tr>
<td>Woo, Jung-Jai</td>
<td>A-34</td>
</tr>
<tr>
<td>Woo, Koaan Sik</td>
<td>J-17</td>
</tr>
<tr>
<td>Woo, Soo-Young</td>
<td>S11-3</td>
</tr>
<tr>
<td>Woo, Sung-I</td>
<td>A-8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xie, Ling</td>
<td>J-24, J-62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yadav, Dil Raj</td>
<td>J-10, J-39, S4-4, S8-4</td>
</tr>
<tr>
<td>Yadav, Subhash</td>
<td>S23-3</td>
</tr>
<tr>
<td>Yamasaki, Sho</td>
<td>PL-1</td>
</tr>
<tr>
<td>Yang, Byung Wook</td>
<td>B-43, J-37, J-41, J-42</td>
</tr>
<tr>
<td>Yang, Chang-Yeo</td>
<td>A-69</td>
</tr>
<tr>
<td>Yang, Chul-Su</td>
<td>S10-3</td>
</tr>
<tr>
<td>Yang, Ji Yeong</td>
<td>F-17, F-18, J-27, J-64</td>
</tr>
<tr>
<td>Yang, Kwang Hye</td>
<td>F-8, F-26</td>
</tr>
<tr>
<td>Yang, Mi-So</td>
<td>G-13</td>
</tr>
<tr>
<td>Yang, Miso</td>
<td>G-9</td>
</tr>
<tr>
<td>Yang, Seung-Hoon</td>
<td>A-3</td>
</tr>
<tr>
<td>Yang, Seung-Jo</td>
<td>A-52</td>
</tr>
<tr>
<td>Yang, Sung-Hyun</td>
<td>S1-2</td>
</tr>
<tr>
<td>Yang, Yoon-Yong</td>
<td>B-67</td>
</tr>
<tr>
<td>Yeo, Joo-Hong</td>
<td>A-31, H-10</td>
</tr>
<tr>
<td>Yeo, Soo-Hwan</td>
<td>D-8, D-10, D-11, D-20, D-27, D-28, H-47</td>
</tr>
<tr>
<td>Yeom, Se Joung</td>
<td>J-13</td>
</tr>
<tr>
<td>Yeon, Ji Yoon</td>
<td>C-26</td>
</tr>
<tr>
<td>Yeon, Min Ji</td>
<td>F-17, F-18, J-27, J-64</td>
</tr>
<tr>
<td>Yeong, Park Ji</td>
<td>I-26</td>
</tr>
<tr>
<td>Yi, Han</td>
<td>A-71, S23-2</td>
</tr>
<tr>
<td>Yi, Pyoungho</td>
<td>A-54</td>
</tr>
<tr>
<td>Yi, Taewoo</td>
<td>H-43</td>
</tr>
<tr>
<td>Yim, Jin-Hyeok</td>
<td>I-28, J-85</td>
</tr>
<tr>
<td>Yim, Jounghan</td>
<td>B-20</td>
</tr>
<tr>
<td>Yim, Sung Sun</td>
<td>S6-1</td>
</tr>
<tr>
<td>Yong, Dongeun</td>
<td>F-83</td>
</tr>
<tr>
<td>Yong, Zhi</td>
<td>F-74</td>
</tr>
<tr>
<td>Yoo, Boung-Hyuk</td>
<td>D-27, H-47</td>
</tr>
<tr>
<td>Yoo, Cheon-Kwon</td>
<td>F-5, F-43, F-44, F-46, F-47, F-53, G-22</td>
</tr>
<tr>
<td>Author Index</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Yoo, Han Sang .......................... F-48, F-80, H-11, S3-3</td>
<td></td>
</tr>
<tr>
<td>Yoo, JaeChem .................................. J-7</td>
<td></td>
</tr>
<tr>
<td>Yoo, Jee-hong ................................ B-46</td>
<td></td>
</tr>
<tr>
<td>Yoo, Jeehyong ................................ B-50</td>
<td></td>
</tr>
<tr>
<td>Yoo, Jangyeon ................................ B-21, B-22</td>
<td></td>
</tr>
<tr>
<td>Yoo, Jamin ................................... E-4, E-5, E-6, E-10</td>
<td></td>
</tr>
<tr>
<td>Yoo, Jeong Rae ................................ S15-4</td>
<td></td>
</tr>
<tr>
<td>Yoo, Jung Sik ................................ B-24</td>
<td></td>
</tr>
<tr>
<td>Yoo, Seung Hwa ................................ E-28</td>
<td></td>
</tr>
<tr>
<td>Yoo, Seung Min ................................ H-22</td>
<td></td>
</tr>
<tr>
<td>Yoo, Seung Min ................................ H-4, YS4-2</td>
<td></td>
</tr>
<tr>
<td>Yoo, Su Jin ................................... C-26</td>
<td></td>
</tr>
<tr>
<td>Yoo, Tae Hyun ................................ J-64</td>
<td></td>
</tr>
<tr>
<td>Yoo, Wanki ................................... C-15</td>
<td></td>
</tr>
<tr>
<td>Yoo, Woongjae ................................ I-29</td>
<td></td>
</tr>
<tr>
<td>Yoo, Yong-Jae ................................ B-47</td>
<td></td>
</tr>
<tr>
<td>Yoon, Ah ...................................... J-67</td>
<td></td>
</tr>
<tr>
<td>Yoon, Chang-Kyu ................................ YS2-1</td>
<td></td>
</tr>
<tr>
<td>Yoon, Cheol-Hee ................................ F-72</td>
<td></td>
</tr>
<tr>
<td>Yoon, EE ...................................... J-23</td>
<td></td>
</tr>
<tr>
<td>Yoon, Han-Hong ................................ I-12</td>
<td></td>
</tr>
<tr>
<td>Yoon, Hee Jung ................................ S5-2</td>
<td></td>
</tr>
<tr>
<td>Yoon, Hyun Jin ................................ B-14</td>
<td></td>
</tr>
<tr>
<td>Yoon, Hyunjin ................................... I-29</td>
<td></td>
</tr>
<tr>
<td>Yoon, In Joong ................................ H-11</td>
<td></td>
</tr>
<tr>
<td>Yoon, In-Kyu ................................... S15-1</td>
<td></td>
</tr>
<tr>
<td>Yoon, Jeong Hwa ................................ E-29</td>
<td></td>
</tr>
<tr>
<td>Yoon, Ji Hee ................................... C-27</td>
<td></td>
</tr>
<tr>
<td>Yoon, Jihye ................................... J-32</td>
<td></td>
</tr>
<tr>
<td>Yoon, Jin Ju .................................... J-22, J-24, J-62</td>
<td></td>
</tr>
<tr>
<td>Yoon, Jong Kwang .................................. J-68, S18-4</td>
<td></td>
</tr>
<tr>
<td>Yoon, Jung-Hoon .................................. J-46</td>
<td></td>
</tr>
<tr>
<td>Yoon, Kyung-A ................................ D-7, H-9</td>
<td></td>
</tr>
<tr>
<td>Yoon, Sang Sun ................................ F-34, F-83, S11-4, YS1-3</td>
<td></td>
</tr>
<tr>
<td>Yoon, Sang-Hong ................................ H-16</td>
<td></td>
</tr>
<tr>
<td>Yoon, Sang-Hwal ................................ H-26</td>
<td></td>
</tr>
<tr>
<td>Yoon, Soon Duck ................................ J-17</td>
<td></td>
</tr>
<tr>
<td>Yoon, Yeo Joong ................................ C-18</td>
<td></td>
</tr>
<tr>
<td>Yoon, Yeo Kyong ................................ J-45</td>
<td></td>
</tr>
<tr>
<td>Yoon, Yohan .................................... J-35, YS3-1</td>
<td></td>
</tr>
<tr>
<td>You, Donglim ................................... B-12</td>
<td></td>
</tr>
<tr>
<td>You, Hyun Ju ................................... B-56, B-57, F-28</td>
<td></td>
</tr>
<tr>
<td>You, Jee-Hyung ................................ S14-4</td>
<td></td>
</tr>
<tr>
<td>You, Young-Hyun ................................ B-54</td>
<td></td>
</tr>
<tr>
<td>Yu, Dong Su ................................... E-28</td>
<td></td>
</tr>
<tr>
<td>Yu, Hyun Jin ................................... J-83</td>
<td></td>
</tr>
<tr>
<td>Yu, Junsun ..................................... A-52</td>
<td></td>
</tr>
<tr>
<td>Yu, Su Mi ...................................... C-11</td>
<td></td>
</tr>
<tr>
<td>Yu, Sung-Lim ................................... S19-3</td>
<td></td>
</tr>
<tr>
<td>Yum, Jae-Min ................................... G-19</td>
<td></td>
</tr>
<tr>
<td>Yum, Su Jin ................................... I-37</td>
<td></td>
</tr>
<tr>
<td>Yun, Bong-Sik ................................ D-21, D-22, D-24</td>
<td></td>
</tr>
<tr>
<td>Yun, Churljong ................................ B-55</td>
<td></td>
</tr>
<tr>
<td>Yun, Ji-Hun ................................... A-58</td>
<td></td>
</tr>
<tr>
<td>Yun, Jungpyo ................................... B-26</td>
<td></td>
</tr>
<tr>
<td>Yun, Soak-Min ................................ F-13, F-36, F-37</td>
<td></td>
</tr>
<tr>
<td>Yun, Suk-Hyun ................................ D-10, D-20, J-8, S12-2</td>
<td></td>
</tr>
<tr>
<td>Yun, Yeo Hong ................................ J-84, YS1-6</td>
<td></td>
</tr>
<tr>
<td>Yun, Yeohong ................................... J-34</td>
<td></td>
</tr>
<tr>
<td>Yun, Young-Sun ................................ F-46</td>
<td></td>
</tr>
<tr>
<td>Zerin, Tamanna ................................ F-86</td>
<td></td>
</tr>
<tr>
<td>Zhao, Lu ........................................ D-17</td>
<td></td>
</tr>
<tr>
<td>Zhao, Xu ......................................... C-30</td>
<td></td>
</tr>
<tr>
<td>Zo, Young-Gun .................................. A-69, B-62, B-64, B-68</td>
<td></td>
</tr>
</tbody>
</table>
**Keyword Index**

<table>
<thead>
<tr>
<th>Keyword</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-DAP</td>
<td>D-2</td>
</tr>
<tr>
<td>1,3-diaminopropane</td>
<td>D-2</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>B-8, B-43</td>
</tr>
<tr>
<td>16S rRNA gene</td>
<td>A-67, A-68, B-3, I-24, J-16, S2-4</td>
</tr>
<tr>
<td>16S rRNA sequence</td>
<td>A-23</td>
</tr>
<tr>
<td>1-deoxynojirimycin (DNJ)</td>
<td>D-12</td>
</tr>
<tr>
<td>21S rRNA sequence</td>
<td>A-22</td>
</tr>
<tr>
<td>26S rDNA</td>
<td>H-17</td>
</tr>
<tr>
<td>28S</td>
<td>A-63</td>
</tr>
<tr>
<td>2D-PAGE</td>
<td>D-8</td>
</tr>
<tr>
<td>2-Hydroxydodecanoate</td>
<td>B-45</td>
</tr>
<tr>
<td>2-Hydroxyhexadecanoate</td>
<td>B-45</td>
</tr>
<tr>
<td>3-Aminopropionic acid</td>
<td>H-21, H-24</td>
</tr>
<tr>
<td>3-Hydroxypropionic Acid</td>
<td>H-24</td>
</tr>
<tr>
<td>4-Hydroxybutyric acid</td>
<td>H-3</td>
</tr>
<tr>
<td>5-Aminolevulinic acid</td>
<td>H-1</td>
</tr>
<tr>
<td>5-aminovalerate</td>
<td>H-23</td>
</tr>
<tr>
<td>5-Hydroxymeloulin</td>
<td>D-17</td>
</tr>
<tr>
<td>600 different phages</td>
<td>J-48</td>
</tr>
<tr>
<td>Aeromonas salmonicida</td>
<td>B-24</td>
</tr>
<tr>
<td>African Horse Sickness</td>
<td>J-15</td>
</tr>
<tr>
<td>Agaricaceae</td>
<td>A-49</td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>E-8</td>
</tr>
<tr>
<td>Agaricus spp.</td>
<td>C-16</td>
</tr>
<tr>
<td>Aggregate</td>
<td>F-2</td>
</tr>
<tr>
<td>Aging</td>
<td>J-29</td>
</tr>
<tr>
<td>Agriculture</td>
<td>B-44</td>
</tr>
<tr>
<td>agricultural soil</td>
<td>B-66</td>
</tr>
<tr>
<td>Air</td>
<td>J-15</td>
</tr>
<tr>
<td>Airborne bacteria</td>
<td>B-44</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>H-31</td>
</tr>
<tr>
<td>Algalid bacteria</td>
<td>B-11, B-63</td>
</tr>
<tr>
<td>Algalid reactor</td>
<td>B-11</td>
</tr>
<tr>
<td>alkaline phosphatase</td>
<td>H-6</td>
</tr>
<tr>
<td>alkaloid secondary metabolites</td>
<td>A-25</td>
</tr>
<tr>
<td>Alkane hydroxylase system</td>
<td>B-1</td>
</tr>
<tr>
<td>Alkyl hydroperoxide reductase</td>
<td>C-6</td>
</tr>
<tr>
<td>Alkyl hydrogenase</td>
<td>C-6</td>
</tr>
<tr>
<td>Allophylolase</td>
<td>C-6</td>
</tr>
<tr>
<td>Allium hookeri root</td>
<td>J-3</td>
</tr>
<tr>
<td>Allium sativum</td>
<td>J-19</td>
</tr>
<tr>
<td>Aloe arborescens</td>
<td>F-20</td>
</tr>
<tr>
<td>Aloe extract</td>
<td>F-20</td>
</tr>
<tr>
<td>Aloe vera</td>
<td>F-20</td>
</tr>
<tr>
<td>Alloerythrobacter</td>
<td>A-43</td>
</tr>
<tr>
<td>Althamaria</td>
<td>B-16</td>
</tr>
<tr>
<td>Althamaria spp.</td>
<td>J-76</td>
</tr>
<tr>
<td>Amanita spp.</td>
<td>C-13</td>
</tr>
<tr>
<td>AMF</td>
<td>B-59</td>
</tr>
<tr>
<td>amphotericin-B</td>
<td>B-18</td>
</tr>
<tr>
<td>AMPK</td>
<td>G-15</td>
</tr>
<tr>
<td>Amylase</td>
<td>D-8</td>
</tr>
<tr>
<td>anaerobic</td>
<td>B-67</td>
</tr>
<tr>
<td>Anamorph stage</td>
<td>A-72</td>
</tr>
<tr>
<td>Angelica sinensis</td>
<td>A-14</td>
</tr>
<tr>
<td>ANI</td>
<td>A-71</td>
</tr>
<tr>
<td>Animal model</td>
<td>F-80, I-32</td>
</tr>
<tr>
<td>Antagonistic microbes</td>
<td>J-25</td>
</tr>
<tr>
<td>Antarctic</td>
<td>A-50, B-20</td>
</tr>
<tr>
<td>Antarctica</td>
<td>S2-4</td>
</tr>
<tr>
<td>anthracnose</td>
<td>E-3</td>
</tr>
<tr>
<td>Anthranilate</td>
<td>D-19</td>
</tr>
<tr>
<td>Antituberculosis activity</td>
<td>J-66</td>
</tr>
<tr>
<td>antibacterial</td>
<td>H-37, YS1-5</td>
</tr>
<tr>
<td>Anti-Bollism</td>
<td>F-52</td>
</tr>
<tr>
<td>Anti-biofilm agents</td>
<td>F-85</td>
</tr>
<tr>
<td>antibiosis</td>
<td>F-17</td>
</tr>
<tr>
<td>antibiotic resistance</td>
<td>F-17</td>
</tr>
</tbody>
</table>

---

**A**

- α-factor | C-22
- a slow growing bacterium | A-13
- A. pleurobranumiae | H-11
- A. subrufescens | C-16
- A2/O reactor | B-52
- Abies koreana | A-31
- Abnormal fruit disease | J-76
- ABTS | C-14
- Acaetin | D-16
- Acetobacter Bacteria | D-28
- Acetylene Reduction Assay | B-40
- Acid stress | C-2
- Acitv Fast Stain | F-1
- Acinetobacter baumannii | YS1-5
- Acne | J-37
- Actinobacteria | A-7
- actinomycetes | D-25
- actinomycin D | F-6
- Acute gastroenteritis | F-47
- Acryl-Homoserine Lactone | F-57
- adamalysin | B-20
- Adaptive laboratory evolution | D-27
- Adaptive mutations | C-10
- Adipogenesis | J-12
- adjuvants | J-69
- Aeromonas | A-28, B-24
Keyword Index

Antibiotic resistance ........................................... 1-37, J-1
Antibiotics ......................................................... YS4-4
antibody .......................................................... J-50
anti-desertification ............................................. B-13
antifungal ........................................................ B-32
Antifungal Activity ............................................ A-5, A-32, B-37, D-13, J-57
Antigen mimic ...................................................... H-5
tagens .............................................................. F-48
anti-gout ............................................................ F-20
antiinflammatory ................................................ F-85
Antimicrobial activity ......................................... A-11, A-26, YS2-4
antimicrobial ...................................................... H-10
Antimicrobial fatty acid ....................................... J-3
antimicrobial peptide ......................................... B-37
antimicrobial resistance ..................................... I-28
Antimicrobial Resistant ....................................... J-54
Antimicrobial susceptibility ................................ F-46
anti-mycobacterial activity ................................ F-30
Antioxidant ........................................................ I-11, I-25
antioxidant activities ......................................... J-81, J-82
Antioxidant activity .......................................... I-26, I-27
Antioxidant defense .......................................... H-19
Antioxidants ..................................................... D-21
anti-tyrosinase activity ....................................... D-12
antiviral ........................................................... F-77
Antiviral Effect .................................................. I-8
Anti-viral response ............................................. G-15
aphidical .......................................................... J-24
Apo-9'-fucoxanthinone ...................................... G-1
Apopep-1 ........................................................ F-59
Apoptosis ........................................................ F-12, G-21
Apple ................................................................... I-36
Apx toxins .......................................................... H-11
Arbovirus .......................................................... A-45, B-15
arboviruses ........................................................ J-40
Arbuscular Mycorrhizal Fungi ................................ B-31, B-48
Arctic ............................................................... E-2
Arctic ocean ....................................................... A-62, A-66
Arctic region ........................................................ A-46
Arg-1 ................................................................. G-13
Arginine .............................................................. H-40
Arthrornia .......................................................... A-4
Arthrobacter sp. PAMC25486 ................................ E-7
Artic region ........................................................ E-7
Artificial cultivation .......................................... A-60
ascomycota ....................................................... E-3
Ascorbic acid 2-glcoside .................................... J-11
Aspartase .......................................................... H-21
Aspergillus ........................................................ D-10, I-14, J-60
Aspergillus awamori ......................................... J-19
Aspergillus candidus .......................................... I-15
Aspergillus flavus ............................................. I-15
Aspergillus fumigatus ......................................... C-3, I-15
Aspergillus niger ............................................... A-56
Aspergillus luchuensis ........................................ I-12
Aspergillus nidulans .......................................... C-3, E-16
Aspergillus oryzae ............................................... I-12
ATMT ............................................................... E-22
ATP synthase I .................................................. F-50
ATP ................................................................. B-6
AUR1 ............................................................... H-47
Aureobasidin A .................................................... H-47
Auto Stainer ...................................................... F-1
Autoinducer ....................................................... F-54
Autoinducer -2 ................................................ J-28, YS1-2
Autophagy ........................................................ F-40
β-lactamase (bla) gene ....................................... F-33
β-mannanase .................................................... I-17, I-21
B. subtilis MORI ................................................. B-12
Bacillus ........................................................... A-32, D-29
Bacillus amyloyquefaciens ................................ J-25, J-57
Bacillus cereus .................................................. F-43, I-19, I-30
Bacillus licheniformis ....................................... H-39
Bacillus methylotrophicus ................................ E-4
Bacillus paralicheniformis ................................ A-21
Bacillus sp. ........................................................... B-19
Bacillus subtilis ................................................ H-50, I-5, J-71
Bacillus subtilis BCNU 1330 ................................ J-79
Bacillus tequilensis 10b .................................... I-23
Bacillus thuringiensis ........................................ B-47, I-19, J-32
Bacteria .......................................................... B-50, B-65, H-41, H-44, J-13, J-54, S2-4
Bacteria bioreporter .......................................... A-33
Bacterial community ......................................... B-5, B-26, B-66
Bacterial fruit blotch (BBF) ................................. J-66
Bacterial interactions ........................................ F-52
Bacterial pigment .............................................. D-23
Bacterial two hybrid system ............................... C-23
Bacterial vaginosis ........................................... F-28
Bacterial wilt ..................................................... J-58
Bactericidal activity ........................................... F-16
Bacitracin ........................................................ A-55, I-22, I-23, I-34
Bacterioideles ................................................ A-52
Bacteriophage .................................................. B-30, B-53, H-41, J-20, J-48
Bacteroides ...................................................... J-73
Baclovirus ........................................................ H-35, J-5, J-69, J-70
bank vole ........................................................ F-25
barcoded pyrosequencing .................................... J-72
barley ............................................................... J-17
Barton Peninsular ............................................. S2-4
Bacillolivibrio bacteriovorus ................................ F-10
Beneficial Microorganism ................................. B-21
Beneficial microorganisms ................................ B-22
bentonite ........................................................... B-35
Beta Alanine ..................................................... H-21
Bifidobacterium spp. ......................................... I-21
bioactive compound ......................................... B-54
bio-ceramics biofilm system ............................ B-63
<table>
<thead>
<tr>
<th>Keyword Index</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochar</td>
<td>B-21</td>
</tr>
<tr>
<td>biochemical analysis</td>
<td>J-85</td>
</tr>
<tr>
<td>Biocontrol</td>
<td>B-47, B-53, I-15, J-56</td>
</tr>
<tr>
<td>biocontrol agent</td>
<td>B-37</td>
</tr>
<tr>
<td>Bioconversion</td>
<td>H-39, H-40</td>
</tr>
<tr>
<td>Biodegradation</td>
<td>B-1, B-46, B-50, B-61, E-8</td>
</tr>
<tr>
<td>Biodiversity</td>
<td>E-28</td>
</tr>
<tr>
<td>Bioethanol</td>
<td>H-8</td>
</tr>
<tr>
<td>biofertilizer</td>
<td>B-19</td>
</tr>
<tr>
<td>Biofilm</td>
<td>B-51, E-30, F-54, F-6, I-4</td>
</tr>
<tr>
<td>Biofilms</td>
<td>B-24, H-37</td>
</tr>
<tr>
<td>Biofilm formation</td>
<td>D-19, F-7</td>
</tr>
<tr>
<td>Biofunctionality</td>
<td>H-39</td>
</tr>
<tr>
<td>Biogenic amine</td>
<td>J-77</td>
</tr>
<tr>
<td>Biogenic amines</td>
<td>J-78, J-80</td>
</tr>
<tr>
<td>biogeochemistry</td>
<td>B-66</td>
</tr>
<tr>
<td>Bioinformatic resource</td>
<td>E-26</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>E-28</td>
</tr>
<tr>
<td>Biological activity</td>
<td>I-26</td>
</tr>
<tr>
<td>biological control</td>
<td>J-38</td>
</tr>
<tr>
<td>Biological resources</td>
<td>J-33</td>
</tr>
<tr>
<td>Biological soil crust</td>
<td>B-13</td>
</tr>
<tr>
<td>biological treatment</td>
<td>B-25, B-52</td>
</tr>
<tr>
<td>Biomass</td>
<td>H-8</td>
</tr>
<tr>
<td>Biomedical material</td>
<td>J-47</td>
</tr>
<tr>
<td>biopreservative</td>
<td>I-22</td>
</tr>
<tr>
<td>Bioremediation</td>
<td>H-38</td>
</tr>
<tr>
<td>bio-reporter strain</td>
<td>F-31</td>
</tr>
<tr>
<td>Bio-resource</td>
<td>B-28</td>
</tr>
<tr>
<td>biosafety</td>
<td>J-50</td>
</tr>
<tr>
<td>Biosensor</td>
<td>E-32, H-28</td>
</tr>
<tr>
<td>biosurfactant</td>
<td>D-5</td>
</tr>
<tr>
<td>Bisotorta vivipara</td>
<td>A-46</td>
</tr>
<tr>
<td>Bitter melon</td>
<td>I-33</td>
</tr>
<tr>
<td>Bjerkandera adusta</td>
<td>H-33</td>
</tr>
<tr>
<td>BL21(DE3)</td>
<td>C-10</td>
</tr>
<tr>
<td>Black garlic</td>
<td>I-36</td>
</tr>
<tr>
<td>Black ginseng</td>
<td>J-37</td>
</tr>
<tr>
<td>BoNT/B</td>
<td>F-58</td>
</tr>
<tr>
<td>Botulinum neurotoxins A</td>
<td>F-59</td>
</tr>
<tr>
<td>Botulinum neuretoplasmic A</td>
<td>A-29</td>
</tr>
<tr>
<td>Bowmanella</td>
<td>A-29</td>
</tr>
<tr>
<td>Bowmanella dokdonensis</td>
<td>A-29</td>
</tr>
<tr>
<td>Brain homogenate</td>
<td>F-39</td>
</tr>
<tr>
<td>Branched-chain amino acids</td>
<td>F-81</td>
</tr>
<tr>
<td>Brassica</td>
<td>A-5</td>
</tr>
<tr>
<td>Breed</td>
<td>A-13</td>
</tr>
<tr>
<td>brown beech</td>
<td>E-21</td>
</tr>
<tr>
<td>brown fruitbody</td>
<td>A-8</td>
</tr>
<tr>
<td>Brown rat</td>
<td>J-31, J-34</td>
</tr>
<tr>
<td>Brown-rot fungi</td>
<td>H-9</td>
</tr>
<tr>
<td>Brucella abortus</td>
<td>F-48</td>
</tr>
<tr>
<td>Burkholderia sp. nov.</td>
<td>A-16</td>
</tr>
<tr>
<td>CA Uti</td>
<td>G-3</td>
</tr>
<tr>
<td>Cacao</td>
<td>G-16</td>
</tr>
<tr>
<td>cadaverine</td>
<td>H-22</td>
</tr>
<tr>
<td>Cadmium</td>
<td>E-32</td>
</tr>
<tr>
<td>Caenorhabditis elegans</td>
<td>C-28</td>
</tr>
<tr>
<td>calcium-binding protein</td>
<td>I-4</td>
</tr>
<tr>
<td>camel</td>
<td>H-13</td>
</tr>
<tr>
<td>Camellia japonica</td>
<td>B-49</td>
</tr>
<tr>
<td>capture ELISA</td>
<td>F-58</td>
</tr>
<tr>
<td>carbapenem-resistance</td>
<td>F-83</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>J-47</td>
</tr>
<tr>
<td>Carbohydrate recognition</td>
<td>G-17</td>
</tr>
<tr>
<td>carboxymethylcellulose</td>
<td>C-14</td>
</tr>
<tr>
<td>Carotenoid</td>
<td>D-23, H-34</td>
</tr>
<tr>
<td>Caryophyline</td>
<td>F-18</td>
</tr>
<tr>
<td>CCAAT-DNA binding domain</td>
<td>E-17</td>
</tr>
<tr>
<td>c-di-GMP</td>
<td>I-4</td>
</tr>
<tr>
<td>CDK inhibitor</td>
<td>C-1</td>
</tr>
<tr>
<td>cDNA library and clone</td>
<td>J-13</td>
</tr>
<tr>
<td>Cecal microbiota</td>
<td>J-16</td>
</tr>
<tr>
<td>Cedecea aerofastigata GTC 346T</td>
<td>B-40</td>
</tr>
<tr>
<td>Celastrol</td>
<td>J-27</td>
</tr>
<tr>
<td>cell cycle</td>
<td>C-1</td>
</tr>
<tr>
<td>Cellulast</td>
<td>J-42</td>
</tr>
<tr>
<td>cellulase</td>
<td>C-16, C-21, H-9, J-71</td>
</tr>
<tr>
<td>cellulolytic bacteria</td>
<td>B-23</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>J-59</td>
</tr>
<tr>
<td>ceramic coated stainless</td>
<td>B-55</td>
</tr>
<tr>
<td>cereal</td>
<td>J-60</td>
</tr>
<tr>
<td>CFTR</td>
<td>F-62</td>
</tr>
<tr>
<td>CFU</td>
<td>F-86</td>
</tr>
<tr>
<td>CGTase</td>
<td>J-11</td>
</tr>
<tr>
<td>Chaperone</td>
<td>C-8, F-82</td>
</tr>
<tr>
<td>Characteristics</td>
<td>A-37</td>
</tr>
<tr>
<td>characterization</td>
<td>I-22</td>
</tr>
<tr>
<td>chaterization</td>
<td>C-25</td>
</tr>
<tr>
<td>Chemokine</td>
<td>G-19</td>
</tr>
<tr>
<td>Chemotherapeutic drug</td>
<td>A-33</td>
</tr>
<tr>
<td>Cheonggyejang</td>
<td>I-27</td>
</tr>
<tr>
<td>chicken carcasses</td>
<td>I-28</td>
</tr>
<tr>
<td>Chicken farm</td>
<td>H-38</td>
</tr>
<tr>
<td>chicken single chain Fv</td>
<td>H-25</td>
</tr>
<tr>
<td>Chlorde village</td>
<td>H-34</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>H-44</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>H-34</td>
</tr>
<tr>
<td>cholera toxin</td>
<td>F-34, YS1-3</td>
</tr>
<tr>
<td>chronic hepatitis B infection</td>
<td>F-61</td>
</tr>
<tr>
<td>Chrysobacterium</td>
<td>A-18</td>
</tr>
<tr>
<td>Chungcheongnam-do</td>
<td>A-27</td>
</tr>
<tr>
<td>Keyword</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Chungkookjang</td>
<td>G-5</td>
</tr>
<tr>
<td>Cinnamon bark oil</td>
<td>F-7</td>
</tr>
<tr>
<td>Circadian rhythm</td>
<td>F-26</td>
</tr>
<tr>
<td>Classification</td>
<td>A-28</td>
</tr>
<tr>
<td>CLEAs</td>
<td>C-15</td>
</tr>
<tr>
<td>Climate Change</td>
<td>B-64</td>
</tr>
<tr>
<td>Climate change</td>
<td>B-41</td>
</tr>
<tr>
<td>Clone 13 vaccine</td>
<td>J-43</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>J-85</td>
</tr>
<tr>
<td>Cipl</td>
<td>C-8</td>
</tr>
<tr>
<td>c-myc peptide</td>
<td>H-13</td>
</tr>
<tr>
<td>coastal plant</td>
<td>B-54</td>
</tr>
<tr>
<td>coastal stream water</td>
<td>B-9</td>
</tr>
<tr>
<td>Cobalt chloride</td>
<td>F-21</td>
</tr>
<tr>
<td>Cochlostomum polykrikoides</td>
<td>B-63</td>
</tr>
<tr>
<td>Co-Culture</td>
<td>YS4-4</td>
</tr>
<tr>
<td>Sodium fragile</td>
<td>H-50</td>
</tr>
<tr>
<td>Codon adaptation index</td>
<td>E-29</td>
</tr>
<tr>
<td>Coffee grounds</td>
<td>B-21</td>
</tr>
<tr>
<td>Corinella collisoli</td>
<td>A-3</td>
</tr>
<tr>
<td>Cold-active</td>
<td>H-16</td>
</tr>
<tr>
<td>Coliphage</td>
<td>B-30</td>
</tr>
<tr>
<td>Collagenase activity</td>
<td>G-6</td>
</tr>
<tr>
<td>Collection</td>
<td>J-49</td>
</tr>
<tr>
<td>Colletotrichum acutatum</td>
<td>E-3</td>
</tr>
<tr>
<td>Colletotrichum sp.</td>
<td>H-10</td>
</tr>
<tr>
<td>collybioid</td>
<td>A-54</td>
</tr>
<tr>
<td>colony forming units (CFUs)</td>
<td>F-49</td>
</tr>
<tr>
<td>Coloro</td>
<td>H-45</td>
</tr>
<tr>
<td>Comamonadaceae</td>
<td>A-71</td>
</tr>
<tr>
<td>Commensal Bacteria</td>
<td>F-42</td>
</tr>
<tr>
<td>Comparative genomics</td>
<td>E-20, E-25, F-84</td>
</tr>
<tr>
<td>Compatible Solute</td>
<td>B-44</td>
</tr>
<tr>
<td>Complete genome sequence</td>
<td>E-30</td>
</tr>
<tr>
<td>compost</td>
<td>B-21</td>
</tr>
<tr>
<td>Concentration</td>
<td>H-40</td>
</tr>
<tr>
<td>Confrontation line</td>
<td>B-33</td>
</tr>
<tr>
<td>condidation</td>
<td>E-31</td>
</tr>
<tr>
<td>contagious equine metritis</td>
<td>J-6</td>
</tr>
<tr>
<td>Contaminated stream</td>
<td>B-22</td>
</tr>
<tr>
<td>Control</td>
<td>H-38, J-58</td>
</tr>
<tr>
<td>control efficacy</td>
<td>J-22</td>
</tr>
<tr>
<td>control value</td>
<td>J-38</td>
</tr>
<tr>
<td>Coprinellus congregatus</td>
<td>C-2</td>
</tr>
<tr>
<td>Coprinopsis cinerea</td>
<td>C-17</td>
</tr>
<tr>
<td>Coprinopsis spp.</td>
<td>C-17</td>
</tr>
<tr>
<td>Coprinus</td>
<td>D-24</td>
</tr>
<tr>
<td>Coprinus echinosorus</td>
<td>D-21</td>
</tr>
<tr>
<td>Coral Beach</td>
<td>B-62</td>
</tr>
<tr>
<td>Coriolis force</td>
<td>J-7</td>
</tr>
<tr>
<td>Corynebacterium glutamicum</td>
<td>D-3, E-18, H-26</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>H-32, H-48, H-49</td>
</tr>
<tr>
<td>Crab</td>
<td>B-62</td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>F-80</td>
</tr>
<tr>
<td>Cronobacter sakazakii</td>
<td>J-20</td>
</tr>
<tr>
<td>Cronobacter sakazaki</td>
<td>YS2-4</td>
</tr>
<tr>
<td>Cropping system</td>
<td>B-8</td>
</tr>
<tr>
<td>crude oil</td>
<td>B-67</td>
</tr>
<tr>
<td>Cryptococcus albidusimilel</td>
<td>J-81</td>
</tr>
<tr>
<td>Cryptococcus pseudolongus</td>
<td>J-34</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>B-58</td>
</tr>
<tr>
<td>Crystal structure</td>
<td>G-17</td>
</tr>
<tr>
<td>CTL</td>
<td>G-18</td>
</tr>
<tr>
<td>CTX</td>
<td>J-83</td>
</tr>
<tr>
<td>Cullin</td>
<td>B-15</td>
</tr>
<tr>
<td>culin RING E3 ligase</td>
<td>YS3-6</td>
</tr>
<tr>
<td>cultivar</td>
<td>J-17</td>
</tr>
<tr>
<td>Cultivars</td>
<td>C-9</td>
</tr>
<tr>
<td>cultivation</td>
<td>B-43, B-65</td>
</tr>
<tr>
<td>Culture Collection</td>
<td>F-79</td>
</tr>
<tr>
<td>culture filtrate</td>
<td>J-24</td>
</tr>
<tr>
<td>Culture filtrate protein</td>
<td>G-11</td>
</tr>
<tr>
<td>culture media</td>
<td>J-62</td>
</tr>
<tr>
<td>Culture</td>
<td>J-49</td>
</tr>
<tr>
<td>cuparene-type sesquiterpene</td>
<td>D-24</td>
</tr>
<tr>
<td>Cu-resistant bacteria</td>
<td>B-36</td>
</tr>
<tr>
<td>cyclic AMP receptor protein</td>
<td>F-34, YS1-3</td>
</tr>
<tr>
<td>Cyclo(l-Phe-L-Pro)</td>
<td>F-71</td>
</tr>
<tr>
<td>Cyclohexane tolerance</td>
<td>H-32</td>
</tr>
<tr>
<td>Cysteine Cysteine redox shuttle</td>
<td>P-20</td>
</tr>
<tr>
<td>Cylindrocarpon</td>
<td>A-32</td>
</tr>
<tr>
<td>cytochrome</td>
<td>C-26</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>F-30, G-9</td>
</tr>
<tr>
<td>Decolorization</td>
<td>H-33</td>
</tr>
<tr>
<td>Decomposition</td>
<td>C-9</td>
</tr>
<tr>
<td>Dectin-1</td>
<td>F-69, G-19</td>
</tr>
<tr>
<td>Defense gene</td>
<td>E-15</td>
</tr>
<tr>
<td>Dehydrogenase activity</td>
<td>B-12</td>
</tr>
<tr>
<td>demucilaging</td>
<td>J-42</td>
</tr>
<tr>
<td>Dendritic cell</td>
<td>G-20</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>G-32</td>
</tr>
<tr>
<td>deoxyxypyrullarine</td>
<td>F-30</td>
</tr>
<tr>
<td>Deoxyviolacein</td>
<td>D-4, YS4-4</td>
</tr>
<tr>
<td>Deposti body</td>
<td>J-33</td>
</tr>
<tr>
<td>derivates</td>
<td>C-30</td>
</tr>
<tr>
<td>Dermabacter vaginalis</td>
<td>A-41</td>
</tr>
<tr>
<td>DesaturaseB</td>
<td>J-35</td>
</tr>
<tr>
<td>Desert lichens</td>
<td>B-38</td>
</tr>
<tr>
<td>Detection</td>
<td>B-60</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>H-11, J-76</td>
</tr>
<tr>
<td>diaminopimelate epimerase</td>
<td>C-29</td>
</tr>
<tr>
<td>diaper</td>
<td>F-85</td>
</tr>
<tr>
<td>diarrhea-causing bacteria</td>
<td>F-44</td>
</tr>
<tr>
<td>Didymopseudomonas</td>
<td>J-39</td>
</tr>
<tr>
<td>Dietary restriction</td>
<td>J-67</td>
</tr>
<tr>
<td>dimeric</td>
<td>H-13</td>
</tr>
<tr>
<td>Keyword Index</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>Dimerization</td>
<td>E-22</td>
</tr>
<tr>
<td>Dimorphic fungi</td>
<td>J-36</td>
</tr>
<tr>
<td>Disinfectant</td>
<td>H-6</td>
</tr>
<tr>
<td>Diversification</td>
<td>A-11</td>
</tr>
<tr>
<td>Diversity</td>
<td>B-4, B-48, B-49, B-65, B-66, J-10, J-39</td>
</tr>
<tr>
<td>DLBCL</td>
<td>J-64</td>
</tr>
<tr>
<td>DMSO</td>
<td>C-20</td>
</tr>
<tr>
<td>DNA vaccine</td>
<td>J-68, J-70, S18-4</td>
</tr>
<tr>
<td>Donghae</td>
<td>D-29, I-19</td>
</tr>
<tr>
<td>Dogbaekdongsan</td>
<td>J-84</td>
</tr>
<tr>
<td>Dokdo</td>
<td>H-16</td>
</tr>
<tr>
<td>Dombaroccus</td>
<td>A-59</td>
</tr>
<tr>
<td>DPG-5</td>
<td>F-86</td>
</tr>
<tr>
<td>DPPH</td>
<td>J-23</td>
</tr>
<tr>
<td>DPPH radical scavenging</td>
<td>C-24</td>
</tr>
<tr>
<td>D-Psicose 3-α-epimerase</td>
<td>H-26</td>
</tr>
<tr>
<td>Drosophila Expression System</td>
<td>F-14</td>
</tr>
<tr>
<td>Drosophila melanogaster</td>
<td>F-8, F-26, J-29, J-67</td>
</tr>
<tr>
<td>drought stress tolerance</td>
<td>B-19</td>
</tr>
<tr>
<td>Dual culture</td>
<td>B-33</td>
</tr>
<tr>
<td>Dyella thiocyanids</td>
<td>E-10</td>
</tr>
<tr>
<td>E. coli</td>
<td>D-9</td>
</tr>
<tr>
<td>ear edema</td>
<td>G-10</td>
</tr>
<tr>
<td>EBER</td>
<td>J-64</td>
</tr>
<tr>
<td>Ebola virus</td>
<td>F-13, F-14</td>
</tr>
<tr>
<td>EBV</td>
<td>F-64</td>
</tr>
<tr>
<td>Ecology</td>
<td>E-28</td>
</tr>
<tr>
<td>Ectromycomitral fungi</td>
<td>C-14</td>
</tr>
<tr>
<td>Effector protein</td>
<td>G-12</td>
</tr>
<tr>
<td>Efficiency</td>
<td>H-40</td>
</tr>
<tr>
<td>Egg liquid products</td>
<td>I-20</td>
</tr>
<tr>
<td>Egg thermotolerant products</td>
<td>I-20</td>
</tr>
<tr>
<td>EHEC</td>
<td>F-32</td>
</tr>
<tr>
<td>ELISA</td>
<td>H-11</td>
</tr>
<tr>
<td>ELISA test</td>
<td>J-15</td>
</tr>
<tr>
<td>emetic toxin</td>
<td>F-43</td>
</tr>
<tr>
<td>Endothelial bacteria</td>
<td>B-38, B-45</td>
</tr>
<tr>
<td>Endothelial fungus</td>
<td>B-17</td>
</tr>
<tr>
<td>endophyte</td>
<td>B-54, H-10</td>
</tr>
<tr>
<td>Endophytic bacteria</td>
<td>A-44</td>
</tr>
<tr>
<td>Endophytic fungi</td>
<td>A-31, A-46, B-49, B-58</td>
</tr>
<tr>
<td>Endoplasmic reticulum stress</td>
<td>F-15</td>
</tr>
<tr>
<td>Endurococcus ginsengi</td>
<td>A-12</td>
</tr>
<tr>
<td>enteric virus</td>
<td>F-47</td>
</tr>
<tr>
<td>Enter-Net</td>
<td>F-44</td>
</tr>
<tr>
<td>Enter-Net Korea</td>
<td>F-43</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>F-83</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>F-26</td>
</tr>
<tr>
<td>Enterococcus faeaeum</td>
<td>G-4, H-39, I-6</td>
</tr>
<tr>
<td>Enterovirus 71</td>
<td>J-5</td>
</tr>
<tr>
<td>entomopathogenic fungi</td>
<td>J-22, J-24, J-62</td>
</tr>
<tr>
<td>environment</td>
<td>A-36, B-31</td>
</tr>
<tr>
<td>environmental DNA</td>
<td>J-46</td>
</tr>
<tr>
<td>Environmental Restoration</td>
<td>J-55</td>
</tr>
<tr>
<td>Enzymatic Index</td>
<td>C-21</td>
</tr>
<tr>
<td>Enzyme inhibition activity</td>
<td>I-25</td>
</tr>
<tr>
<td>enzymes</td>
<td>I-31</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>F-19</td>
</tr>
<tr>
<td>epoxy-coated cement</td>
<td>B-55</td>
</tr>
<tr>
<td>Equine Infectious Anaemia</td>
<td>J-15</td>
</tr>
<tr>
<td>Equine Viral Arteritis</td>
<td>J-15</td>
</tr>
<tr>
<td>ESBL</td>
<td>J-1</td>
</tr>
<tr>
<td>Essential oil</td>
<td>C-11</td>
</tr>
<tr>
<td>essential oil extract</td>
<td>F-18</td>
</tr>
<tr>
<td>Essential oils</td>
<td>J-66</td>
</tr>
<tr>
<td>estrogen</td>
<td>F-61</td>
</tr>
<tr>
<td>ETEC</td>
<td>G-22</td>
</tr>
<tr>
<td>Eugenol</td>
<td>F-7</td>
</tr>
<tr>
<td>Eurotium</td>
<td>I-14</td>
</tr>
<tr>
<td>Exoelectrogen</td>
<td>A-29</td>
</tr>
<tr>
<td>Exogenous glycerol</td>
<td>H-30</td>
</tr>
<tr>
<td>Exogenous trehalose</td>
<td>H-15, H-29</td>
</tr>
<tr>
<td>exopolysaccharide</td>
<td>A-14</td>
</tr>
<tr>
<td>Exotic Plant Invasion</td>
<td>B-31</td>
</tr>
<tr>
<td>Exotoxin</td>
<td>I-37</td>
</tr>
<tr>
<td>Expression</td>
<td>E-15</td>
</tr>
<tr>
<td>extracellular protease</td>
<td>B-20</td>
</tr>
<tr>
<td>Extreme city environments</td>
<td>B-59</td>
</tr>
<tr>
<td>FACS</td>
<td>H-36</td>
</tr>
<tr>
<td>FACS319</td>
<td>H-7</td>
</tr>
<tr>
<td>Falsiaeromonas</td>
<td>A-28</td>
</tr>
<tr>
<td>Fecal microbiota</td>
<td>J-16</td>
</tr>
<tr>
<td>ferment</td>
<td>A-33</td>
</tr>
<tr>
<td>Fermentable sugars</td>
<td>H-8</td>
</tr>
<tr>
<td>Fermentation</td>
<td>D-10, D-11, D-16, D-18, D-28, I-9, I-27</td>
</tr>
<tr>
<td>Fermented foods</td>
<td>I-6</td>
</tr>
<tr>
<td>Fermented soy milk</td>
<td>I-25</td>
</tr>
<tr>
<td>Fimbrial-uptake regulator</td>
<td>C-6</td>
</tr>
<tr>
<td>fibrinogen</td>
<td>F-74</td>
</tr>
<tr>
<td>Fibrinolytic enzyme</td>
<td>I-5</td>
</tr>
<tr>
<td>Filamentous fungi</td>
<td>J-4</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>A-3, A-59, D-20</td>
</tr>
<tr>
<td>First report</td>
<td>J-34</td>
</tr>
<tr>
<td>fission yeast</td>
<td>C-1</td>
</tr>
<tr>
<td>fixed virus</td>
<td>YS3-2</td>
</tr>
<tr>
<td>FK506</td>
<td>C-18, C-30</td>
</tr>
<tr>
<td>Flaviviridae</td>
<td>A-22</td>
</tr>
</tbody>
</table>
flavivirus ................................................................. H-42
Flavobacteriaceae ..................................................... A-18
Flavobacterium halomollic .......................................... A-15
fluorescence ................................................................ C-22
Flux Optimization ....................................................... H-20
FMD Vaccine ............................................................. J-69
food safety ................................................................. I-19, A-55, I-34
food waste ................................................................. B-7
Food-borne bacteria ..................................................... I-24
Food-borne pathogens ............................................... I-16
foodborne pathogens ................................................ A-55, I-34
forest soil ................................................................. A-16
forestry soil ............................................................... J-74
Formosa algae ........................................................... I-3
freshwater ................................................................. A-67, A-68
Friedmanniella aerolata ............................................. A-22
FrsA ........................................................................ E-19
Fructose ..................................................................... H-26
Fruit body ................................................................. I-13, J-34
Fruit waste ................................................................. H-8
Fruiting bodies .......................................................... A-60
FruR ........................................................................ YS2-1
Fucoidan ................................................................. I-3
Fucoidanase ............................................................. I-3
Fusarium oxysporum .................................................. I-3
Fumaginic Acid ........................................................ H-20, H-21, H-24
fungal development .................................................... E-31
Fungal genome .......................................................... E-27
Fungal identification ................................................... J-19
fungal ITS ................................................................. B-32
Fungal Pathogen ....................................................... B-64
Fungal Pathogens ..................................................... J-57
Fungi ......................................................................... E-27, I-31, J-13, J-45, J-49
Fusacidin ................................................................. D-13
Fusarium ................................................................. J-25
Fusarium graminearum ............................................. E-17, E-33
Fusarium oxysporum ................................................ A-5, J-38
Fusarium sp. ............................................................. H-10
Fusion peptide .......................................................... H-5
FXIa ........................................................................ J-61

γ-Aminobutyric acid ................................................. I-6
γ-Butyrolactone ........................................................ H-3
γ-Proteobacteria ....................................................... A-12
Gait/S transition ........................................................ C-1
Galaclin ................................................................. G-17
Gallic acid reaction .................................................... H-33
Gamma herpesvirus ................................................... F-45, F-78
Gardnerella vaginalis ................................................ F-28
Gasolin ................................................................. H-2
gasteroid ................................................................. A-49
Gastrodia elata Blume ............................................. I-7

GC-MS ........................................................................ J-3
GenBank ................................................................. J-51
Gene annotation ....................................................... E-27
Gene expression ....................................................... C-5, C-6, D-7
Gene prediction pipeline ........................................ E-27
Gene typing ............................................................. A-10
genera ..................................................................... A-51
generic analysis ....................................................... A-57
genetic circuits ......................................................... E-32
genetic diversity ....................................................... A-20, A-31
genetic engineering .................................................. C-18
genetic evolution ...................................................... E-7
genetic heterogeneity ............................................... E-12
genetic information ................................................ J-50
Genetic resources ..................................................... J-33
Genetic variation ...................................................... A-61
Genetically modified crop ........................................ B-26
Genome ................................................................. A-21, B-2, E-4, E-5, E-6, E-9, E-10, E-21
Genome annotation ................................................ E-26
Genome browser ..................................................... E-26
Genome sequence ................................................... E-23
Genome sequencing ................................................ E-7
genomic analysis .................................................... J-20
Genomic data analysis ............................................. E-26
Genomic diversity .................................................... F-35
genotype ................................................................. F-53
genus Leucothrix ..................................................... A-62
Genus Planktotaela ................................................ A-66
Germinated soybean ................................................ I-26
Germination ............................................................ C-7
GM ........................................................................... I-21
ginseng ................................................................. A-57, B-43, I-10, J-57
ginseng berry extract ............................................... I-10
Gliadelia splendens ................................................... D-22
Global repressor Mlc ................................. C-27
Glucose metabolism ................................................ F-34, YS1-3
Glucose starvation ................................................... YS2-2
Glucose transporter ................................................... Y-12
Glucose uptake ......................................................... J-12
Glutamate decarboxylase ......................................... I-6
Glutamate ................................................................. H-23
Glutathione ............................................................. C-26
Glycan ................................................................. H-51
Glycine ................................................................. H-1
glycoprotein .......................................................... F-13
GMP ................................................................. J-68
Gonadotrophin ........................................................ H-35
Goljawal ................................................................. J-84
Goljawal forest soil ................................................... S2-3
GPCR ................................................................. C-22, H-36
GPI-anchored protein ................................................. J-11
Gram-negative ........................................................ A-25, A-38
Granular activated carbon ........................................ B-6

www.fkms.kr | 391
Keyword Index

Green environmental city .............................................. B-59
Green seaweed .......................................................... H-50
Greenhouse .............................................................. J-14
Grifia frondosa ........................................................ G-10
Group B Streptococci .................................................. F-73, F-74
GST pull down assay .................................................... C-23
Gut Homeostasis ....................................................... J-29
Gut microbes ............................................................ J-29
Gut microbiota .......................................................... A-11, J-28, YS1-2
gyrA ......................................................................... F-17
HIV-1 Tat .................................................................. F-72
HIV-1 Tat .................................................................. F-72
HNS ........................................................................ F-70
HOK-16B Cells .......................................................... F-55
Horses ..................................................................... J-6, J-40
host immune response .................................................. G-11
host restriction ......................................................... F-72
Host specification ...................................................... F-36
Host-microbes interaction .......................................... J-67
hot water extraction .................................................. J-41
HPr ........................................................................ YS2-1
HSP 100 family ........................................................ C-8
hsps65 genotype ........................................................ J-63
human airway epithelial cells ...................................... F-69
human norovirus ...................................................... I-32
human skin ............................................................... C-25
Humibacter soil ......................................................... A-7
humoral immune response ........................................ F-73
HY-209 .................................................................. F-62
Hydrometaphaga ....................................................... A-71
Hydrocarbons .......................................................... B-61
Hydrodynamic method .............................................. F-60
hydrogel ................................................................. B-35
hydrophobicity ........................................................ F-6
hypa length ............................................................. D-14
hypal growth ........................................................... E-31
Hypoxia-mimicking agent ......................................... F-21
Hypsizigus marmoreus ............................................... E-21
Hypsizigus marmoreus ............................................... E-21
Hyrtios sp. ................................................................ F-77
HQQ4me3 ................................................................. J-2
Haemaphysalis longicornis ......................................... F-36
Halliclona sp. ........................................................... F-77
halo sensitive bacterium ........................................... A-15
Halomonas ............................................................... A-17
halophilic bacterium ................................................ A-17
Halotolerant bacteria ................................................ B-44
Hansenula polymorpha ................................................ C-26
Hantavirus ............................................................... A-20, F-19
Hantavirus ............................................................... A-24, F-19
Harmful algal blooms ............................................... B-11, B-63
HBsAg ...................................................................... F-60
HBx ......................................................................... YS3-6
HCDC ........................................................................ D-4
HCMV ................................................................. F-4, F-40, F-41, G-2
health risk ............................................................... A-69
heat shock protein 70(DnaK) ...................................... C-23
Helicobacter pylori .................................................. F-17, F-18, J-27
hemolysis .................................................................. F-6, F-7
hepatitis A .............................................................. F-53
hepatitis A virus ........................................................ F-53
Hepatitis B virus .................................................... F-60, YS3-6
Hepatocellular carcinoma ......................................... F-61
Herbicide ................................................................... D-26
herbicidin A ............................................................. D-14
Heteroclitum erinaceus ............................................ J-58
Heterologous expression ......................................... E-11
Heterotrophic cultures .............................................. D-23
Heterozygosity ........................................................ B-33
Hexamedine ............................................................ H-45
hexane fraction ........................................................ G-6
hexane tolerance ........................................................ H-15
HIF-1a ..................................................................... F-21
High copy plasmid ..................................................... H-7
high throughput screening ......................................... H-25
high passaged strain .................................................. E-29
His tag ....................................................................... C-4
Histidine Kinase ....................................................... B-51
Histone modification ................................................. J-2
HIV-1 ................................................................. F-71, F-72
HIV-1 p24 ................................................................ G-18
IBC ........................................................................ J-65
Identification .......................................................... J-4
IE1 ........................................................................ G-14
IFNbeta ................................................................. G-14
IgG .......................................................................... H-36
IL-6 ........................................................................ F-66, F-67, G-5
Ilyonectria radicicola ............................................... A-57
Imid pathway ........................................................... F-8
Imijn Virus ................................................................ A-24
Immune evasion ........................................................ G-12
immune regulation .................................................... F-64
Immune response .................................................. J-68, J-70, S18-4
immune responses .................................................. F-48
Imunization ............................................................ J-70
immunocytochemistry staining ................................ H-25
Immunomodulatory activity ..................................... G-4
Indole ....................................................................... D-19
indoor cultivation .................................................... A-69
Industrialization ........................................................ I-5
Infection ..................................................................... F-26
Infectious clone ........................................................ F-63
Inflammation .......................................................... F-55, G-1, G-8, G-23
<table>
<thead>
<tr>
<th>Keyword</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory bowel disease</td>
<td>F-42</td>
</tr>
<tr>
<td>Inflammatory monocyte</td>
<td>F-42</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>F-62</td>
</tr>
<tr>
<td>Influenza</td>
<td>J-68, S18-4</td>
</tr>
<tr>
<td>Influenza A</td>
<td>F-35</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>G-15, I-8</td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>F-56</td>
</tr>
<tr>
<td>Influenza hemagglutinin</td>
<td>H-5</td>
</tr>
<tr>
<td>Ingredients of Kimchi</td>
<td>I-8</td>
</tr>
<tr>
<td>innate immune response</td>
<td>F-8</td>
</tr>
<tr>
<td>iNOS</td>
<td>G-13</td>
</tr>
<tr>
<td>Insecticidal activity</td>
<td>J-32</td>
</tr>
<tr>
<td>inside of ginseng root</td>
<td>A-13</td>
</tr>
<tr>
<td>Insulinotropic effect</td>
<td>J-9</td>
</tr>
<tr>
<td>Integration Helper Plasmid</td>
<td>E-14</td>
</tr>
<tr>
<td>Interacting partners</td>
<td>F-4</td>
</tr>
<tr>
<td>Interleukin-1 beta</td>
<td>F-42</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>F-61</td>
</tr>
<tr>
<td>intermediates</td>
<td>C-30</td>
</tr>
<tr>
<td>Intestinal microbe</td>
<td>J-67</td>
</tr>
<tr>
<td>inventory</td>
<td>A-48</td>
</tr>
<tr>
<td>ionogenic amine reduction</td>
<td>J-79</td>
</tr>
<tr>
<td>Ion Torrent PGM</td>
<td>E-2</td>
</tr>
<tr>
<td>Iron</td>
<td>F-27</td>
</tr>
<tr>
<td>Iron–sulfur cluster enzyme</td>
<td>F-81</td>
</tr>
<tr>
<td>ISG15</td>
<td>F-41</td>
</tr>
<tr>
<td>ISGylation</td>
<td>F-41</td>
</tr>
<tr>
<td>Isoflavone</td>
<td>I-9, I-25, I-26, I-27</td>
</tr>
<tr>
<td>Isolation</td>
<td>A-27, B-61, F-36</td>
</tr>
<tr>
<td>ITS</td>
<td>A-48, A-63, A-64</td>
</tr>
<tr>
<td>ITS sequence</td>
<td>A-53</td>
</tr>
<tr>
<td>IVIG</td>
<td>J-61</td>
</tr>
<tr>
<td>J.lividum</td>
<td>B-34</td>
</tr>
<tr>
<td>Japanese eel</td>
<td>H-35</td>
</tr>
<tr>
<td>Japanese encephalitis virus</td>
<td>H-42</td>
</tr>
<tr>
<td>jeotgal</td>
<td>J-78, J-80</td>
</tr>
<tr>
<td>JEV</td>
<td>J-40</td>
</tr>
<tr>
<td>Johne’s disease</td>
<td>F-80</td>
</tr>
<tr>
<td>Kallir</td>
<td>YS2-4</td>
</tr>
<tr>
<td>KEGG metabolic pathway</td>
<td>F-84</td>
</tr>
<tr>
<td>KGH strain</td>
<td>F-3, YS3-2</td>
</tr>
<tr>
<td>Kimchi</td>
<td>I-1</td>
</tr>
<tr>
<td>Kimchi cabbage</td>
<td>A-61</td>
</tr>
<tr>
<td>kinetic</td>
<td>I-30</td>
</tr>
<tr>
<td>Kitasatospora</td>
<td>E-9</td>
</tr>
<tr>
<td>Korea</td>
<td>A-4</td>
</tr>
<tr>
<td>Korea Mushroom Resource Bank</td>
<td>J-49</td>
</tr>
<tr>
<td>Korea National Arboretum</td>
<td>A-54</td>
</tr>
<tr>
<td>Korean Beijing strain</td>
<td>E-1</td>
</tr>
<tr>
<td>KSHV</td>
<td>F-64, F-65, F-66, F-67, F-68</td>
</tr>
<tr>
<td>LAB</td>
<td>I-1</td>
</tr>
<tr>
<td>Lab-on-a-Disc(LOD)</td>
<td>J-7</td>
</tr>
<tr>
<td>laboratory surveillance</td>
<td>F-47</td>
</tr>
<tr>
<td>Laccase</td>
<td>C-17, C-2, E-11, H-9</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>D-16, G-13, H-7, I-7, J-78</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>F-28</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>A-11</td>
</tr>
<tr>
<td>Lactobacillus brevis</td>
<td>I-7</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>I-25</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>I-17</td>
</tr>
<tr>
<td>lag phase</td>
<td>C-10</td>
</tr>
<tr>
<td>Laminarin</td>
<td>J-16</td>
</tr>
<tr>
<td>LAMMER kinase</td>
<td>C-1</td>
</tr>
<tr>
<td>LAMP</td>
<td>F-83, J-18</td>
</tr>
<tr>
<td>large scale fermentation</td>
<td>D-9</td>
</tr>
<tr>
<td>large-scale detection</td>
<td>F-33</td>
</tr>
<tr>
<td>L-arginine</td>
<td>D-3</td>
</tr>
<tr>
<td>lava forest</td>
<td>A-3</td>
</tr>
<tr>
<td>Legionella pneumonia</td>
<td>B-60</td>
</tr>
<tr>
<td>Lentilina edodes</td>
<td>B-33, C-9, I-11, I-13, I-8, J-34</td>
</tr>
<tr>
<td>Leptodinum orchidicola</td>
<td>B-58</td>
</tr>
<tr>
<td>Lettuce</td>
<td>J-56</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>H-7</td>
</tr>
<tr>
<td>Leuconostoc mesenteroides</td>
<td>I-1</td>
</tr>
<tr>
<td>Leucothrix arctica sp. nov.</td>
<td>A-62</td>
</tr>
<tr>
<td>liruO</td>
<td>F-70</td>
</tr>
<tr>
<td>levofloxacin</td>
<td>F-17</td>
</tr>
<tr>
<td>library</td>
<td>J-46</td>
</tr>
<tr>
<td>Lichen-forming fungi</td>
<td>A-19, E-20, J-52</td>
</tr>
<tr>
<td>Lichen-forming fungus</td>
<td>E-22</td>
</tr>
<tr>
<td>Lichenicolous fungi</td>
<td>A-19</td>
</tr>
<tr>
<td>Lichenized fungi</td>
<td>A-4</td>
</tr>
<tr>
<td>lichen</td>
<td>J-52</td>
</tr>
<tr>
<td>Li-F type antibiotics</td>
<td>D-13</td>
</tr>
<tr>
<td>Lifespan extension</td>
<td>C-28</td>
</tr>
<tr>
<td>Lignocellulolytic Enzyme</td>
<td>H-9</td>
</tr>
<tr>
<td>lignocellulose degradation</td>
<td>B-23</td>
</tr>
<tr>
<td>lignocellulose</td>
<td>E-8</td>
</tr>
<tr>
<td>Limonene</td>
<td>H-8</td>
</tr>
<tr>
<td>Lipase</td>
<td>H-16</td>
</tr>
<tr>
<td><a href="http://www.fkms.kr">www.fkms.kr</a></td>
<td>393</td>
</tr>
</tbody>
</table>
Keyword Index

Lipid .............................................. H-34
Lipocalin 2 ...................................... F-15
Lipolysis ........................................ J-12
lipopeptides ................................... A-32
Lipopoly saccharide ............................ F-29
Listeria monocytogenes ....................... I-16
Living Modified Organisms ................... J-65
I-lysine .......................................... H-23
LMO ............................................... J-65
Longevity ....................................... G-4, J-67
Low temperature ................................ D-11
low-dose radiation ............................. F-8
Luciferase assay ................................ F-13
Lumintor ........................................ B-6
Lung cancer ..................................... G-9
Lysophosphatidylcholine ..................... F-16
lytic replication ................................ F-68

Metabolic gene .................................. F-50
Metabolic reprogramming ..................... F-50
Metabolism reprogramming ................... F-9, YS4-4
Metagenome ................................... B-5, E-2, H-16, I-24, J-46
Metagenomic Analysis ....................... B-68
Metagenomics .................................. B-43, J-44
metal stress ..................................... B-36
Methane emission ............................. J-44
Methane oxidizing bacteria ................. A-65
methanogen .................................... B-67
Methanotroph .................................. A-65
Methylobacterium sp. ......................... A-65
Methyl .............................. Microbe J-47
Methylated ..................................... H-12
Methylotrophs ................................. J-44
Methionine ..................................... D-6
Microarray (GeoChip) ......................... S2-3
Microbacterium ............................... A-7
Microbial ....................................... J-47
Microbial association ......................... H-43
Microbial community ......................... B-41, B-43, B-62, E-2, H-43
Microbial community structure ............. S2-3
Microbial contamination ..................... I-20
Microbial control ............................. YS2-4
microbial diversity ........................... B-10
Microbial dynamics .......................... B-26
Microbial fuel cell ........................... A-29
Microbial Source Tracking ................. B-30
Microbiome .................................... B-62
Microorganism ............................... B-7, J-55
Microsatellite .................................. B-33
Microscopic .................................... A-37
MidtreLoenviernien .......................... E-2
milking degree ................................ J-17
Minimum temperature ....................... C-14
MiSeq ........................................... J-16
Mitogenome .................................... E-3
Mixing .......................................... J-7
Mju ............................................. I-14
mitude ........................................... J-32
Monoclonal antibodies ....................... F-58
Monoclonal antibody ......................... F-59, H-42

M. phyllosphaerae ............................ E-5
Macroluval diversity ......................... B-27
Macroluva ..................................... B-28
Macrophage ................................... F-15, G-21
Macrophages polarization .................... G-13
Magnaporthe grise ................................ H-12
Makgeolli ..................................... I-10, I-31, J-17
MALDI-TOF/TOF ............................. I-23
Mangrove Rhizosphere ......................... B-62
mannanase ..................................... H-50
Mannheimia suconicprodusens ............. H-3
Manual Staining ............................... F-1
MAPK .......................................... E-16
MAPKs ......................................... G-10
Marasmus magnus magnus .................... A-1
Marburg virus .................................. F-13
Marine bacteria ................................ I-3
Marine fungi ................................... J-33
marine invertebrate ........................... A-71
marine sediment .............................. B-25
Mass production ................................ C-4
Matrine .......................................... J-32
MDA-MB-231 ................................... G-5
Mdr pseudomonas aureginosa ............... A-26
MDR ............................................. F-86
MDRO .......................................... D-15
Meet retailer ................................... J-85
Medium optimization ......................... D-26
Meli .................. Microarray (GeoChip) S2-3
Meningitis ..................................... F-74
Moniliaceae .................................... A-50
meta-analysis ................................ B-3

394 | 2015 International Meeting of FKMS
Keyword Index

Monosodium glutamate ........................................... H-46
Morphogenesis ................................................. J-2
Morphological characteristics ........................... A-56, A-72, C-12, C-13
Mosquitoes .................................................. A-45
Motility .......................................................... B-51
Mouse-adaptation .................................................. F-56
mptB ............................................................ E-16
MRSA .......................................................... A-55, I-34
msbb ............................................................ F-29
MTB .......................................................... F-86
MTT assay ......................................................... G-9, H-25
Mucilaginibacter .................................................. A-70
Mucoraceae .................................................. A-64
mucosal vaccine .................................................. G-18
Multi-Drug Resistance ......................................... YS1-5
Multi-lamination .................................................. J-7
Multilocus sequence analysis ............................... A-10
Murine norovirus .............................................. I-35
mushroom diversity ............................................. J-84
mushroom fruiting body ........................................ H-51
Mushroom ......................................................... A-64, D-22, D-24, E-11, E-21, H-51, J-49
Mutant ............................................................ J-45
Myc ............................................................. YS3-6
Mycelial .......................................................... A-37
Mycelial colony .................................................. C-12, C-13
Mycoerial growth ................................................ C-14
mycobacteria ...................................................... C-5, C-6
mycobacterial infection ......................................... F-80
Mycobacterium abscessus ..................................... J-63
Mycobacterium avium ........................................... G-21
Mycobacterium chelonae ....................................... G-19
Mycobacterium fortuitum ....................................... F-12
Mycobacterium massil ......................................... F-49
Mycobacterium massilenterse ................................ J-63
Mycobacterium tuberculosis ................................ E-1, F-1, F-15, F-16, F-69, G-11, G-20
mycoflora .......................................................... D-10
mycology .......................................................... I-31
Mycosphaerella nawae ........................................ A-72, J-75
Mycoxin .......................................................... B-16, J-25
mycovirus .......................................................... J-8

Neurite outgrowth .............................................. F-24
neuronal cell line ................................................ F-25
Neurospora crassa ............................................. E-31
Neutralization .................................................... F-59
new cephalosporin ............................................. A-26
new genus ........................................................ A-40
New record ...................................................... A-34
New species ..................................................... A-19, A-48
New strain ....................................................... I-13
New subfamily .................................................. A-51
nitrate ............................................................ B-17, B-39
nitric oxide ...................................................... E-31, F-81
Nitrogen fixation ............................................. B-40
nitrogen source ................................................ I-11
Nitrogen-related enzyme IIA ............................... I-29
NleB2 ............................................................ G-12
NLRLP10 ......................................................... F-55
NLRL3 inflammassome ........................................ F-42
Nm21 ............................................................. C-15
NNV capsid protein ........................................... H-17
Nokkome Oream ................................................. A-3
Nonfusogenic .................................................... H-5
Non-ionic Detergents ........................................ F-38
nonstructural protein 1 ....................................... H-42
Noroovirus source tracking ................................. B-14
Noroovirus ...................................................... B-29
novel bacteria ................................................... A-36
novel human norovirus GII.17 .............................. B-9
novel species ................................................... A-40, A-70
Novel strain KACC 93057P ................................ A-60
NRPS ........................................................... C-19
N-SPERSE(10-HSA) ........................................... D-9
nuclear factor kappa B ......................................... G-10
Nucleotide composition ....................................... F-35
Nuruk molds .................................................... I-12
nuruk ............................................................. D-8, D-10, D-20, I-31

O

Oconobacillus sojae ............................................. J-80
Odor control ..................................................... B-22
O-GlcNAc transferase (OGT) ................................ H-12
Oil-degrading bacteria ......................................... B-61
Olate hydratase .................................................. D-9
Oligotrophic ..................................................... A-58
OmpF ............................................................. J-59
Optimization ..................................................... I-6
Opuntia ficus-indica ........................................... J-12, J-41, J-42
oral bacteria ..................................................... J-53

www.fkms.kr | 395
<table>
<thead>
<tr>
<th>Term</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-mining Area</td>
<td>B-48</td>
</tr>
<tr>
<td>post-transcription</td>
<td>E-19</td>
</tr>
<tr>
<td>Potato</td>
<td>J-31</td>
</tr>
<tr>
<td>powdered infant formula</td>
<td>YS2-4</td>
</tr>
<tr>
<td>ppGpp</td>
<td>YS2-2</td>
</tr>
<tr>
<td>probiotics</td>
<td>H-50</td>
</tr>
<tr>
<td>predation</td>
<td>F-10</td>
</tr>
<tr>
<td>predatory bacteria</td>
<td>F-10</td>
</tr>
<tr>
<td>ProS1 mutation</td>
<td>F-61</td>
</tr>
<tr>
<td>Preservation</td>
<td>C-21, J-33</td>
</tr>
<tr>
<td>prevalence</td>
<td>J-85</td>
</tr>
<tr>
<td>Prickly pear cactus</td>
<td>J-9</td>
</tr>
<tr>
<td>Primordia</td>
<td>E-11</td>
</tr>
<tr>
<td>Prion</td>
<td>C-4</td>
</tr>
<tr>
<td>Prion disease</td>
<td>F-25, F-39</td>
</tr>
<tr>
<td>Prion protein</td>
<td>F-2, F-24</td>
</tr>
<tr>
<td>prp</td>
<td>F-25</td>
</tr>
<tr>
<td>Probiotic bacteria</td>
<td>J-77, J-79, J-80</td>
</tr>
<tr>
<td>Probiotic supplement</td>
<td>G-4</td>
</tr>
<tr>
<td>Probiotics</td>
<td>A-11, J-28, J-72, YS1-2</td>
</tr>
<tr>
<td>Processed Egg Products</td>
<td>I-20</td>
</tr>
<tr>
<td>Procoagulant</td>
<td>J-61</td>
</tr>
<tr>
<td>Pro-inflammatory cytokines</td>
<td>G-1</td>
</tr>
<tr>
<td>Pro-inflammatory cytokines</td>
<td>G-8</td>
</tr>
<tr>
<td>propionate catabolism operon</td>
<td>I-29</td>
</tr>
<tr>
<td>propionate</td>
<td>I-29</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>J-37</td>
</tr>
<tr>
<td>Prosthecococcobacterium sp</td>
<td>A-65</td>
</tr>
<tr>
<td>protease activity</td>
<td>D-29</td>
</tr>
<tr>
<td>Protease IV</td>
<td>F-51</td>
</tr>
<tr>
<td>Protection</td>
<td>F-29</td>
</tr>
<tr>
<td>Protein aggregate method</td>
<td>F-2</td>
</tr>
<tr>
<td>protein complementation assay</td>
<td>H-25</td>
</tr>
<tr>
<td>protein interaction</td>
<td>C-23</td>
</tr>
<tr>
<td>Protein–protein interaction</td>
<td>C-27, C-29, YS2-1</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>D-20</td>
</tr>
<tr>
<td>Proteolytic enzymes</td>
<td>I-23</td>
</tr>
<tr>
<td>Proteome</td>
<td>F-9, YS3-4</td>
</tr>
<tr>
<td>Proteomic analysis</td>
<td>I-8</td>
</tr>
<tr>
<td>Protomics</td>
<td>D-8, G-3</td>
</tr>
<tr>
<td>PRRSV</td>
<td>J-70</td>
</tr>
<tr>
<td>Psathyrella ammophylla</td>
<td>A-1</td>
</tr>
<tr>
<td>Pseudokatenomorina</td>
<td>B-20</td>
</tr>
<tr>
<td>Pseudoduganella</td>
<td>D-15</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>B-51</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>D-19, F-8, F-51, F-57, J-35</td>
</tr>
<tr>
<td>Pseudomonas cititidis</td>
<td>B-1</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>H-28</td>
</tr>
<tr>
<td>Pseudomonas sp</td>
<td>B-45, H-15, H-29, H-30</td>
</tr>
<tr>
<td>Pseudomonas sp, strains</td>
<td>H-18, H-19</td>
</tr>
<tr>
<td>Pseudovirus</td>
<td>F-13</td>
</tr>
<tr>
<td>Psicose</td>
<td>H-26</td>
</tr>
<tr>
<td>PSP-1</td>
<td>F-59</td>
</tr>
<tr>
<td>Psychrophila</td>
<td>E-7</td>
</tr>
<tr>
<td>psychrotroph</td>
<td>B-20</td>
</tr>
<tr>
<td>pUL26</td>
<td>F-41</td>
</tr>
<tr>
<td>Purification</td>
<td>C-4, H-51, I-22</td>
</tr>
<tr>
<td>Putative drug target</td>
<td>F-84</td>
</tr>
<tr>
<td>putrescine</td>
<td>H-4, YS4-2</td>
</tr>
<tr>
<td>Pyracnicarpous</td>
<td>A-47</td>
</tr>
<tr>
<td>pyrophosphohydrolase</td>
<td>C-29</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>B-8, B-23, B-68, I-24</td>
</tr>
<tr>
<td>qPCR</td>
<td>E-15</td>
</tr>
<tr>
<td>QORR</td>
<td>J-1</td>
</tr>
<tr>
<td>Qrr sRNAs</td>
<td>F-27</td>
</tr>
<tr>
<td>Quantitative proteomics</td>
<td>F-62</td>
</tr>
<tr>
<td>quasispecies</td>
<td>E-12</td>
</tr>
<tr>
<td>Quercus spp.</td>
<td>C-9</td>
</tr>
<tr>
<td>Quorur sensing</td>
<td>F-10, F-27, F-31, F-32, F-51, F-54, G-3, J-28, YS1-2</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>F-3, YS3-2</td>
</tr>
<tr>
<td>Race</td>
<td>A-61</td>
</tr>
<tr>
<td>radiation</td>
<td>F-73</td>
</tr>
<tr>
<td>Ralstonia solanacearum</td>
<td>B-42, J-31</td>
</tr>
<tr>
<td>RAPD analysis</td>
<td>A-61</td>
</tr>
<tr>
<td>Rational design</td>
<td>H-31</td>
</tr>
<tr>
<td>RC-3 strain</td>
<td>F-3, YS3-2</td>
</tr>
<tr>
<td>reactivation</td>
<td>F-67</td>
</tr>
<tr>
<td>Reactive Blue 4</td>
<td>H-33</td>
</tr>
<tr>
<td>reactive nitrogen species</td>
<td>F-82</td>
</tr>
<tr>
<td>Reactive oxygen species</td>
<td>F-30, F-69</td>
</tr>
<tr>
<td>reactive oxygen species</td>
<td>F-82</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>B-60, J-63</td>
</tr>
<tr>
<td>Real time RT-PCR</td>
<td>J-43</td>
</tr>
<tr>
<td>recA</td>
<td>H-41</td>
</tr>
<tr>
<td>receptor affinity</td>
<td>C-22</td>
</tr>
<tr>
<td>reclassification</td>
<td>A-57</td>
</tr>
<tr>
<td>Recombinant baculovirus</td>
<td>J-68, S18-4</td>
</tr>
<tr>
<td>Recombinant DNA experiments</td>
<td>J-65</td>
</tr>
<tr>
<td>Recombinant Protein</td>
<td>C-4, H-35</td>
</tr>
<tr>
<td>Recombinant proteins</td>
<td>H-11</td>
</tr>
<tr>
<td>Recombinant vaccine</td>
<td>H-5</td>
</tr>
<tr>
<td>Recovery efficiency</td>
<td>B-29</td>
</tr>
<tr>
<td>Recovery of Ecosystem</td>
<td>J-65</td>
</tr>
<tr>
<td>RED lambda recombination</td>
<td>F-45</td>
</tr>
<tr>
<td>Red tides bloom (HAB)</td>
<td>B-52</td>
</tr>
<tr>
<td>regulatory gene</td>
<td>C-18</td>
</tr>
<tr>
<td>removal efficiency bacteria</td>
<td>B-25</td>
</tr>
<tr>
<td>replication</td>
<td>F-66</td>
</tr>
<tr>
<td>Republic of Korea</td>
<td>F-36, F-37</td>
</tr>
<tr>
<td>Research Resource Center</td>
<td>J-54</td>
</tr>
<tr>
<td>residential facility</td>
<td>F-53</td>
</tr>
</tbody>
</table>
Keyword Index

Resourc e center ........................................... J-51, J-53
response surface methodology ........................................ J-41
reverse genetic system .............................................. F-3, YS3-2
Revisonal study .................................................. A-4
Rhizobacterial communities ........................................ B-8
rhizosphere ......................................................... E-4, E-6, E-10
rhizosphere soil .................................................. A-16, A-67
RhoA ................................................................. E-24
Rhodanobacter pantaprisneas ...................................... A-12
Rhodobacter capsulatus ........................................... D-23
Rhodospirillaceae ................................................ A-44
Rhodotorula glutinis ................................................ J-82
Rhus verniciflua stocks ........................................... D-28
Ribosomal Database Project ...................................... B-3
Rice ................................................................. I-15, J-44
Rift valley fever virus ............................................ J-43
RIP1 ................................................................. G-2
RNA virus ........................................................ A-24
RNA–sequencing analysis ......................................... H-18
root rot .............................................................. A-57
ROS ................................................................. F-26, G-19
rotavirus .......................................................... F-77
RpfE ............................................................... G-20
RP–HPLC ........................................................ I-23
RT ................................................................. F-45
RT–PCR ........................................................ A-45, J-64
ruminal archaea ................................................... B-3
Runella ............................................................. A-67
Russula section Foetentinae ....................................... A-48

S aureus .......................................................... B-34, D-15, F-52
S cerevisiae ......................................................... C-22
Saccharomyces cerevisiae .......................................... B-18
Saccharomyces .................................................. A-53
Saccharomyces cerevisiae .......................................... D-27
Saccharomyces cerevisiae .......................................... D-11
Safety .............................................................. I-12
Safflower seeds .................................................. D-16
Sallinic Acid ....................................................... J-59
Salix polaris ....................................................... A-46
Salmonella ......................................................... F-46, F-82, I-28, J-1
Salmonella enterica ............................................... I-16
Salmonella Enteritidis ............................................ F-29
Salmonella I 4,[5],12:i ............................................ F-46
Salmonella Typhimurium ......................................... F-21, F-81, F-84, J-59
salt ................................................................. B-10
Salt accumulation ................................................ B-8
Sampling methods ................................................ B-29
sanghuang mushroom ........................................... A-30
Sanghuangrus sanghuang ......................................... A-30
Saprotoph ........................................................ A-1
Sarcodon asparatus ................................................. C-12
Sarcoscyphaeae .................................................. A-2
Sargassum micranthum ........................................... G-6
Sargassum muticum ............................................. G-1
Sawdust Cultivation .............................................. I-13
SBR system ...................................................... B-25
ScFv–AP fusion protein .......................................... H-6
ScFv ............................................................. H-14
Sclerotinia Rot ..................................................... J-56
Sclerotina ......................................................... B-47
scraper .............................................................. F-25
Screening method ................................................ D-5, D-12, D-29
screening ........................................................ D-5, D-12, D-29
seasonal difference .............................................. J-22
Seawater ........................................................ A-6, A-29, A-42, A-52
secondary metabolite ............................................ D-25
Secondary metabolites .......................................... D-22, D-26
sediment ........................................................ A-17, A-36, A-38
sedimentation basin ............................................. B-55
Sediminibacterium ................................................. A-36
Seedborne ........................................................ B-16
Separation ........................................................ D-18
Sepsis .............................................................. F-62
serine O-acetyl transferase ....................................... C-26
Serological study ................................................ F-19
Serotype .......................................................... F-46
Ser-Thr protein kinase ........................................... C-5
Sesquiterpenes ................................................... D-21
Seti ................................................................. J-2
Sexual development ............................................... C-3
SFTSV ............................................................ F-36
SGNH hydrolase ................................................... C-15
Shiitake .......................................................... I-11, J-8
Shrew .............................................................. A-24
Silene acaulis ...................................................... A-46
Silkworm .......................................................... H-35
simulated gastric fluid .......................................... I-30
singlechain variable fragment ................................ H-6
SIR–2,1 ........................................................... C-28
Site–directed mutagenesis ....................................... H-31, H-47
Site–specific survey ............................................... B-27
size–exclusion chromatography ................................ H-14
Skp2 ............................................................... YS3-6
slaughterhouse waste water ...................................... J-36
sludge ............................................................. J-36
small RNA ........................................................ E-19, H-4, H-22, YS4-2
Sneathia .......................................................... F-28
SNP ................................................................. A-8
Soil Fungi ........................................................ J-39
Soil microorganism ................................................ B-12
Soil stabilization ................................................ B-13
solid salt .......................................................... A-52
solar saltpond ..................................................... A-17
solute dietary fiber ............................................... J-41
Keyword Index

<table>
<thead>
<tr>
<th>Keyword</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent-tolerant bacterium</td>
<td>H-19</td>
</tr>
<tr>
<td>South Korea</td>
<td>A-19, A-35, A-47</td>
</tr>
<tr>
<td>Soybean</td>
<td>I-9, I-27</td>
</tr>
<tr>
<td>Sparassis latifo</td>
<td>D-6</td>
</tr>
<tr>
<td>Sphingobium polysaccharaeus</td>
<td>A-14</td>
</tr>
<tr>
<td>Spookoptera exigua</td>
<td>J-32</td>
</tr>
<tr>
<td>sponge</td>
<td>F-77</td>
</tr>
<tr>
<td>Spy</td>
<td>F-82</td>
</tr>
<tr>
<td>sRNA</td>
<td>D-1, H-4, YS4-2</td>
</tr>
<tr>
<td>SRP pathway</td>
<td>H-36</td>
</tr>
<tr>
<td>stainless steel</td>
<td>B-55</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>A-55, F-6, I-34, YS1-5</td>
</tr>
<tr>
<td>Staphylococcus equorum</td>
<td>J-78</td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>E-30</td>
</tr>
<tr>
<td>starter</td>
<td>D-29</td>
</tr>
<tr>
<td>starter culture</td>
<td>J-77</td>
</tr>
<tr>
<td>Steatorspore gene cluster</td>
<td>E-23</td>
</tr>
<tr>
<td>Stereospecificity</td>
<td>H-31</td>
</tr>
<tr>
<td>Sterigmatocystin</td>
<td>E-16</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>F-73, F-74</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>C-8</td>
</tr>
<tr>
<td>Streptomyces</td>
<td>C-18, D-5, D-26, E-9</td>
</tr>
<tr>
<td>Streptomyces extract</td>
<td>F-31</td>
</tr>
<tr>
<td>Streptomyces rubrolavendulae</td>
<td>E-23</td>
</tr>
<tr>
<td>Streptomyces scopolinitis</td>
<td>D-14</td>
</tr>
<tr>
<td>Streptomyces vellosus</td>
<td>B-37</td>
</tr>
<tr>
<td>Streptomycetae</td>
<td>E-9</td>
</tr>
<tr>
<td>Stress condition</td>
<td>D-27</td>
</tr>
<tr>
<td>Stress tolerance</td>
<td>H-15, H-29, H-30</td>
</tr>
<tr>
<td>stringent response</td>
<td>YS2-2</td>
</tr>
<tr>
<td>Struvite</td>
<td>B-17, B-35, B-39</td>
</tr>
<tr>
<td>submerged culture</td>
<td>D-14</td>
</tr>
<tr>
<td>sub-MIC</td>
<td>B-18</td>
</tr>
<tr>
<td>Substrate</td>
<td>H-40</td>
</tr>
<tr>
<td>Substrate specificity</td>
<td>H-31</td>
</tr>
<tr>
<td>Succinate minimal medium</td>
<td>C-10</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>H-3</td>
</tr>
<tr>
<td>Succinyl-CoA</td>
<td>H-1</td>
</tr>
<tr>
<td>Sugar transport</td>
<td>H-26</td>
</tr>
<tr>
<td>Sugar transport system</td>
<td>C-27</td>
</tr>
<tr>
<td>Sulfate-reducing bacteria</td>
<td>B-67</td>
</tr>
<tr>
<td>sulfur pathway</td>
<td>C-26</td>
</tr>
<tr>
<td>surface coating</td>
<td>H-37</td>
</tr>
<tr>
<td>Surface display</td>
<td>J-11</td>
</tr>
<tr>
<td>Surveillance</td>
<td>F-44</td>
</tr>
<tr>
<td>survival</td>
<td>I-30</td>
</tr>
<tr>
<td>swine wastes</td>
<td>B-46</td>
</tr>
<tr>
<td>Symbiont</td>
<td>B-48</td>
</tr>
<tr>
<td>Symbiotic algae</td>
<td>E-20</td>
</tr>
<tr>
<td>Synthetic CdC–T7</td>
<td>E-32</td>
</tr>
<tr>
<td>Systems biology</td>
<td>F-9, YS3-4</td>
</tr>
<tr>
<td>Systems metabolic engineering</td>
<td>H-1</td>
</tr>
<tr>
<td>Taxonomic characterization</td>
<td>I-12</td>
</tr>
<tr>
<td>Taylorea equigenitisa</td>
<td>J-6</td>
</tr>
<tr>
<td>TB clinical strain</td>
<td>E-1</td>
</tr>
<tr>
<td>TBK1</td>
<td>F-78</td>
</tr>
<tr>
<td>Tegument protein pp28</td>
<td>F-4</td>
</tr>
<tr>
<td>Teloschistaceae</td>
<td>A-51</td>
</tr>
<tr>
<td>terpene metabolism</td>
<td>D-7</td>
</tr>
<tr>
<td>Terra Nova Bay</td>
<td>S-24</td>
</tr>
<tr>
<td>tetramerization</td>
<td>H-14</td>
</tr>
<tr>
<td>TH-2 cells</td>
<td>G-23</td>
</tr>
<tr>
<td>Thermococcus onnurineus NA1</td>
<td>C-20</td>
</tr>
<tr>
<td>Thrips</td>
<td>B-64</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>J-61</td>
</tr>
<tr>
<td>Th-T binding assay</td>
<td>F-2</td>
</tr>
<tr>
<td>Tick-borne viruses</td>
<td>F-37</td>
</tr>
<tr>
<td>Tight junction</td>
<td>G-16</td>
</tr>
<tr>
<td>TLR</td>
<td>G-23</td>
</tr>
<tr>
<td>TLR2</td>
<td>G-19</td>
</tr>
<tr>
<td>Tobacco whitefly</td>
<td>J-22</td>
</tr>
<tr>
<td>TollST two-component system</td>
<td>H-28</td>
</tr>
<tr>
<td>Toll-like receptor 2</td>
<td>F-69</td>
</tr>
<tr>
<td>Toluene adaptation</td>
<td>H-19</td>
</tr>
<tr>
<td>Toluene tolerance</td>
<td>H-30</td>
</tr>
<tr>
<td>Tomato</td>
<td>J-58</td>
</tr>
<tr>
<td>tomato root</td>
<td>B-65</td>
</tr>
<tr>
<td>tonsillar B cells</td>
<td>F-68</td>
</tr>
<tr>
<td>Total active biomass</td>
<td>B-6</td>
</tr>
<tr>
<td>Total polyphenol</td>
<td>C-24</td>
</tr>
<tr>
<td>Toxascaris leonina</td>
<td>G-17</td>
</tr>
<tr>
<td>toxin gene</td>
<td>I-19</td>
</tr>
<tr>
<td>toxin production</td>
<td>F-7</td>
</tr>
<tr>
<td>toxin profiling</td>
<td>J-85</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>E-17, E-22</td>
</tr>
<tr>
<td>Transcriptional profiles</td>
<td>F-80</td>
</tr>
<tr>
<td>Transcriptome</td>
<td>B-42, F-9, YS3-4</td>
</tr>
<tr>
<td>transgenic mouse</td>
<td>J-32</td>
</tr>
<tr>
<td>transational repression</td>
<td>E-19</td>
</tr>
<tr>
<td>Transwell plate</td>
<td>B-61</td>
</tr>
<tr>
<td>Trehalose</td>
<td>H-15, H-29</td>
</tr>
</tbody>
</table>
### Keyword Index

<table>
<thead>
<tr>
<th>Keyword</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triazole fungicide</td>
<td>B-50</td>
</tr>
<tr>
<td>Trichoderma</td>
<td>J-8, J-56</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>I-17</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>J-38</td>
</tr>
<tr>
<td>Trichotecanes</td>
<td>J-25</td>
</tr>
<tr>
<td>trimetric</td>
<td>H-13</td>
</tr>
<tr>
<td>Trimethylamine N-oxide</td>
<td>F-34, YS1-3</td>
</tr>
<tr>
<td>Tropical Regions</td>
<td>B-64</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>H-48</td>
</tr>
<tr>
<td>Tulostoma fremontium</td>
<td>A-1</td>
</tr>
<tr>
<td>two-component system</td>
<td>C-5</td>
</tr>
<tr>
<td>Type II genotype</td>
<td>F-49</td>
</tr>
<tr>
<td>Tyrosinase inhibitor</td>
<td>J-81, J-82</td>
</tr>
<tr>
<td>tyrosine</td>
<td>J-22</td>
</tr>
<tr>
<td>water curtain greenhouse</td>
<td>A-69</td>
</tr>
<tr>
<td>Water extract</td>
<td>E-15</td>
</tr>
<tr>
<td>Water Treatment Plants</td>
<td>B-24</td>
</tr>
<tr>
<td>wetland</td>
<td>A-67, A-68</td>
</tr>
<tr>
<td>wetland freshwater</td>
<td>A-43</td>
</tr>
<tr>
<td>white fruitbody</td>
<td>E-18</td>
</tr>
<tr>
<td>Whole-genome sequencing</td>
<td>E-20, E-25</td>
</tr>
<tr>
<td>Wild mushroom</td>
<td>J-84</td>
</tr>
<tr>
<td>Wild yeasts</td>
<td>A-27</td>
</tr>
<tr>
<td>Wilt disease</td>
<td>A-5</td>
</tr>
<tr>
<td>WNv</td>
<td>J-40</td>
</tr>
<tr>
<td>Wogonin</td>
<td>G-15</td>
</tr>
<tr>
<td>Wood</td>
<td>C-9</td>
</tr>
<tr>
<td>wood rot fungi</td>
<td>D-7</td>
</tr>
<tr>
<td>VacA</td>
<td>J-27</td>
</tr>
<tr>
<td>vaccine</td>
<td>F-73, H-17</td>
</tr>
<tr>
<td>Vaccine escape mutation</td>
<td>F-60</td>
</tr>
<tr>
<td>vaccine-specific sites</td>
<td>E-12</td>
</tr>
<tr>
<td>Vanilla flavor</td>
<td>H-49</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>E-12, E-29</td>
</tr>
<tr>
<td>Varic永不well.sp. nov.</td>
<td>J-74</td>
</tr>
<tr>
<td>VdpA</td>
<td>C-3</td>
</tr>
<tr>
<td>VeA</td>
<td>C-3</td>
</tr>
<tr>
<td>Vesicle</td>
<td>B-34</td>
</tr>
<tr>
<td>VHH nanobody</td>
<td>H-13</td>
</tr>
<tr>
<td>viable but nonculturable</td>
<td>B-42</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>F-34</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>YS1-3</td>
</tr>
<tr>
<td>Vibrio vulgaris</td>
<td>B-2, B-4, F-9, F-27, F-50, F-70, F-71, I-4, I-37, YS3-4, C-27</td>
</tr>
<tr>
<td>Vietnam</td>
<td>A-34</td>
</tr>
<tr>
<td>Vinegar</td>
<td>D-28, I-36</td>
</tr>
<tr>
<td>Vialcoain</td>
<td>B-34, D-4, D-15, YS4-4</td>
</tr>
<tr>
<td>Viperin</td>
<td>F-4</td>
</tr>
<tr>
<td>viral disease</td>
<td>J-50</td>
</tr>
<tr>
<td>viral replication</td>
<td>F-65</td>
</tr>
<tr>
<td>viral vector</td>
<td>G-18</td>
</tr>
<tr>
<td>virulence</td>
<td>F-29, F-51, F-56, F-57</td>
</tr>
<tr>
<td>Virus</td>
<td>J-13</td>
</tr>
<tr>
<td>virus neutralisation test</td>
<td>J-15</td>
</tr>
<tr>
<td>Virus-like particle</td>
<td>J-68, S18-4</td>
</tr>
<tr>
<td>Virus-like particles</td>
<td>J-5</td>
</tr>
<tr>
<td>viscosity</td>
<td>J-42</td>
</tr>
<tr>
<td>Viscozyma</td>
<td>J-42</td>
</tr>
<tr>
<td>VLP</td>
<td>F-14</td>
</tr>
<tr>
<td>xanthine oxidase</td>
<td>F-20</td>
</tr>
<tr>
<td>Xanthomonas</td>
<td>A-10</td>
</tr>
<tr>
<td>XDR</td>
<td>F-86</td>
</tr>
<tr>
<td>Xenobiotic compounds</td>
<td>B-45</td>
</tr>
<tr>
<td>Xylanase</td>
<td>J-71</td>
</tr>
<tr>
<td>Xyloglone aphaerospora</td>
<td>I-21, J-10</td>
</tr>
<tr>
<td>xylophagous insect</td>
<td>B-23</td>
</tr>
<tr>
<td>Yakju</td>
<td>D-11</td>
</tr>
<tr>
<td>Yarrowia lipolytica</td>
<td>H-12, H-17</td>
</tr>
<tr>
<td>yeast</td>
<td>A-53</td>
</tr>
<tr>
<td>Zoogloea family</td>
<td>A-38</td>
</tr>
</tbody>
</table>