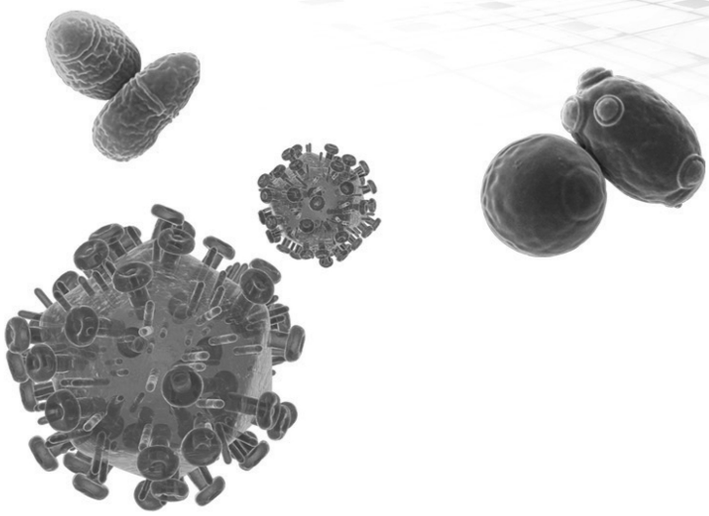
Proceedings

2014 International Meeting of the Microbiological Society of Korea

- April 30 ~ May 2, 2014
- EXCO, Daegu, Korea

–Next Generation Microbiology for the Future–



Hosted by

The Microbiological Society of Korea

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Contents

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This work was supported by the Korean Federation of Science and Technology Societies Grant funded by the Korean Government.

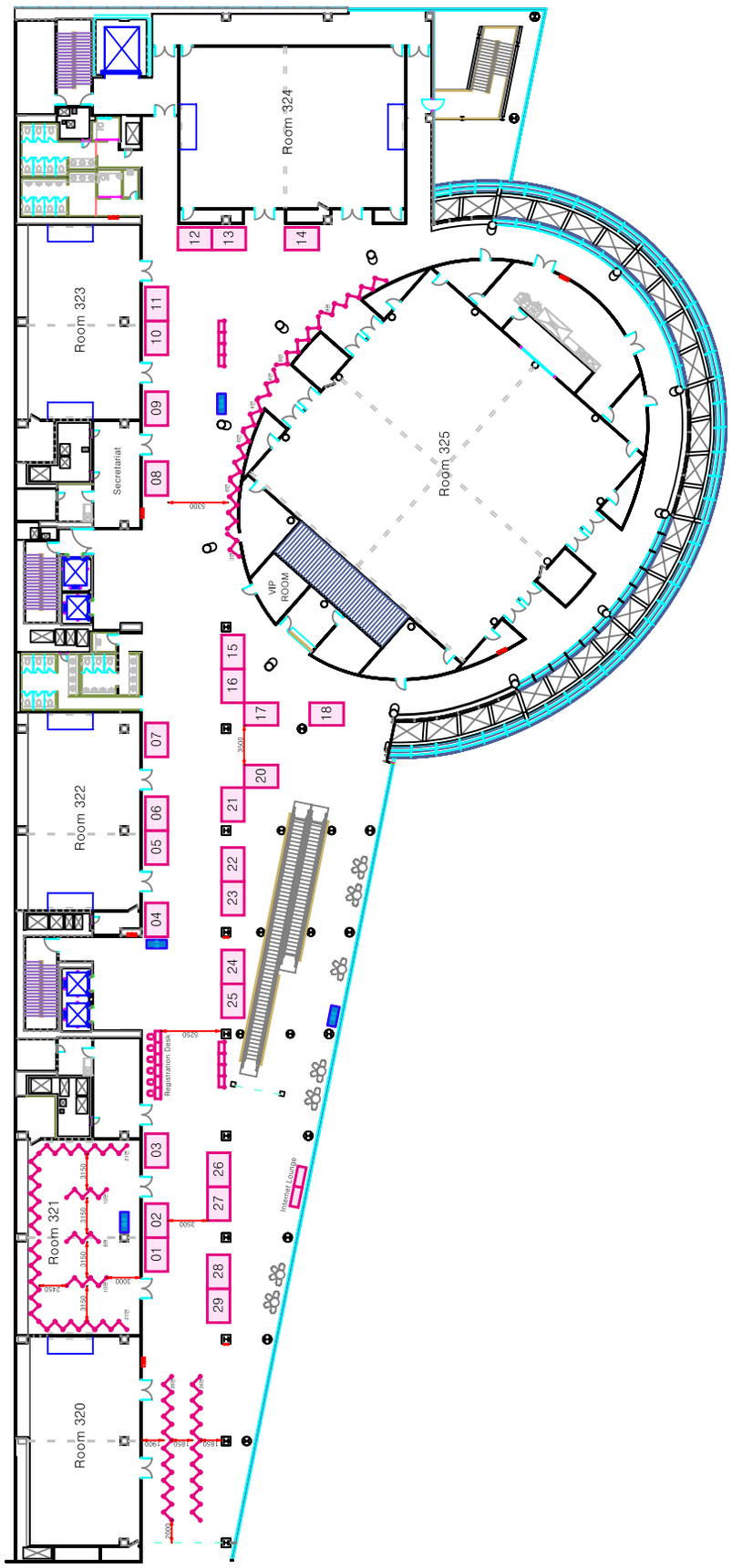
Timetable

April 30 (Wed.)	Room 324	Room 323	Room 322
12:30~	Registration		
13:30-13:45	Opening Ceremony (Room 324)		
13:45-14:30	Plenary Lecture 1 (Room 324_James W. Kronstad)		
14:30-16:30	TW1 Technology Workshop 1	S1 Life in Cold Habitats	YS1 Young Scientists' Session 1
17:00-18:30	관광 및 체험		

May 1 (Thu.)	Room 324	Room 323	Room 322
09:00-09:45	Plenary Lecture 2 (Room 324_Timothy J. Donohue)		
09:45-11:45	S2 Biochemistry and Molecular Biology of Prokaryotes	S3 Novel Insights for Microbial Ecology	S4 Signal Transduction and Gene Expression in Fungi
11:45-12:15		Poster Presentation 1	
12:15-12:30			
12:30-13:30	Workshop 1(ChunLab)	Lunch	
13:30-15:30	S5 Microbial Systems Biology and Genome Engineering	S6 Korea Traditional Fermented Food	S7 Host-Fungal Pathogen Interactions
15:30-16:15	Plenary Lecture 3 (Room 324_Pei-Yuan Qian)		
16:15-18:15	S8 New Approaches in Understanding of Microbial Pathogenesis	S9 Novel Concepts in Gene Expression System	YS2 Young Scientists' Session 2
18:30-21:00	Welcome Reception (Hotel Inter-Burgo EXCO, Grand Ballroom A)		

May 2 (Fri.)	Room 324	Room 323	Room 322
09:00-11:00	S10 Structural Microbiology and Pathogenesis	GS Graduate Students' Presentation Session	S11 Molecular Epidemiology and Control of Enteric Virus
11:00-13:00	S12 Microbial and Toxicological Burdens in Biomaterials : How to Measure and Remove?	S13 Animal Gut Microbiome	S14 Molecular & Cellular Biology of Yeast
13:00-14:00	General Meeting of MSK	Lunch	
14:00-14:45	Plenary Lecture 4 (Room 324_Nils Gunnar Hansson von Heijne)		HS High School Students' Presentation Session (14:00-16:00)
14:45-15:30	Poster Presentation 2		
15:30-16:00	S15 Lactic Acid Bacteria and Human Health	TW2 Technology Workshop 2	
16:00-17:00			Special Lecture for High School Students (16:00-17:00)
17:00-17:30			
17:30-18:00	Closing Ceremony (Room 324)		

Floor Plans



Room 321	Poster Sessions
Room 322	Symposia, Young Scientists' Sessions, High School Students' Presentation Session, Special Lecture for High School Students
Room 323	Symposia, Graduate Students' Presentation Session, Technology Workshop 2
Room 324	Opening Ceremony, Plenary Lectures, Technology Workshop 1, Symposia, Workshop, MSK General Meeting, Closing Ceremony
Lobby	Registration Desk, Exhibition, Poster Sessions

Scientific Program

■■■ Plenary Lectures

PL1 Plenary Lecture 1

April 30 (Wed.), Room 324

Chair: Hyun Ah Kang, Chung-Ang University

13:45-14:30

Targeting the Acquisition of Iron and Other Nutrients in Fungal Pathogens of Humans

James W. Kronstad, University of British Columbia, Canada

PL2 Plenary Lecture 2

May 1 (Thu.), Room 324

Chair: Jung-Hye Roe, Seoul National University

09:00-09:45

Gaining Biological Insights from Mining Bacterial Genomes & Pathways

Timothy J. Donohue, University of Wisconsin-Madison, USA

PL3 Plenary Lecture 3

May 1 (Thu.), Room 324

Chair: Sang-Jin Kim, Korea Institute of Ocean Science and Technology

15:30-16:15

Genome Mining for Drug Lead Discovery from Marine Bacteria

Pei-Yuan Qian, The Hong Kong University of Science and Technology, Hong Kong

PL4 Plenary Lecture 4

May 2 (Fri.), Room 324

Chair: Hyun Kim, Seoul National University

14:00-14:45

Co-translational Insertion and Folding of Membrane Proteins *In Vivo*

Nils Gunnar Hansson von Heijne, Stockholm University, Sweden

■■■ Symposia

S1 Life in Cold Habitats

April 30 (Wed.), Room 323

Chair: Hong Kum Lee, Korea Polar Research Institute

S1-1 **14:30-15:00**

Exploring Microbial Diversity in Antarctic Terrestrial Ecosystems

Ok-Sun Kim, Korea Polar Research Institute

S1-2 **15:00-15:30**

Bacterial Community Structure of Tundra Soils

Yoo Kyung Lee, Korea Polar Research Institute

S1-3 **15:30-16:00**

DNA-based Fecal Analyses for Trophic Relations of Svalbard Reindeer *(Rangifer tarandus platyrhynchus)*

Sungbae Joo, Ajou University

S1-4 **16:00-16:30**

Divergence of Endosymbiotic Bacteria of Deep-sea Hydrothermal Vent Bivalve Mussels

Yong-Jin Won, Ewha Womans University

S2 Biochemistry and Molecular Biology of Prokaryotes

May 1 (Thu.), Room 324

Chair: Soon-Jung Park, Yonsei University

S2-1 **09:45-10:15**

A *Salmonella* Virulence Protein Promotes Pathogenicity by Altering Intracellular ATP Levels

Eun-Jin Lee, Kyung Hee University

S2-2 **10:15-10:45**

Strategy of Survival of *E. coli* and Translational System

Nobuo Shimamoto, Kyoto Sangyo University, Japan

S2-3 **10:45-11:15**

O_2 Dependent Transcriptional Networks of *Escherichia coli*

Patricia J. Kiley, University of Wisconsin-Madison, USA

S2-4 **11:15-11:45**

Indole Inhibits Predation by *Bdellovibrio bacteriovorus* and Bdelloplast Lysis: A Transcriptomic Analysis

Robert J. Mitchell, Ulsan National Institute of Science and Technology

S2-5 **11:45-12:15**

Bacterial Cancer Therapy: Antitumor Effect of *Salmonellae* Expressing L-Asparaginase

Hyon E Choy, Chonnam National University Medical School

S3 Novel Insights for Microbial Ecology

May 1 (Thu.), Room 323

Chair: Kyoung-Ho Kim, Pukyong National University

S3-1 **09:45-10:15**

Bioinformatics Effort to Better Understand Microbial Community

Kyung Mo Kim, Korea Research Institute of Bioscience and Biotechnology

S3-2 **10:15-10:45**

Cyanobacterial Bloom: from Micro to Macro Aspect

Chi-Yong Ahn, Korea Research Institute of Bioscience and Biotechnology

S3-3 **10:45-11:15**

Identification and Single-Cell Isolation of Active N_2O Reducers in Environments

Satoshi Ishii, Hokkaido University, Japan

S3-4 **11:15-11:45**

Nocardiopsis: from Its Biodiversity, Biogeography to Genetic Mechanisms of Environmental Adaptability

Wen-Jun Li, Yunnan University, China

S4 Signal Transduction and Gene Expression in Fungi

May 1 (Thu.), Room 322

Chair: Hee-Moon Park, Chungnam National University

S4-1 **09:45-10:15**

Characterization of the RGS Protein GprK and RgsC in *Aspergillus fumigatus*

Kwang-Soo Shin, Daejeon University

S4-2 **10:15-10:45**

Septation and Conidiation in *Aspergillus nidulans*

Ling Lu, Nanjing Normal University, China

Chair: Hyang Burm Lee, Chonnam National University

S4-3 **10:45-11:15**

Regulation of Pathogenesis by Light in *Cercospora zeae-maydis*

Hun Kim, Seoul National University

S4-4 **11:15-11:45**

Gene Expression in Yeast During Drug Synergy with Iron Chelating Agents

Dee Carter, University of Sydney, Australia

S5 Microbial Systems Biology and Genome Engineering

May 1 (Thu.), Room 324

Chair: Byung-Kwan Cho, Korea Advanced Institute of Science and Technology

S5-1 13:30-14:00

Microbial Relationships Uncovered–Community Systems Biology Approaches in Microbial Ecology

Karsten Zengler, University of California San Diego, USA

S5-2 14:00-14:30

Eubacterium limosum KIST612 as a Model Strain for C1 Biorefinery

In Seop Chang, Gwangju Institute of Science and Technology

S5-3 14:30-15:00

Development of Oxygen-independent *E. coli* Strain for Overproduction of Commodity Chemicals

Vasiliy Portnoy, BP Biofuels, USA

S5-4 15:00-15:30

Multiomics-guided Bacterial Genome Analysis

Byung-Kwan Cho, Korea Advanced Institute of Science and Technology

S6 Korea Traditional Fermented Food

May 1 (Thu.), Room 323

Sponsored by Microbial Institute for Fermentation Industry

Chair: Young Soo Kim, Chonbuk National University

S6-1 13:30-14:00

Caenorhabditis elegans Conditioning with The Probiotic Bacterium *Lactobacillus acidophilus* Strain A4 Enhances Longevity and Resistance to Foodborne Pathogen Infections

Younghoon Kim, Chonbuk National University

S6-2 14:00-14:30

Indigenous Yeasts Isolated from Traditional Fermented Soy-Sauce Can Prevent Pathogenic Bacteria Occurred at Low-Salt Fermentation Process

Sang Ho Baik, Chonbuk National University

S6-3 14:30-15:00

Changes in Transcriptional Level of Subtilisin-like Proteases of *Bacillus licheniformis* during Fermentation of Fast-fermented Soybean Paste

Tai-Boong Uhm, Chonbuk National University

S6-4 15:00-15:30

Antimicrobial Activity of *Bacillus licheniformis* Isolated From Korean Traditional Food Sources against Porcine Enteropathogenic Bacteria

Ho-Seong Cho, Chonbuk National University

S7 Host-Fungal Pathogen Interactions

May 1 (Thu.), Room 322

Chair: Won Hee Jung, Chung-Ang University

S7-1 **13:30-14:00**

Development of System-wide Functional Analysis Platform for Pathogenicity Genes in The Rice Blast Fungus

Sook-Young Park, Seoul National University

S7-2 **14:00-14:30**

Population Structure of the Plant Pathogenic Fungus *Fusarium graminearum* in Korea

Jungkwan Lee, Dong-A University

S7-3 **14:30-15:00**

Copper Homeostasis as a Virulence Factor in Systemic Infection by The Human Fungal Pathogen *Cryptococcus neoformans*

Chen Ding, Northeastern University, China

S7-4 **15:00-15:30**

Candida Infection and Antifungal Drug Resistance

Mi-Kyung Lee, Chung-Ang University

S8 New Approaches in Understanding of Microbial Pathogenesis

May 1 (Thu.), Room 324

Chair: Dong Wook Kim, Hanyang University

S8-1 **16:15-16:45**

Investigating *Salmonella* Pathogenesis for The Development of Targeted Intervention Strategies

Bradley L. Bearson, National Laboratory for Agriculture and the Environment, USA

S8-2 **16:45-17:15**

Structural and Biophysical Insights into Flagellin-mediated Activation of TLR5 Signaling

Sung-il Yoon, Kangwon National University

S8-3 **17:15-17:45**

FoxP3+ Tregs, PD-1 and CTLA4: Negative Immune Regulatory Pathways in Patients with Chronic HIV and/or HCV Infection

Hyosun Cho, Duksung Women's University

S8-4 **17:45-18:15**

Tumor Cell Modulation by Mucosa-associated *Escherichia coli* as an Internal Exposome via Macrophage Inhibitory Cytokine 1

Yuseok Moon, Pusan National University

S9 Novel Concepts in Gene Expression System

May 1 (Thu.), Room 323

Chair: Eung-Soo Kim, Inha University

S9-1 16:15-16:45

Proof of Concept Trials for Functional Overexpression and High Throughput Assay of Proteins

Geun-Joong Kim, Chonnam National University

S9-2 16:45-17:15

Incorporation of Unnatural Amino Acids into Proteins in *Escherichia coli* and Their Applications

Hyungdon Yun, Konkuk University

S9-3 17:15-17:45

New Strategy for Enhancing Heterologous Protein Expression through the Alternating N-terminal Codons

Jong Hyun Choi, Korea Research Institute of Bioscience and Biotechnology

S9-4 17:45-18:15

Strategy to Overexpress a Large Biosynthetic Gene Cluster in *Streptomyces* Species

Eung-Soo Kim, Inha University

S10 Structural Microbiology and Pathogenesis

May 2 (Fri.), Room 324

Co-organized by Systems & Synthetic Agrobiotech Center

Chair: Myung Hee Kim, Korea Research Institute of Bioscience and Biotechnology

S10-1 09:00-09:30

Crystal Structures of Bifunctional Penicillin-Binding Protein 4 from *Listeria monocytogenes*

Yeon-Gil Kim, Pohang University of Science and Technology

S10-2 09:30-10:00

Structural Basis for The Recognition of Peptidoglycan Tripeptide by *Helicobacter pylori* Csd4, a D,L-carboxypeptidase Controlling The Helical Cell Shape

Byung Il Lee, Research Institute National Cancer Center

Chair: Sun-Shin Cha, Korea Institute of Ocean Science and Technology

S10-3 10:00-10:30

Structure and Function of Fungal Zn Finger Transcription Factor in Sterol Homeostasis and Antifungal Resistance

Young Jun Im, Chonnam National University

S10-4 10:30-11:00

Dxo1, a Novel Eukaryotic Enzyme with Both Decapping and 5′-3′ Exoribonuclease Activity

Jeong Ho Chang, Kyungpook National University

S11 Molecular Epidemiology and Control of Enteric Virus

May 2 (Fri.), Room 322

Chair: Jin Hyun Ahn, Sungkyunkwan University School of Medicine

S11-1 09:00-09:30

Management Strategy of Norovirus in South Korea

In-Sun Joo, National Institute of Food and Drug Safety Evaluation

S11-2 09:30-10:00

Public Health Impact of Human Noroviruses

Jan Vinjé, Centers for Disease Control and Prevention, USA

S11-3 10:00-10:30

Norovirus: The Main Target for Food Safety and Control

GwangPyo Ko, Seoul National University

S11-4 10:30-11:00

Antiviral Activity and Its Mechanism of Ginsenoisdes against Norovirus Surrogates

Changsun Choi, Chung-Ang University

S12 Microbial and Toxicological Burdens in Biomaterials : How to Measure and Remove?

May 2 (Fri.), Room 324

Chair: In Seop Kim, Hannam University

S12-1 11:00-11:30

Nonclinical Safety Research and Related Matters for Drug Development

Tarumoto Yasuo, Genia

S12-2 11:30-12:00

Virological Safety Aspects of Biopharmaceuticals Produced in Mammalian Cell Cultures

In Seop Kim, Hannam University

S12-3 12:00-12:30

What Testings Should be Done to Confirm The Safety of Biologics such as Gene Medicines?

Jae-Gyun Jeong, ViroMed Co., Ltd.

S12-4 12:30-13:00

Toxicology Tests of Microbial and Agrochemical Pesticides

Heon Ju Lee, Korea Human Resource Development Institute For Health & Welfare

S13 Animal Gut Microbiome

May 2 (Fri.), Room 323

Chair: Woojun Park, Korea University

S13-1 11:00-11:30

Gut Microbiota of *Tenebrio molitor* and Their Responses to Environmental Changes

Woojun Park, Korea University

S13-2 11:30-12:00

Change of Gut Bacterial Communities Based on Evolution of Animal Host Species

Jin-Woo Bae, Kyung Hee University

S13-3 12:00-12:30

Genetic Basis for Intestinal Colonization by Gut Microbes Revealed by a Metagenomic Screen

Sang Sun Yoon, Yonsei University

S13-4 12:30-13:00

The Biological Functions of Novel Symbiotic Factors in *Riptortus–Burkholderia* Symbiotic System

Bok Luel Lee, Pusan National University

S14 Molecular and Cellular Biology of Yeast

May 2 (Fri.), Room 322

Chair: Won-Ki Huh, Seoul National University

S14-1 11:00-11:30

Nst1 Functions as an Adapting Protein to Mediates a Crosstalk of Cell Wall Integrity and HOG MAPK Pathways in Response to Heat Stress in Budding Yeast *Saccharomyces cerevisiae*

Kiwon Song, Yonsei University

S14-2 11:30-12:00

Control of Gene Induction Kinetics by Set3 HDAC and Overlapping Non-coding RNA Transcription

TaeSoo Kim, Ewha Womans University

S14-3 12:00-12:30

The Sec62/Sec63 Translocon Mediates Topogenesis of Membrane Proteins

Hyun Kim, Seoul National University

S14-4 12:30-13:00

Multistep Functions of Dna2 Nuclease in DNA Double-strand Break Repair by Homologous Recombination

Woo-Hyun Chung, Duksung Women's University

S15 Lactic Acid Bacteria and Human Health

May 2 (Fri.), Room 324

Sponsored by Korea Yakult

Chair: Ju-Hoon Lee, Kyung Hee University

S15-1 15:30-16:00

Lactic Acid Bacteria: An Overview of Beneficial Effects

Dong-Hyun Kim, Kyung Hee University

S15-2 16:00-16:30

Analysis of Human Milk Oligosaccharides and Their Utilization by *Bifidobacterium*

Jaehan Kim, Chungnam National University

Chair: Dong-Hyun Kim, Kyung Hee University

S15-3 16:30-17:00

Comparative and Functional Genomic Analysis of Bifidobacteria Reveals Its Genomic Adaptation into Human Intestinal Habitat

Ju-Hoon Lee, Kyung Hee University

S15-4 17:00-17:30

Probiotics as an Immune Modulator for Hyper-immune Disorders

Sin-Hyeog Im, Academy of Immunology and Microbiology (AIM), Institute for Basic Science (IBS)/ Pohang University of Science and Technology

■■■ Young Scientists' Sessions

YS1 Young Scientists' Session 1

April 30 (Wed.), Room 322

Chair: Jung-Shin Lee, Kangwon National University

YS1-1 14:30-14:42

The Role of a Specific Hemagglutinin Residue as an Indicator of The Evolution Dynamics of Human Influenza A H1N1 Viruses

Jin Il Kim, Korea University

YS1-2 14:42-14:54

Inverse Regulation of Fe- and Ni-containing SOD Genes by a Fur Family Regulator Nur Through Small RNA Processed From 3'UTR of The *sodF* mRNA

Hae Mi Kim, Seoul National University

YS1-3 14:54-15:06

Characterization of Catalytic Functions of Bacterial CYP191A1

Sun-Ha Park, Korea Atomic Energy Research Institute

YS1-4 15:06-15:18

Identification of Colistin Resistance Mechanism Using Transcriptome Anlaysis in *Acinetobacter baumannii*

Young Kyoung Park, Sungkyunkwan University

YS1-5 15:18-15:30

HPr Antagonizes the Anti-σ^{70} Activity of Rsd in *Escherichia coli*

Young-Ha Park, Seoul National University

YS1-6 15:30-15:42

Fitness of Plasmid Bearing $bla_{CTX\text{-}M\text{-}15}$ Gene in *Klebsiella pneumonia*

Juyoun Shin, Sungkyunkwan University

YS1-7 15:42-15:54

Altered Gut Microbiota Composition Affects Mouse Susceptibility to *Vibrio cholerae* Infection

Mi Young Yoon, Yonsei Universtiy

YS1-8 15:54-16:06

Genomic Variations Between Colistin-susceptible and -resistant *Pseudomonas aeruginosa* Clinical Isolates and Their Effects on Colistin Resistance

Ji-Young Lee, Sungkyunkwan University

YS1-9 16:06-16:18

Tn7 Transposition: Importance of Protein-Protein Interactions Between TnsABCD

Ki Young Choi, Johns Hopkins University

YS2 Young Scientists' Session 2

May 1 (Thu.), Room 322

Chair: Won Hee Jung, Chung-Ang University

YS2-1 16:15-16:27

Characterization and *In Vitro* Inhibition Studies of *Bacillus anthracis* FtsZ: A Potential Antibacterial Target

Hae-Chul Park, Animal and Plant Quarantine Agency

YS2-2 16:27-16:39

Induction of Apoptosis by a *Vibrio vulnificus* Metalloprotease

Mi-Ae Lee, Sogang University

YS2-3 16:39-16:51

Translation of Leaderless Transcripts are Regulated by Non-coding RNAs in *Thermococcus onnurineus* NA1

Bo-Rahm Lee, Korea Advanced Institute of Science and Technology

YS2-4 16:51-17:03

Inhibition of HCV Replication with HCV NS5B Specific RNA Aptamer

Chang Ho Lee, Dankook University

YS2-5 17:03-17:15

Novel Na^+-Dependent Respiration in Hyperthermophilic Archaeon, *Thermococcus onnurineus* NA1

Jae Kyu Lim, Korea Institute of Ocean Science and Technology

YS2-6 17:15-17:27

Application of a New Cultivation Technology, I-tip, for Studying Microbial Diversity in Freshwater Sponges of Lake Baikal, Russia

Dawoon Jung, Kangwon National University

YS2-7 17:27-17:39

Comparative Genomic and Transcriptomic Analyses of *Acinetobacter* and *Alishewanella* Species Adapted to Different Habitats

Jaejoon Jung, Korea University

YS2-8 17:39-17:51

Genome-scale Probing of *In Vivo* Organization of Bacterial Transcription Initiation Complexes

Suhyung Cho, Korea Advanced Institute of Science and Technology

YS2-9 17:51-18:03

RNA-mediated Regulation of Photosynthesis in *Synechocystis* sp. PCC6803

Yoo-Bok Cho, Korea Advanced Institute of Science and Technology

■■■ Graduate Students' Presentation Session

GS

Graduate Students' Presentation Session

May 2 (Fri.), Room 323

Chair: Woojun Park, Korea University, Sang Sun Yoon, Yonsei University

GS-1 **09:00-09:10**

Multiple Resistance Mechanisms of High-level Fluoroquinolone Resistant *Aeromonas* sp. Strain C3 Isolated from Waste Water Treatment Plant

Cung Nawl Thawng, Chung-Ang University

GS-2 **09:10-09:20**

Metabolic Pathway Analysis for Efficient Succinic Acid Production

Won Jun Kim, Korea Advanced Institute of Science and Technology

GS-3 **09:20-09:30**

Quorum Sensing for Biofilm Formation and Oil Degradation in *Acinetobacter oleivorans* DR1

Jisun Kim, Korea University

GS-4 **09:30-09:40**

Community Structure Analysis and Characterization of Soil Humic Substances-Degrading Bacteria from Cold Environments

Ha Ju Park, Korea Polar Research Institute

GS-5 **09:40-09:50**

Development of Rapid One-Step Inactivation Tool and Engineering of *E. coli* to Produce Fumaric Acid

Chan Woo Song, Korea Advanced Institute of Science and Technology

GS-6 **10:00-10:10**

Genetic Bases of Enhanced *Pseudomonas aeruginosa* Biofilm Development by Sub-Minimum Inhibitory Concentration Treatment of Antibiotics

Keehoon Lee, Yonsei University

GS-7 **10:10-10:20**

Comparison of CO-dependent H_2 Production with Strong Promoters in *Thermococcus onnurineus* NA1

Seong Hyuk Lee, Korea Institute of Ocean Science and Technology

GS-8 **10:20-10:30**

Characterization of Plasmid pEMB1 Harboring a β-Lactamase Gene and a Toxin-antitoxin System

Hyo Jung Lee, Chung-Ang University

GS-9 **10:30-10:40**

Identification and Role of the DNA-Damage Response Two Component System, DrtR/S, in *Deinococcus radiodurans*

Seonghun Im, Korea Atomic Energy Research Institute

GS-10 **10:40-10:50**

Potassium Ion-Mediated Regulation of Biofilm Formation via Controlling Cellular Level of Sigma S

Yu-Sook Chung, Sogang University

GS-11 **10:40-10:50**

Metagenomic and Metatranscriptomic Analysis of Kimchi, a Traditional Korean Fermented Food

Ji Young Jung, Chung-Ang University

GS-12 **10:50-11:00**

Staphylococcus aureus Vesicles Modulate the Surface Hydrophobicity Which Inhibits Other ESKAPE Pathogens from Forming Biofilms

Hansol Im, Ulsan National Institute of Science and Technology

■■■ Technology Workshops

TW1

Technology Workshop 1

April 30 (Wed.), Room 324

Sponsored by Korea National Research Resource Center

Chair: Byung-Kwan Cho, Korea Advanced Institute of Science and Technology

TW1-1 14:30-14:50

Next-generation Sequencing 기술의 원리와 응용

Yong-Joon Cho, ChunLab Inc.

TW1-2 14:50-15:10

Next-generation Sequencing 기술을 이용한 세균의 분류동정

Mincheol Kim, Korea Polar Research Institute

TW1-3 15:10-15:30

Genome Resequencing을 이용한 세균 적응진화연구

Byung-Kwan Cho, Korea Advanced Institute of Science and Technology

TW1-4 15:30-15:50

Transcriptome Analysis and Its Applications by Using Stranded/Differential RNA-sequencing Technologies

Bo-Rahm Lee, Korea Advanced Institute of Science and Technology

TW1-5 15:50-16:10

Principle and Application of ChIP-seq to Understand Transcriptional Regulation

Yoo-Bok Cho, Korea Advanced Institute of Science and Technology

TW1-6 16:10-16:30

Translatomic Analysis by Using Ribo-seq Technique

Yujin Jeong, Korea Advanced Institute of Science and Technology

TW2 Technology Workshop 2

May 2 (Fri.), Room 323

Sponsored by Korea National Research Resource Center

Chair: Byung-Kwan Cho, Korea Advanced Institute of Science and Technology

TW2-1 15:30-15:50

Introduction to Metabolomics: Methods, Protocols and Applications

Young-Sang Jung, Korea Basic Science Institute

TW2-2 15:50-16:10

Glycosylation 분석을 위한 질량분석기 원리와 응용

Kyoung-Soon Jang, Korea Basic Science Institute

TW2-3 16:10-16:30

Principle and Application of Bimolecular Fluorescence Complementation Assay for Protein-protein Interaction Study

Yong Bhum Song, Seoul National University

TW2-4 16:30-16:50

Synthetic Regulatory Small RNA for Fine-tuning Gene Expression and Its Application

Seung Min Yoo, Korea Advanced Institute of Science and Technology

TW2-5 16:50-17:10

The Principle and Application of RNA-guided Nuclease Based Genome Editing

Sooin Lee, Korea Advanced Institute of Science and Technology

TW2-6 17:10-17:30

Bio-Imaging: How to Make a Good Digital Image

Nuri Kim, Korea Advanced Institute of Science and Technology

■■■ Workshop

W ChunLab, Inc.

May 1 (Thu.), Room 324

Chair: Byung-Yong Kim, ChunLab, Inc.

12:30-13:30

RNA-Seq Analysis: Current Methods and Its Applications

Namil Kim, ChunLab, Inc.

제3회 미생물 탐구 페스티벌

■■■ The 3rd Microbiology Research Festival for High School Students

HS

High School Students' Presentation Session

May 2 (Fri.), Room 322

Sponsored by R&D Center Maeil Dairies Co., Ltd.

좌장: 한양대학교 김동욱 교수

14:00-14:05 **개회**

HS-1 **14:05-14:15**

철세균을 이용한 토양박테리아의 생장을 저해하는 박테리오파지의 억제효과에 대하여
양준용, 중산고등학교

HS-2 **14:15-14:25**

기후 변화에 따른 토양의 온도변화가 항생제 내성 박테리아의 토양번식에 미치는 영향
장서현, 세종과학고등학교

HS-3 **14:25-14:35**

효모를 이용한 돌외 및 10종 천연물의 수명 및 노화에 미치는 영향 및 관련 기전 분석
김재현, 개포고등학교

HS-4 **14:35-14:45**

유분 환경에 따른 *Houttuynia cordata* 의 *Propionibacterium acnes* 생장 억제 효과 탐구
김원휘, 송민호, 서울과학고등학교

HS-5 **14:45-14:55**

후추의 피페리딘 성분의 박테리아의 면역 저하 반응유도에 의한 유해 박테리아(항생제 내성 대장균)의 억제 방안
이동근, 신도림고등학교

HS-6 **14:55-15:05**

폐식용유가 토양에 존재하는 토양박테리아와 유해세균의 증식에 미치는 영향에 대하여
박지연, 동덕여자고등학교

HS-7 **15:05-15:15**

세포벽을 손상시킨 토양박테리아를 이용한 토양의 방사성 스트론륨의 제거방안
조지원, 은광여자고등학교

HS-8 **15:15-15:25**

저염 환경에 적합한 유산균 선발 및 이를 이용한 저염 김치 생산
심지호, 배재고등학교

HS-9 **15:25-15:35**

정제당류 및 인공당류에 노출된 세균의 항생제 민감성 변화
이가은, 아시아퍼시픽국제 외국인학교(APIS)

HS-10 **15:35-15:45**

다양한 식품군의 섭취에 따른 햄스터 장내 세균의 분포 변화
이현정, 은광여자고등학교

HS-11 **15:45-15:55**

비타민 C의 세포증식 억제 효과를 이용한 박테리아의 항생제 내성발생의 감소방안 및 원리의 규명
방민정, 박나현, 민족사관고등학교

HS-12~HS-31

포스터발표(14:00-16:00, 장소: 포스터 세션장 321호)

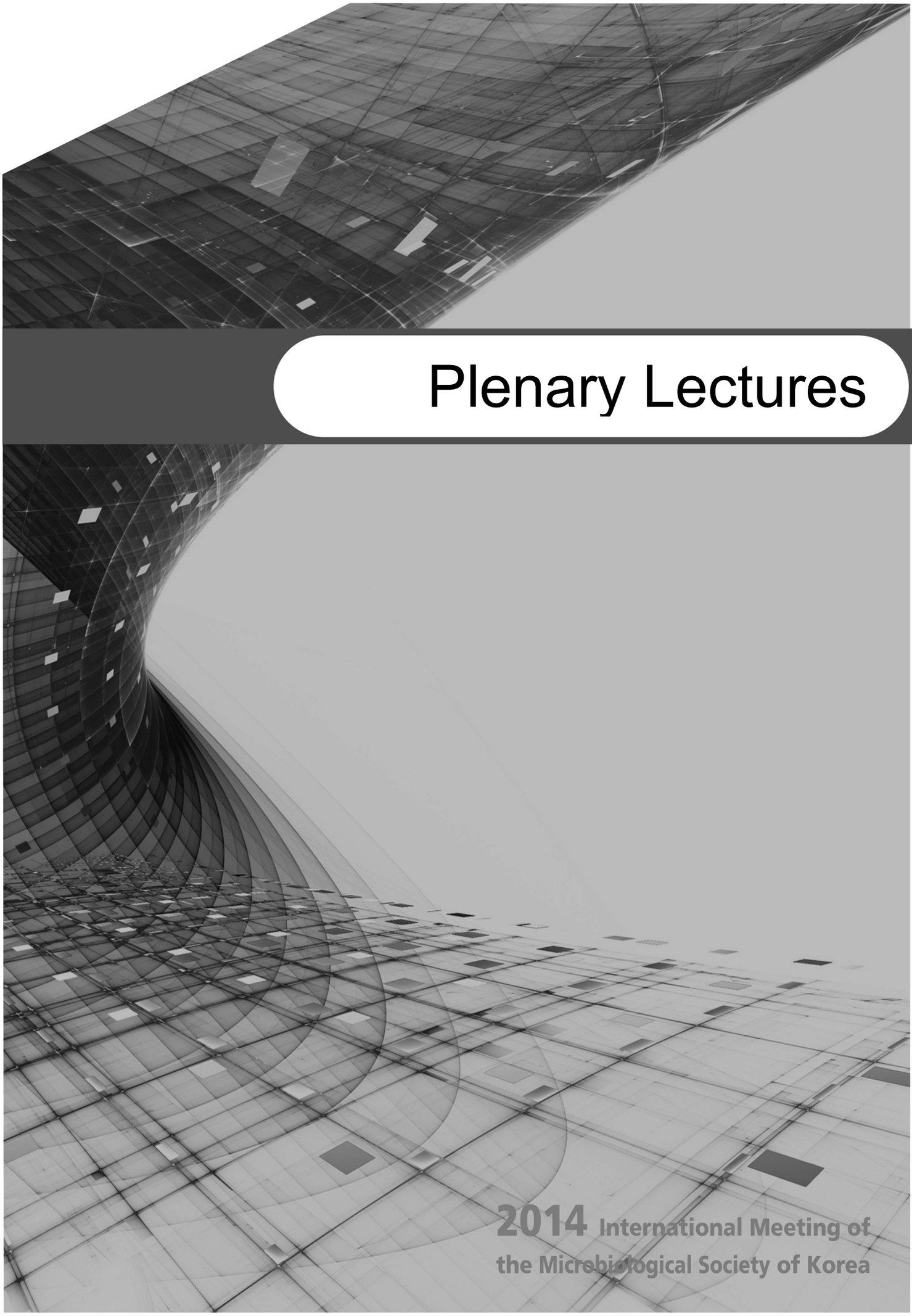
SS Special Lecture for High School Students

May 2 (Fri.), Room 322

좌장: 서울대학교 천종식 교수

SS **16:00-17:00**

진로탐색 특강: 과학자의 삶 '공부해서 무엇하나?'
노정혜, 서울대학교



Plenary Lectures

2014 International Meeting of
the Microbiological Society of Korea

PL1

Targeting the Acquisition of Iron and Other Nutrients in Fungal Pathogens of Humans

Jim Kronstad[1*], Guanggan Hu[1], Sanjay Saikia[1], Melissa Caza[1], Rodgoun Attarian[1], Daniel Croll[1], Brigitte Cadieux[1], Matthias Kretschmer[1], Won Hee Jung[2], Eunsoo Do[2], and Jennifer Geddes[1]

[1]Michael Smith Laboratories, University of British Columbia, Canada,
[2]Department of Systems Biotechnology, Chung-Ang University

Fungal pathogens are major health threats for people with compromised immune systems. An important example is the pathogenic yeast, *Cryptococcus neoformans*, which annually causes approximately one million cases of life-threatening meningitis in people with immunodeficiency due to HIV/AIDS. Globally this disease results in an estimated 625,000 deaths every year. In addition, a related species *Cryptococcus gattii* has emerged as a pathogen of people with normal immune systems, as demonstrated by an outbreak in British Columbia, Canada and the Pacific Northwest region of the United States over the past 15 years. Although antifungal drugs are available, there is a clear need for new drugs and strategies to treat cryptococcal and other fungal diseases. Over the past 15 years, we have been investigating the mechanisms by which *C. neoformans* acquires iron and other nutrients during the infection of mammalian hosts. We hypothesize that an understanding of nutrient acquisition will allow us to identify potential therapeutic targets treating fungal diseases. Iron appears to be a particularly critical nutrient for *C. neoformans* because it is essential for proliferation and the metal also regulate the elaboration of key virulence factors such as the polysaccharide capsule. The capsule protects the fungus from the host immune system and mutants that lack the capsule cannot cause disease. We have used genomic and transcriptomic approaches to identify components of iron acquisition and the regulatory factors that control their expression. We then used this information to construct targeted deletion mutations and we tested the resulting mutants for defects in virulence. This approach has provided a detailed view of the mechanisms of iron acquisition from the host sources transferrin and heme. The ability to use transferrin is particularly important for fungal colonization of the central nervous system. However, it is clear that *C. neoformans* has several mechanisms for iron acquisition including cell surface proteins for iron reduction and high affinity uptake, and proteins for heme binding and endocytosis. Each of these systems makes a partial contribution to disease. Therefore, a complete understanding of the relevance of iron acquisition to cryptococcosis will require systematic deletion of multiple systems and detailed virulence tests. Along with iron uptake, we have also analyzed the roles of other nutrients and nutrient-sensing systems in cryptococcal disease. For example, we recently discovered that phosphate sensing and uptake is important for the virulence of *C. neoformans*.

PL2

Gaining Biological Insights from Mining Bacterial Genomes & Pathways

Saheed Imam[1,2], Kimberly C. Lemmer-Christenson[2,3], Daniel R. Noguera[2,4], and Timothy J. Donohue[2,3]*

[1]Cell & Molecular Biology Graduate Program, University of Wisconsin-Madison, Madison WI, USA,
[2]Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison WI, USA,
[3]Bacteriology Department, University of Wisconsin-Madison, Madison WI, USA,
[4]Department of Civil & Environmental Engineering, University of Wisconsin-Madison, Madison WI, USA

Until recently, our knowledge of bacterial activities has been acquired by detailed biochemical, genetic and physiological analyses of its macromolecules, cofactors, metabolites and inorganic constituents. With the ever-growing access to genomic information, new experimental and computational approaches are available to gain insight into pathways and networks across the bacterial phylogeny. Photosynthetic microbes, with their ability to harness light energy and fix atmospheric carbon dioxide, are the major contributor to global carbon cycling and can be instrumental in development of industrial processes. We are interested in merging experimental and computational approaches to obtain a systems-level understanding of photosynthetic microbes. *Rhodobacter sphaeroides,* an α-proteobacterium that is arguably the best studied member of this group, has provided key insights on photon capture, light-driven energy metabolism and other aspects of the photosynthetic lifestyle. This metabolically versatile microbe is also capable of aerobic and anaerobic respiration, CO_2 and N_2 fixation, or production of H_2, polyhydroxybutyrate or other compounds of industrial importance. We have integrated experimental and computational approaches to construct models that provide new insight into the integration of metabolic and transcriptional aspects of *R. sphaeroides* lifestyles. Experimental validation of these models reveals the existence of a suite of interconnected metabolic, energetic and transcriptional networks that coordinate the lifestyles of this facultative bacterium. Based on the occurrence of individual nodes of these networks across the bacterial phylogeny these models also predict the existence of related metabolic, energetic and transcriptional systems in related α-proteobacteria.

PL3

Genome Mining for Drug Lead Discovery from Marine Bacteria

Pei-Yuan Qian

Division of Life Science and Environmental Science Programs, The Hong Kong University of Science and Technology, Hong Kong

The ocean possesses vast natural products. Many of these products are ultimately derived from marine bacteria. It is estimated that many thousands of different species of such bacteria remain to be identified and characterized and that marine microbes are a gold mine of marine bioactive molecules for various commercial applications. In this presentation, I will discuss some of our recent findings in drug lead screening. The first part of my presentation will discuss our discovery of a didemnins-producing marine α-proteobacteria *Tistrella mobilis* from the Red Sea and the biosynthesis pathways of didemnins. Then, I will present our discovery of a group of calpian inhibitors from the same bacterial strain and other microbes, based on genome mining and of the relevant their biosynthesis pathways. I will conclude this talk by giving a brief discussion on possible implication of genome mine and biosynthesis pathways manipulation in providing a long-term solution to the supply problem that presently hinders commercialization of marine natural products and in paving a new way to generate more analogous for screening and studying mode of action of compounds.

PL4

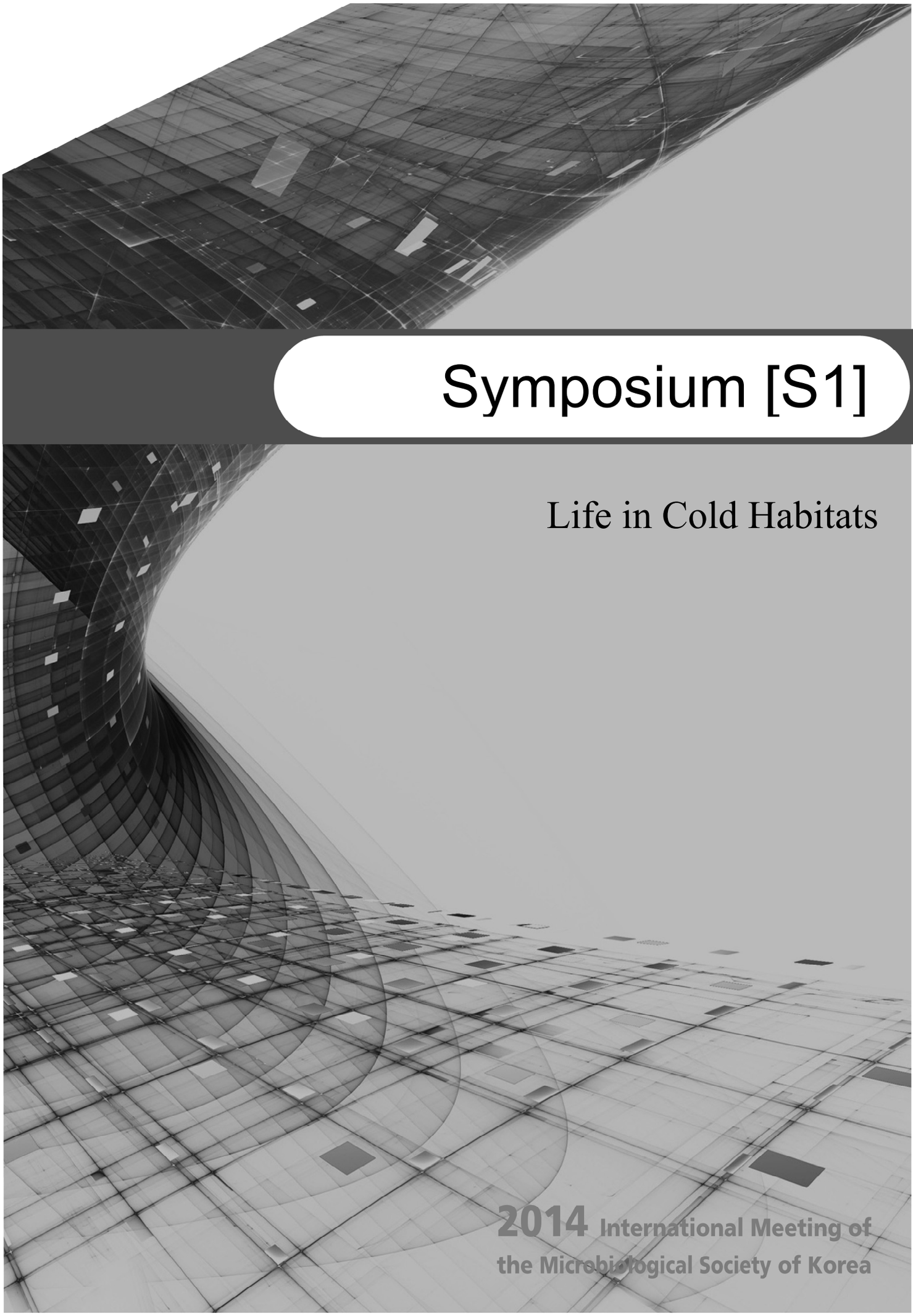
Co-translational Insertion and Folding of Membrane Proteins *In Vivo*

Nils Gunnar Hansson von Heijne

Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Sweden

Nearly all integral membrane proteins are inserted into their target membrane with the aid of a translocon, i.e., a protein-conducting channel that can mediate both translocation of polypeptides across membranes as well as insertion of proteins into membranes. Studies in our lab have defined the basic energetics of membrane insertion of transmembrane helices (*1, 2*), and our current work aims to map the early steps of tertiary structure formation in membrane proteins (*3, 4*). The lecture will review the basic steps of membrane protein integration and folding *in vivo*.

1. Hessa, T., Kim., H., Bihlmaier, K., Lundin, C., Boekel, J., Andersson, H., Nilsson, I.M., White, S.H., and von Heijne, G. (2005) Recognition of transmembrane helices by the endoplasmic reticulum translocon. Nature 433, 377-381.
2. Hessa, T., Meindl-Beinker, N.M., Bernsel, A., Kim, H., Sato, Y., Lerch-Bader, M., Nilsson, IM., White, S.H., and von Heijne, G. (2007) Molecular code for transmembrane-helix recognition by the Sec61 translocon. Nature 450, 1026-1030.
3. Cymer, F., and von Heijne, G. (2013) Co-translational folding of membrane proteins probed by arrest-peptide mediated force measurements. Proc Natl Acad Sci USA 110, 14640-14645.
4. Ismail, N., Hedman, R., Schiller, N., and von Heijne, G. (2012) A bi-phasic pulling force acts on transmembrane helices during translocon-mediated membrane integration. Nature Struct. Molec. Biol 19, 1018-1023.



Symposium [S1]

Life in Cold Habitats

2014 International Meeting of
the Microbiological Society of Korea

S1-1

Exploring Microbial Diversity in Antarctic Terrestrial Ecosystems

Ok-Sun Kim

Division of Polar Life Sciences, Korea Polar Research Institute, Incheon

Antarctica, the fifth-largest continent is the coldest and driest continent. For several decades, terrestrial environments in this continent had been believed as sterilized habitats without any life formsbecause of the harsh condition. With the dedicated terrestrial biological research, we are now beginning to understand the uniqueness and complexity of these fragile ecosystems. Molecular methods in microbiology have revolutionized the field and our understanding. Through the use of these techniques, it is revealed that microorganisms are much more diverse than we expected before and play important roles in these harsh environments. Our main research projects focus on understanding microbial life in terrestrial ecosystems in Antarctica. Ongoing project, the first, is to study microbial diversity on King George Island, around Terra Nova Bay, in Victoria Land along the latitudinal gradient and in Lakes of the Dry Valleys. Here in this talk, some preliminary results will be presented and discussed.

S1-2

Bacterial Community Structure of Tundra Soils

Ji Young Jung[1], Hye Min Kim[1,2], Sung Jin Nam[1], Hye Young Kwon[1], and Yoo Kyung Lee[1]*

[1]Arctic Research Center, Korea Polar Research Institute, KIOST,
[2]School of Biological Science, Seoul National University

The arctic region is highly responsive and vulnerable to climate change. Global warming has accelerated glacial retreat in the high Arctic and permafrost thawing. Understanding the structure of arctic soil microbial communities is essential for predicting the response of the permafrost environment to climate change. To determine the composition of the bacterial community and its relationship with soil properties, we investigated the bacterial community structure and properties of surface soil from the moist acidic tussock tundra in Council, Alaska (64°N), and the foreland of the Midtre Lovénbreen glacier in Svalbard (79°N). The bacterial community was analyzed by pyrosequencing of 16S rRNA genes, and the following soil properties were analyzed: soil moisture content (MC), pH, total carbon (TC), total nitrogen (TN), and inorganic nitrogen (NH_4^+ and NO_3^-). Bacterial community similarity based on jackknifed unweighted UniFrac distance showed greater similarity across horizontal layers than through the vertical depth in Council, Alaska. Among the soil properties measured, soil pH was the most significant factor correlating with bacterial community in both upper- and lower-layer soils. This study showed that soil depth and pH were the most important soil properties determining bacterial community structure of the subarctic tundra soil in Council, Alaska. The further away from the glacier, the more clay and soil organic carbon contents were observed. In addition, *Cyanobacteria*, *Firmicutes*, and *Actinobacteria* were dominant in soil samples taken close to the glacier, whereas *Acidobacteria* were abundant further away from the glacier. Diversity indices indicated that the bacterial community changed from a homogeneous ecosystem to a heterogeneous one along the glacier chronosequence/distance from the glacier. Although the bacterial community structure differed on basis of the presence or absence of plants, the soil properties varied depending on soil age. These findings suggest that bacterial succession occurs over time in glacier forelands but on a timescale that is different from that of soil development. The physical and chemical properties of the soil varied significantly along the distance from the Midtre Lovénbreen glacier. The further away from the glacier, the more clay and soil organic carbon contents were observed. In addition, *Cyanobacteria*, *Firmicutes*, and *Actinobacteria* were dominant in soil samples taken close to the glacier, whereas *Acidobacteria* were abundant further away from the glacier. Diversity indices indicated that the bacterial community changed from a homogeneous ecosystem to a heterogeneous one along the glacier chronosequence/distance from the glacier. Although the bacterial community structure differed on basis of the presence or absence of plants, the soil properties varied depending on soil age. These findings suggest that bacterial succession occurs over time in glacier forelands but on a timescale that is different from that of soil development.

[This study was supported by the National Research Foundation of Korea, which is funded by the Korean Government (MSIP) (NRF-2011-0021067) (PN13082, KOPRI).]

S1-3

DNA-based Fecal Analyses for Trophic Relations of Svalbard Reindeer (*Rangifer tarandus platyrhynchus*)

Sungbae Joo

Basic Science Research Institute, Ajou University, Department of Biological Science, Ajou University

The knowledge of transfer of energy to higher trophic level on food webs is essential to understanding ecosystem functioning, health and evolutions. The first step to understanding the energy transfer in ecosystems is the answer of question, "Who eats whom and how much?" Various approaches have been attempt to investigate diets of predators, however, there were many limitations to obtain accurate information related with diet components or feeding preferences. Recently, development of molecular techniques and improvement of DNA barcoding database lead to recover degraded and fragmented DNA from environmental samples and to identify species both predator and prey with higher resolution.

Svalbard reindeer (*Rangifer tarandus platyrhynchus*) lives on the high-arctic archipelago of Svalbard (74.80°N lat.) where snow and ice cover most of the local vegetation for 8 months of the year. Because of the long winter period resulting in relatively lower forage availability and poor food quality, the reindeer have to replenish fat reserves for winter survival and fetus development during the summer period. In addition, extreme seasonal variations in the high-arctic region impose strong pressures on arctic herbivores to feed on vegetation in a highly efficient manner to satisfy their energy requirements. Previous studies have reported that reindeer are highly selective feeders and prefer lichens, mosses, graminoids, and various other plant species as food sources in the summer period. These preferences might be associated with their special nutritional needs (food quality) or plant biomass represented as food quantity.

In this study, to efficiently investigate the forage preference of Svalbard reindeer (*Rangifer tarandus platyrhynchus*), we applied length heterogeneity polymerase chain reaction (LH-PCR) based on length differences of internal transcribed spacer (ITS) regions of ribosomal RNA (rRNA) to fecal samples from *R. tarandus platyrhynchus*. A length-heterogeneity (LH) database was constructed using both collected potential food sources of Svalbard reindeer and fecal samples, followed by PCR, cloning and sequencing. In total, eighteen fecal samples were collected between 2011 and 2012 from 2 geographic regions and 15 samples were successfully amplified by PCR. The LH-PCR analysis detected abundant peaks, 18.6 peaks on an average per sample, ranging from 100 to 500 bp in size and showing distinct patterns associated with both regions and years of sample collection. Principal component analysis (PCA) resulted in clustering of 15 fecal samples into 3 groups by the year of collection and region with a statistically significant difference at 99.9% level. The first 2 principal components (PCs) explained 71.1% of the total variation among the samples. Through comparison with LH database and identification by cloning and sequencing, lichens (*Stereocaulon* sp. and *Ochrolechia* sp.) and plant species (*Salix polaris* and *Saxifraga oppositifolia*) were detected as the food sources that contributed most to the Svalbard reindeer diet. Our results suggest that the use of LH-PCR analysis would be a non-invasive and efficient monitoring tool for characterizing the foraging strategy of Svalbard reindeer. Additionally, combining sequence information would increase its resolving power in identification of foraged diet components.

S1-4

Divergence of Endosymbiotic Bacteria of Deep-sea Hydrothermal Vent Bivalve Mussels

Yong-Jin Won[1,2*], Eunji Park[1], Phuong Thao Ho[2], Kang-Chon Kim[2], Ye-Seul Kwan[1], Sook-Jin Jang[2], Soon Gyu Hong[3], and Robert C. Vrijenhoek[4]

[1]Division of EcoScience, Ewha Womans University, [2]Division of EcoCreative, Ewha Womans University,
[3]Division of Polar Life Sciences, Korea Polar Research Institute, [4]Monterey Bay Aquarium Research Institute, USA

Deep-sea hydrothermal vents along the global mid-ocean ridge systems have provided unique chemosynthetic habitats to a variety of invertebrate animals that depend on chemosynthetic bacteria for their nourishment. This symbiotic association between host animals and their bacterial symbionts keeps intriguing us on their nature and evolution. While there has been a remarkable progress in our understanding of geographic distributional structure of host invertebrates and its implication, the same quest toward their endosymbionts is very limited, mostly due to a lack of relevant markers and/or inconvenient PCR-cloning method for bacterial population study.

Here we present a population genetic approach to understand the geography of genetic variation of sulfur-oxidizing endosymbionts of *Bathymodiolus* mussels in the East Pacific Rise (EPR) and the Pacific-Antarctic (PAR) Ridge. This investigation includes experimental development of multiple polymorphic markers of bacterial protein-coding genes, their application to all populations simultaneously by parallel 454 pyrosequencing method based on barcodes and creation of scripts of automatic processing of the huge amount of multiple loci sequence reads for subsequent population genetic analyses.

Our examination of endosymbionts identified a salient genetic disconnection at the Easter Microplate across where host mussels species and other vent invertebrates previously have shown marked divergence, and monotonous connection at either side of the genetic break. Such a corresponding genetic pattern at both vent invertebrates and the present endosymbionts around the boundary of the EPR and PAR suggest that the two recognized biogeographic provinces have a profound influence on not only vent animals but also vent microbes.

Symposium [S2]

Biochemistry and Molecular Biology of Prokaryotes

2014 International Meeting of the Microbiological Society of Korea

S2-1

A *Salmonella* Virulence Protein Promotes Pathogenicity by Altering Intracellular ATP Levels

Eun-Jin Lee

Department of Genetic Engineering and Graduate School of Biotechnology, School of Life Sciences, Kyung Hee University

Several intracellular pathogens including *Salmonella enterica* and *Mycobacterium tuberculosis* require the virulence protein MgtC to survive within macrophages and to cause a lethal infection in mice. We now report that, unlike secreted virulence factors that target the host vacuolar ATPase to withstand phagosomal acidity, the MgtC protein acts on *Salmonella*'s own F_1F_o ATP synthase. This complex couples proton translocation to ATP synthesis/hydrolysis and is itself required for virulence. We establish that MgtC interacts with the *a* subunit of the F_1F_o ATP synthase, hindering proton translocation and ATP hydrolysis in inverted vesicles. An *mgtC* null mutant displayed heightened ATP levels and an acidic cytoplasm whereas *mgtC* overexpression decreased ATP levels. A single amino acid substitution in MgtC that prevented binding to the F_1F_o ATP synthase abolished control of ATP levels and attenuated pathogenicity. MgtC provides a singular example of a virulence protein that promotes pathogenicity by interfering with another virulence protein.

S2-2

Strategy of Survival of *E. coli* and Translational System

Nobuo Shimamoto* and Hideki Nakayama

Faculty of Life Sciences, Kyoto Sangyo University, JAPAN

Bacteria can survive in non-growing conditions. In evolutional selection, this survival is as critical as their growth, but the underlying mechanism is poorly understood because of the complexity. However, the knowledge on *E. coli* may have been accumulated enough to overcome the complexity. We at first focused on the relationship between tmRNA and the protein turnover, in which used proteins are degraded to salvage amino acids for the survival. The tmRNA (*ssrA*) and SmpB induce the degradation of nascent polypeptide by *ssrA*-tagging in response to a reduced level of amino acids in stationary phase. We prepared the disruptants of genes relating to the tmRNA system as well as major chaperons and proteases. Unexpectedly and probably fortunately, most examined protease/chaperon genes are synthetic lethal with Δ*ssrA* or Δ*smpB*, demonstrating that there are two parallel pathways in the surviving mechanism. The tmRNA system is essential to one pathway, and at least nine genes, *clpA*, *clpP*, *clpX*, *dnaJ*, *hslU*, *hslV*, *htpG*, *prc*, and *sspB*, are essential to the other. Among the examined genes, non-synthetic-lethal genes were only *clpB* and *lon*, which could be thus involved the tmRNA pathway. Another unexpected conclusion is that the tmRNA-dependent degradation is not a major protein turnover, because the main proteases in the system, ClpXP, ClpAP, and Prc are synthetic lethal to Δ*ssrA*. These 9 synthetic lethalities were all complemented in LBaa or M9aa medium, which are LB or M9 medium containing nutrient levels (g/L) of 20 amino acids, respectively, suggesting the essential contribution of protein turnover for the survival.

Since the observed lethality, in principle, could be due to any growth stages other than survival period. We thus examined the time course of the CFU decay for single disruptants in LB and LBaa. The obtained results showed that some genes contribute to the survival through their effects on the production/degradation of specific proteins. For example, ClpXP must degrade some specific proteins overproduced by the exogenous amino acids in LBaa before 18 hr. Since the period before 18 hr is the period of the inhibited translation by the dimerization of 70S ribosome, 100S, we examined the 100S-related factors, Rmf, Hpf, and YfiA. Their gene disruptants die as early as Δ*clpX*, suggesting that the checkpoint activated by ClpXP is ribosome digestion through 100S formation. We then invented a new GFP, B-maggio, a brightest GFP with an extremely slow photobleaching. It changes color when it is fused to S10 protein and when ribosome forms 100S or aggregated. In dead cells, S10-B maggio forms inclusion body, while in living cells, it exist in cytosol, demonstrating a correlation between viability and the intact ribosome.

S2-3

O_2 Dependent Transcriptional Networks of *Escherichia coli*

Kevin Myers, Nicole Beauchene, Dan Park, and Patricia Kiley*

Department of Biomolecular Chemistry, University of Wisconsin-Madison, USA

In *E. coli*, 15% of the genes change expression upon O_2 limitation. The most well studied response of *E. coli* and other facultative bacteria to O_2 limitation is the reprogramming of metabolism to utilize alternative energy pathways when O_2 is not available. The two transcription factors in *E. coli* that play a major role in this reprogramming are ArcA and FNR. It is well known that DNA binding of the response regulator ArcA is increased upon phosphorylation by a membrane bound kinase that responds to O_2-dependent changes in electron transport chain flux. FNR contains an O_2 labile [4Fe-4S] cluster that is required for dimerization and DNA binding. Our genome-wide studies indicated that ArcA and FNR accounted for only ~40% of the genes regulated by O_2, suggesting that there must be other major O_2-responsive control systems. Our genome wide data suggest that two other transcription factors, Fur and IscR, known to be involved in Fe and Fe-S cluster homeostasis, respectively, are also involved in the global response to O_2 deprivation.

Comparisons of genome-wide RNA levels from *E. coli* wild type or ΔFur mutant strains with ChIP-Seq data identified differentially expressed operons directly bound by Fur under aerobic and anaerobic conditions. These studies showed unexpectedly that Fur binding is increased under anaerobic conditions, indicating that Fur-DNA interactions and expression of some Fur target genes are also regulated by O_2 availability. Because Fur activity is proposed to be regulated by the availability of the "labile" Fe^{2+} pool, our results suggest that the labile Fe^{2+} pool is increased under anaerobic conditions, increasing the concentration of Fe^{2+}-bound Fur.

The transcription factor IscR also contributes to global reprogramming of gene expression in response to O_2 limitation, although the mechanism is more complex. DNA binding specificity of IscR is broadened by ligation of a [2Fe-2S] cluster; apo-IscR binds only a type 2 DNA motif, whereas [2Fe-2S]-IscR binds both type 1 and 2 motifs. The absence of O_2 increases the *in vivo* Fe-S occupancy of IscR, so genes in the IscR regulon show major changes in expression under aerobic and anaerobic conditions, depending on whether they have type 1 or type 2 sites controlling their expression.

In summary, our data reveal multiple conserved transcription factors govern how O_2 availability controls gene expression in bacteria. By using primarily Fe or Fe-S dependent regulators to control gene expression, these cofactors also provide cells a mechanism to both sense O_2 availability and integrate this signal with iron homeostasis. Since iron and O_2 availability have a major influence on bacteria in their natural environments, our results are critical to understanding a key bacterial adaptation strategy.

S2-4

Indole Inhibits Predation by *Bdellovibrio bacteriovorus* and Bdelloplast Lysis: A Transcriptomic Analysis

Mohammed Dwidar, Dougu Nam, and Robert J. Mitchell*

School of Life Sciences, Ulsan National Institute of Science and Technology (UNIST)

S2-5

Bacterial Cancer Therapy: Antitumor Effect of *Salmonellae* Expressing L-Asparaginase

Kwangsoo Kim and Hyon E Choy*

Department of Microbiology, Chonnam National University Medical School, Kwang-Ju 501-746

Over the last several decades, a number of microorganisms have been shown to display selective replication or preferential accumulation in tumor lesion. Facultative anaerobes such as *Escherichia coli* and *Salmonella* spp. also localize to transplanted tumors in animals and can grow in viable as well as necrotic areas of the tumor, a potential important advantage. This feature has been exploited to treat cancer directly or in combination with conventional chemotherapy. Thus, although the mechanism underlying the initial accumulation or tumor destruction remains to be understood, attempts have been made to treat tumors using various microorganisms. Although bacteria targeted to tumor tissue reduces tumor growth, the bacteria equipped with tumorlytic protein are often more effective in tumor suppression. This is because the concentration of the tumorlytic protein expressed from the bacteria would be much greater within the tumor than in systemic blood. Therefore, it is essential to control expression of the tumorlytic protein expressed selectively only when bacteria are predominantly found in the tumor tissue over other organs, *i.e.*, reticulorendothelial organs. To this end, inducible systems that utilize different external gene "triggers" have been developed: *i.e.*, the L-arabinose system. Using this system, we have shown that the engineered bacteria significantly suppressed both primary and metastatic tumors and prolonged survival in mice.

Symposium [S3]

Novel Insights for Microbial Ecology

2014 International Meeting of
the Microbiological Society of Korea

S3-1

Bioinformatics Effort to Better Understand Microbial Community

Kyung Mo Kim[1]*, Jeongsu Oh[1], Kyuin Hwang[2], Byung Kwon Kim[3], Hanna Choe[1], and Soon Gyu Hong[2]*

[1]Microbial Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon,
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Microorganisms play critical roles in regulating the biogeochemistry of our planet. Microbial communities largely influence the relationships of biotic and abiotic environments. Assessing microbial diversity is the first step in understanding the role of microbes in biogeochemical evolution. Since a universal taxonomic structure of life based on ribosomal RNA (rRNA) was established in mid-1980s, ribosomal genes have been used as 'gold standard' to identify species and build higher-level taxonomies. Based on this taxonomical structure, the sequencing of rRNA genes (e.g., 16S in prokaryotes; 28S in eukaryotic microbes) derived from environmental samples led to the discovery of unprecedented diversity of both cultured and uncultured microbes. In addition, next generation sequencing (NGS) technologies such as pyrosequencing and Illumina are producing high-volume information at DNA level, facilitating the unprecedented detection of new phylotypes. However, the analysis of rRNA data generated from high-throughput sequencing experiments represents a bioinformatics challenge that requires accurate and efficient handling of large-scale data. We here describe our recent bioinformatics effort that includes developments of algorithm, software and database for NGS-based microbial community study.

The availability of 16S rRNA gene sequences from a multitude of natural environments now offers a unique opportunity to study microbial diversity. The large volume of sequencing data however makes it time consuming to assign individual sequences to phylotypes. Since ribosomal sequences have diverged across phylotypes, they can be grouped into clusters. However, available clustering programs suffer from overlap of sequence spaces in adjacent clusters. In natural environments, gene sequences are homogenous within species but divergent between species. This evolutionary constraint results in an uneven distribution of genetic distances of genes in sequence space. To cluster 16S rRNA sequences more accurately, it is therefore essential to select core sequences that are located at the centers of the distributions represented by the genetic distance of sequences in taxonomic units. Based on this idea, we here describe a novel sequence clustering algorithm named CLUSTOM that minimizes the overlaps between adjacent clusters. The performance of this algorithm was evaluated in a comparative exercise with existing programs, using the reference sequences of the SILVA database as well as published pyrosequencing datasets. The test revealed that our algorithm achieves higher accuracy than ESPRIT-Tree and mothur, few of the best clustering algorithms. Results indicate that the concept of an uneven distribution of sequence distances can effectively and successfully cluster 16S rRNA gene sequences. The algorithm of CLUSTOM has been implemented both as a web and as a standalone command line application, which are available at http://clustom.kribb.re.kr.

The 454 pyrosequencing platform produces the longest reads among the most widely used next generation sequencing platforms. Since the relatively longer reads of the 454 platform provide more information for identification of microbial sequences, this platform is dedicated to microbial community and population studies.

In order to accurately perform the downstream analysis of the 454 multiplex datasets, it is necessary to remove artificially designed sequences located at either ends of individual reads and to correct low quality sequences. We have developed a program called PyroTrimmer that removes the barcodes, linkers, and primers, trims sequence regions with low quality scores, and filters out low-quality sequence reads. Although these functions have previously been implemented in other programs as well, PyroTrimmer has novelty in terms of the following features: i) more sensitive primer detection using Levenstein distance and global pairwise alignment, ii) the first stand-alone software with a graphic user interface, and iii) various options for trimming and filtering out the low-quality sequence reads. PyroTrimmer, written in JAVA, is compatible with multiple operating systems and can be downloaded free at http://pyrotrimmer.kribb.re.kr.

The large-volume DNA information that is driven from recent advances in sequencing technology helps us better understand microbial community structures in diverse environments when coupled with a well-curated reference sequence database. Databases such as RDP, Silva, Greengenes, and EzTaxon-e provide reliable 16S rRNA sequences for prokaryotes. However, a similar reference database is absent for the study of fungal diversity and ecology. Although the UNITE system provides ITS sequences across almost all fungal groups, the ITS region is highly variable and therefore yields poor sequence alignments between distant fungal species. Considering the remarkable fungal diversity in nature, we chose the more conserved LSU rRNA gene as a standard genetic marker to study metagenome-based fungal diversity and ecology. In order to build a fungal reference sequence database called MycoDE, tens of thousands of LSU sequences were collected, filtered, aligned and phylogenetically analyzed. The manual phylogenetic inspection showed that the majority of fungal taxonomic groups are polyphyletic. The taxonomic names of monophyletic fungal groups were determined by referring to the current nomenclature system. On the other hand, non-monophyletic fungal groups whose appropriate scientific names were not available were temporarily named using our own rules of nomenclature, which was developed from an ecological point of view. Now, a new fungal taxonomic hierarchy with reliably aligned LSU sequences is available at the MycoDE website (http://mycode.kopri.re.kr). In addition, this website provides an identification tool to assign taxonomic names to the large-scale fungal LSU query sequences.

S3-2

Cyanobacterial Bloom: from Micro to Macro Aspect

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Cyanobacteria are ancient bacteria, their first appearance dating to 3.6 billion years ago. Cyanobacteria provided oxygen gas to primitive atmosphere and formed fundamental conditions for so much life diversity to arise. However, they are regarded as harmful organisms these days, due to bloom formation. Microcystins, representative cyanobacteria toxin, can be threat to animals, including human. Although a lot of studies have been done to control cyanobacterial blooms, no universal solutions have been obtained. One reason for such difficulties seems to be originated from cyanobacterial genetic diversity. Development of genomic tools revealed that the genome of *Microcystis* (major bloom former) is very plastic in genetic rearrangement. Even in one lake, genetic diversity and their composition continuously changed with seasons. Different strains responded differently to the same environmental factor, making it more difficult to prepare defense strategy for cyanobacterial bloom. Toxic and nontoxic strains are mixed in a water body, but toxic proportion increased with bloom formation and decreased with bloom extinction. Global warming could aggravate bloom toxicity. Cyanobacterial bloom can be predicted, using model techniques. Artificial neural networks were applied to Daechung Reservoir. Prediction models showed that blooms could be predicted and water temperature was the most critical factor for bloom formation, rather than phosphorus, which was considered traditionally as the major cause. Data accumulated for years in each reservoir need to be used for modeling and data mining to get some new insights. Cyanobacterial blooms cannot be completely suppressed but can be managed in a moderate level.

S3-3

Identification and Single-Cell Isolation of Active N_2O Reducers in Environments

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Nitrous oxide (N_2O) is considered a major greenhouse gas and is a significant contributor to ozone layer destruction. N_2O can be produced as the end product of denitrification or as the byproduct of nitrification, but N_2O can also serve as an electron acceptor for microbial respiration (i.e., N_2O reduction). However, identity of the N_2O reducers active in environments is largely unknown. In this study, we employed both culture-dependent and culture-independent approaches to identify N_2O reducers in rice paddy soil where N_2O reduction actively occurs. In a soil microcosm, N_2O and succinate were added as the electron acceptor and donor, respectively, for N_2O reduction. For the stable isotope probing (SIP) experiment, ^{13}C-labeled succinate was used to identify succinate-assimilating microbes under N_2O-reducing conditions. DNA was extracted 24 h after incubation, and heavy and light DNA fractions were separated by density gradient ultracentrifugation. Microbial community structures in each fraction were analyzed based on the 16S rRNA gene and the N_2O reductase gene sequences. For culture-dependent analysis, the microbes that elongated under N_2O-reducing conditions in the presence of cell-division inhibitors were individually captured by a micromanipulator and transferred to a low-nutrient medium. The N_2O-reducing ability of these strains was examined by gas chromatography/mass spectrometry. Results of the SIP analysis suggested that *Burkholderiales* and *Rhodospirillales* bacteria dominated the population under N_2O-reducing conditions, in contrast to the control sample (soil incubated with only ^{13}C-succinate). Results of the single-cell isolation technique also indicated that the majority of the N_2O-reducing strains belonged to the genera *Herbaspirillum* (*Burkholderiales*) and *Azospirillum* (*Rhodospirillales*). In addition, *Herbaspirillum* strains reduced N_2O faster than *Azospirillum* strains. These results suggest that *Herbaspirillum* spp. may have an important role in N_2O reduction in rice paddy soils. As shown in our study, combination of culture-independent and culture-dependent approach is useful to identify and characterize ecologically important microbes in environments.

S3-4

Nocardiopsis: from Its Biodiversity, Biogeography to Genetic Mechanisms of Environmental Adaptability

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The genus *Nocardiopsis*, which is a widespread group of the phylum *Actinobacteria*, has received great attention owing to its ecological versatility, pathogenicity and ability to produce a rich array of bioactive metabolites. Before the year of 2000, there are only seven validly published species in this genus. However, now it has expanded to 38 species and two subspecies. And recently, more and more strains of the genus *Nocardiopsis* have been isolated from saline soils or saline-alkali soils by our group and other colleagues in the world, and some of them being strictly halophilic.

According to the fundamental assumption that *'everything is everywhere: but the environment selects'*, it would be inferred that this group actinobacteria is more easily dispersed and better suited for colonizing in new environments, leading to more cosmopolitan distribution. In this study, we tested this hypothesis using five *Nocardiopsis* OTUs isolated from sediments collected from Yunnan salt mines and Xinjiang saline soils, western of China. The phylogenetic analyses based on 16S rRNA, *gyrB*, *rpoB* and *sodA* gene sequences were employed to determine the geographic profiles and evolution of these *Nocardiopsis* subpopulations. The phylogenetic analysis of five *Nocardiopsis* OTUs based on 16S rRNA gene showed that five *Nocardiopsis* OTUs were divided into many phylotypes and majority of these phylotypes presented geographic patterns between Yunnan and Xinjiang in China, without any phylotypes in an OTU being shared by Yunnan and Xinjiang. However, most phylotypes were little exclusive to a site or sites close to each other within Yunnan or Xinjiang. Perhaps the generation of geographic phylotypes in 16S rRNA gene phylogeny mainly resulted from theimpact of geographic isolation on *Nocardiopsis* strains' dispersal and led to their geographic distributions between Yunnan and Xinjiang. Furthermore, five *Nocardiopsis* OTUs were divided into many endemic genotypes and most of them were exclusive to a site or sites close to each other which demonstrated proofs for *Nocardiopsis* population endemism and some evolutionary divergences among these three genes. According to Z-test of Darwinian Selection for *gyrB*, *rpoB* and *sodA*, it suggested these divergences on *sodA*, *rpoB* and *gyrB* may result from neutral evolution of *gyrB* and *rpoB*, but environmental selection of *sodA* evolution. Therefore evolutions of *gyrB* and *rpoB* were not influenced by any environmental disturbances, but evolution of *sodA* was impacted by contemporary environmental forces because of its involvement in *Nocardiopsis* strains adaption and resistances to environmental oxygen toxicity.

In addition, to shed light on speciation, gene content evolution, and environmental adaptation in these unique actinobacteria, we sequenced draft genomes for 16 representative species of the genus and compared them with

that of the type species *N. dassonvillei* subsp. *dassonvillei* DSM 43111^{T}. The core genome of 1,993 orthologous and paralogous gene clusters was identified, and the pan-genomic reservoir was found not only to accommodate more than 22,000 genes, but also to be open. The top ten paralogous genes in terms of copy number could be referred to three functional categories: transcription regulators, transporters, and synthases related to bioactive metabolites. Based on phylogenomic reconstruction, we inferred past evolutionary events, such as gene gains and losses, and identified a list of clade-specific genes implicated in environmental adaptation. These results provided insights into the genetic causes of environmental adaptability in this cosmopolitan actinobacterial group and the contributions made by its inherent features, including genome dynamics and the constituents of core and accessory proteins.

Symposium [S4]

Signal Transduction and Gene Expression in Fungi

2014 International Meeting of
the Microbiological Society of Korea

S4-1

Characterization of the RGS Protein GprK and RgsC in *Aspergillus fumigatus*

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G proteins function as switches that are activated by G protein-coupled receptors (GPCRs) and negatively regulated by regulator of G protein signaling (RGS) proteins. In this study, we characterize the two RGS proteins in the opportunistic human pathogen *Aspergillus fumigatus*. While the deletion of *AfugprK* causes the increased vegetative growth, the growth of *AfurgsC* deletion mutant is inversely. The conidiation of both mutants are repressed and the mRNA accumulation of asexual development related genes (*AfubrlA*, *AfuabaA*, *AfuwetA*, and *AfuvosA*) are delayed and/or decreased. The absence of *AfugprK* and *AfurgsC* results increased *AfubrlA* and *AfuvosA* in liquid submerged culture. The conidial germination is accelerated by the deletion of *AfugprK* and *AfurgsC*, suggesting that they act as repressors of germination. The deletion of *AfurgsC* causes a reduction of vegetative growth in the presence of osmotic stress, cell wall stress, and oxidative stress. Protein kinase A (PKA) activity is assayed using kemptide and the PKA activity pattern of *AfugprK* deletion mutant is different from those of the wild type and Δ*AfurgsC* mutant, as PKA activity is detectable even without the addition of cAMP. Gliotoxin is not detected in the chloroform extract of culture filtrates of both deletion mutants. Conidia invasiveness of two deletion mutants in the type II human alveolar cell line (A549) is reduced compared to that of wild type.

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S4-2

Septation and Conidiation in *Aspergillus nidulans*

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Timely cytokinesis/septation is essential for hyphal growth and conidiation in *Aspergillus nidulans*. Genetic analysis have identified that *A. nidulans* has components of the septum initiation network (SIN) pathway; one of these, SEPH, is a key player for early events during cytokinesis. However, little is known about how the SEPH kinase cascade is regulated by other components. Here, through UV mutagenesis, independent mutants were obtained that could restore cytokinesis in the absence of *sep*H. Among them, the phosphoribosyl pyrophosphate synthetase family acts antagonistically against the SIN so that the downregulation of AnPRS family can bypass the requirements of the SIN for septum formation and conidiation. The transcription defect of the *prs*A gene family accompanied with the reduction of AnPRS activity causes the formation of hyper-septation as well as the restoration of septation and conidiation in the absence of SEPH. Moreover, we demonstrated that AnPRS members are able to form the heterodimers for functional interacting entities but they appear to contribute so unequal that deletion of *prs*1 displays relatively normal septation, but deletion of either *prs*2 or *prs*3 is lethal. *Anprs2* is essential probably through the whole development period during germination and the hyphal growth, whereas *Anprs3* is essential only in the germination process.

In addition, two regulatory subunits of protein serine/threonine type 2A phosphatases (PP2A)-ParA and PabA, whose orthologs are suppressors of SIN in yeasts had been found to be required for conidiation and septation. Deletion of *par*A caused the hyper-septation in hyphal cells, especially in conidiophore cells while deletion of *pab*A abolished or delayed the septation. Different from PP2A-Pab1 and PP2A-Par1 in yeast that are negative regulators to inactivate the SIN, loss of ParA or PabA function failed to suppress defects of the temperature-sensitive mutants of SEPH kinase.

Therefore, SIN, as a major signaling pathway in regulating cytokinesis, might have to work together with multiple other protein complexes.

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S4-3

Regulation of Pathogenesis by Light in *Cercospora zeae-maydis*

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Cercospora zeae-maydis causes gray leaf spot of maize, which has become one of the most widespread and destructive diseases of maize in the world. *C. zeae-maydis* infects leaves through stomata, which is predicated on the ability of the pathogen to perceive stomata and reorient growth accordingly. Thus, we became interested in understanding how fungi perceive and penetrate stomata while studying the maize foliar pathogen *C. zeae-maydis*. In this study, we observed that germ tubes of *C. zeae-maydis* require light in order to find stomata and produce appressoria, which led to the identification of *CRP1*, a gene encoding a putative blue-light photoreceptor homologous to White Collar-1 (WC-1) of *Neurospora crassa*. Disruption of *CRP1* revealed roles in multiple aspects of pathogenesis, including tropism of hyphae to stomata, the formation of appressoria, conidiation, and the biosynthesis of cercosporin. *CRP1* was also required for photoreactivation after a lethal dose of UV exposure. *CRP1* is the first gene known to regulate non-thigmotropic stomatal infection in fungi and thus provides specific insight into how light regulates pathogenesis in *C. zeae-maydis*. Furthermore, we have recently investigated light-responsive phenotypes of *Fusarium graminearum* that causes Fusarium head blight (FHB) on cereal crops and ear rot on maize. This presentation also includes the current knowledge of the roles of WC genes in secondary metabolite synthesis and development of *F. graminearum.*

S4-4

Gene Expression in Yeast During Drug Synergy with Iron Chelating Agents

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The pathogenic yeast species *Cryptococcus neoformans* and *C. gattii* cause cryptococcosis, a normally self-limiting pulmonary infection that can develop into severe, life-threatening meningitis and meningoencephalitis, particulary in HIV/AIDS, cancer and transplant patients. Cryptococcal meningitis is uniformly fatal if not treated, and even with best practice mortality is 20-30%. Current recommendations for therapy are induction with amphotericin B (AMB) and 5-flucytosine (5FC), followed by maintenance on fluconazole (FLC) or other azole-based drugs. However, AMB is highly toxic and requires vigilant monitoring, 5FC and new generation triazoles are expensive and out of the reach of most low income countries, and FLC is less efficacious and induces resistance. Finding and developing new antifungals is very difficult, therefore a promising avenue of antifungal research is to enhance existing therapies using synergistic agents. Iron chelators administered with certain antifungals have been found to improve the clearance of some fungal infections. However, mechanistic data on exactly how these work, and why they sometimes do not, are lacking. In addition, iron depletion can be damaging to the host. The aims of the current study are therefore 1) to find antifungals + iron chelator combinations that result in synergy when used to treat *Cryptococcus;* and 2) to use RNA-Seq and co-expression networks to analyse the synergistic response at the level of transcription and identify important mediators of synergy. The hypothesis is that by using transcriptome analysis during drug-chelator synergy we can identify important, differentially regulated pathways or process that we can target with new therapies that produce synergy without the need to administer chelators.

Checkerboard assays were used to assess synergy between the antifungals AMB, FLC, itraconazole (ITZ), voriconazole (VRZ), and caspofungin (CAS) with iron chelators lactoferrin, deferasirox, deferiprone, deferoxamine, cyclopirox olamine and EDTA. Fungal species included *Cryptococcus neoformans* genome strain H99, *C. gattii* genome strain R265 and *C. gattii* strain 97/170, which is intrinsically resistant to FLC. *Saccharomyces cereviseae* genome strain S288C was also included for interactome construction. Significant synergy was uncommon and was only seen across all species when AMB was combined with lactoferrin, a milk protein with iron chelating properties. Interestingly, while VRZ + EDTA produced a synergistic response against *C. gattii,* this combination was antagonistic when used against *C. neoformans*, as was ITZ when combined with deferasirox, deferiprone and EDTA, highlighting a need for caution when using iron chelators.

Transcriptional analysis by RNA-Seq was performed on *S. cerevisiae* treated with i) AMB only; ii) AMB + lactoferrin, iii & iv) corresponding controls matched for growth but without antifungals or chelators. Visualisation using the network program Cytoscape, and co-expression analysis with self organizing maps (SOMs) suggested transcription factors AFT1 and YAP5 were important during AMB + lactoferrin synergy. AMB treatment alone caused down-regulation of nine genes involved in ergosterol biosynthesis and up-regulation of AFT1, a transcription factor involved in iron transport. AMB + lactoferrin halted the

up-regulation of AFT1 and down-regulated genes involved in iron transport. The latter were co-expressed with YAP5, a second transcription factor that co-ordinates the expression of genes controlling the nuclear localization of AFT1. The influence of these will be further studied using qPCR and gene deletion/complementation. We are currently exploring the role of homologous factors in *Cryptococcus* using additional RNA-Seq assays.

Symposium [S5]

Microbial Systems Biology and Genome Engineering

2014 International Meeting of the Microbiological Society of Korea

S5-1

Microbial Relationships Uncovered-Community Systems Biology Approaches in Microbial Ecology

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Many biogeochemical processes involved in the global geochemical cycles are not performed by individual organisms, but rather by microbial communities - collaborative partnerships between two or more microbes. These partnerships often involve consumption of carbon compounds that cannot be used by any individual organism, but yield sufficient energy for growth when paired organisms couple their metabolic capabilities. These associations are critical to carbon decomposition processes and are particularly important in oxygen-limited environments such as wetlands, sediments, and subsurface aquifers. We developed a novel genome-scale, multi-omics based modeling approach to investigate the systems biology of syntrophic microbial partnerships to shed new light on a poorly understood aspect of carbon cycle processes. The work represents a significant advance in our ability to extend genome-scale systems biology modeling approaches to multispecies microbial consortia.

S5-2

Eubacterium limosum KIST612 as A Model Strain for C1 Biorefinery

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Eubacterium limosum KIST612 is a strict anaerobic, Gram-positive, acetogenic bacterium that uses synthesis gas components (H_2, CO) as carbon and energy source *via* the Wood-Ljungdahl (WL) pathway. This strain was first isolated from anaerobic digestor fluid in sewage treatment process more than two decades ago. When it was isolated this strain showed relatively high organic acid production rates, either acetate and butyrate, from CO oxidation as well as fast growth rate compared to other (homo)acetogens. KIST612 strain has 0.17-0.25 h^{-1} of specific growth rate on phosphate-buffered basal medium with 1 atm of CO partial pressure in vial culture. When the CO partial pressure was less than 0.6 atm (as 0.5 mM dissolved CO) in serum vial (with typical agitation) CO consumption was limiting during the cultivation indicating that the growth rate of KIST612 is dependent on CO partial pressure. This result significantly proves that CO consumption by KIST612 is due to catabolic reaction which generates ATP for microbial growth. Although, CO can be utilized as carbon and energy sources, higher CO concentration derived from CO pressurizing also showed substrate inhibitory effect indicating that CO concentration should be controlled in bioprocess operation to maximize CO conversion rate. KIST612 produces acetate as major product on either CO or H_2 cultivation. Likewise, butyrate and ethanol are also produced as minor products, and butyrate production was stimulated under certain culture conditions such as low pH and especially during stationary phase. The complete genome sequence of this strain consisted of 4,276,902 bp in a single circular chromosome with an average G+C content of 47.5%. Approximately 91% of the nucleotides were predicted as 4,516 protein-coding regions. Metabolic pathway analysis revealed that *E. limosum* KIST612 uses the WL pathway to fix CO (or CO_2) and converts it into acetyl-CoA, like other syngas-utilizing acetogens. It was also found to contain genes annotated as subunits of hydrogenases that may provide reducing equivalents for CO_2 reduction to organic carbons. Further the genome of *E. limosum* KIST612 contains genes that encode key enzymes that convert acetyl-CoA into potential bioenergy-compatible acids/alcohols (acetate, butyrate, and ethanol). In addition to these genes, key genes for growing on syngas can be a platform of synthetic biology to construct carbon fixation pathways for the production of biofuels or chemicals from syngas. However, the design of "new biocircuit" of the strain for the production (or increase) of desired chemicals should be approached with utmost caution because most precursors of these chemicals are essential compounds on catabolic metabolism. Recently, we have tried to obtain deletion mutant which was knocking out the butyryl-CoA dehydrogenase (Bcd) encoding gene (*bcd*) on chromosomal DNA of the strain using designed PCR product through homologous recombination. The deletion of the Bcd encoding gene in the mutant was confirmed by PCR and DNA sequencing. However, the mutant only grew on glucose substrate but not on CO. This result indicates that Bcd is an essential component for energy metabolism of *E. limosum* KIST612 during chemolithotrophic growth on C1 compound, as well, it shows possibility to knock in a foreign gene on chromosomal DNA of this strain. There are a few numbers of acetogens producing acetate as well as other products such as butyrate and ethanol, and most strains only possess acetate producing activity. Ethanol and butanol are more preferred chemicals other than fatty acids of same carbon number. From this point of view, *E. limosum* KIST612 has a diversity of products, therefore, it is a powerful candidate for C1 biorefinery.

S5-3

Development of Oxygen-independent *E. coli* Strain for Overproduction of Commodity Chemicals

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The microbial production of commodity chemicals is a promising avenue for the development of sustainable processes for the utilization of renewable resources and reducing our dependency on foreign oil. In order to become cost and energy effective, the process must utilize an organism that is optimized for production of a number of reduced by-products from variety of feedstocks. *Escherichia coli* is one of the most commonly used host organisms for metabolic engineering and overproduction of metabolites due to its metabolic versatility, amenability to genetic manipulation, and the ability to produce a wide variety of reduced by-products such as bio-ethanol and organic acids. *E. coli* has also been extensively characterized with respect to its metabolic physiology. It is capable of surviving in a variety of environmental conditions, such as oxic and anoxic; however the different growth rates and different secretion profiles under oxic and anoxic conditions poses a significant challenge for metabolic engineering processes in which environmental perturbations will influence the outcome of the bio-catalytic process. Therefore, the utilization of the oxygen-independent strain for bio-catalysis eliminates the need for the stringent control over the fermentation environment with respect to oxygenation, thus significantly reducing the cost of the entire bio-catalytic process. Therefore, it is of interest to develop an *E. coli* strain incapable of oxygen utilization, to be used as a platform strain for metabolic engineering. This talk will outline the development of the *E. coli* strain, not able to utilize oxygen and engineered to overproduce organic acids though a redox-coupling of the production pathway and central metabolism.

S5-4

Multiomics-guided Bacterial Genome Analysis

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Over the past decade or so, dramatic developments in our ability to experimentally determine the contents and functions of genomes have taken place. In particular, high-throughput technologies are now inspiring a new understanding of the bacterial genome on a global scale. In particular, predicting and understanding metabolic network of unfamiliar species have been difficult part of biology. Using the conventional methods, constructing a whole metabolic network has been recognized as a long term and labor intense project. However, with the birth of next-generation sequencing (NGS), not only genomic and transcriptomic approach to biology has reformed but also metabolic network construction has changed as well. Here, we present assembling and constructing a genome-scale metabolic pathway of non-model organism, *Clostridium aceticum*. It is an anaerobic homoacetogen, able to reduce CO_2 to multi-carbon products using the reductive acetyl-CoA pathway. Metabolic pathway mapping using the functional annotation obtained from the assembled contigs identified the majority of central metabolic pathways, such as the glycolysis and TCA cycle. Further, these analyses elucidated the enzymes consisting of Wood-Ljungdahl pathway, in which CO_2 is fixed into acetyl-CoA. Thus, the metabolic reconstruction based on the draft genome assembly provides a foundation for the functional genomics required to engineer *C. aceticum*. Also, we measured the frequency of actual members of a heterogeneous transposon mutant pool to determine the contribution of every essential and non-essential element in the *E. coli* genome under a given growth condition. This high-throughput insertion tracking method (Tn-seq) uses deep sequencing to accurately track the quantitative genetic interactions on genome-wide scale. Using this approach, we scanned *E. coli* transposon mutant libraries at 7 bp resolution, which is comprised of about 10^6 mutants generated using a derivative of the Tn5 transposon. We found hundreds of essential or strongly advantageous genes for growth in rich medium, which encode fundamental biological processes in DNA replication, transcription, and translation. Interestingly, we found two important groups of genomic elements. First, many of transposon insertions were found in non-coding genomic elements, including promoters, regulatory sequences, and function unknown intergenic sequences. Second, the transposon insertions were preferentially positioned according to the protein domains, which enable us to determine the essentiality or advantage of protein domains under a given physiological condition. This comprehensive genetic information will provide the foundation for designing and rewriting an artificial genome.

Symposium [S6]

Korea Traditional Fermented Food

Sponsored by Microbial Institute for Fermentation Industry

2014 International Meeting of
the Microbiological Society of Korea

S6-1

Caenorhabditis elegans Conditioning with The Probiotic Bacterium *Lactobacillus acidophilus* Strain A4 Enhances Longevity and Resistance to Foodborne Pathogen Infections

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Despites the immune response of *Caenorhabditis elegans*, surrogate *in vivo* host model, to pathogen infections is well-established, very limited information is discovered about the impacts of health promoting-probiotic bacteria on host responses [1]. Here we investigate on the potential probiotic activity of *Lactobacillus acidophilus* strain A4 that isolated from human [2] on *in vivo* host using *C. elegans* nematode model.

Initially, we determined on the survivals of *C. elegans* using solid-killing assay and examined the bacterial colonization in *C. elegans* gut by plate method and transmission electron microscopy (TEM). Our results showed that the probiotic *L. acidophilus* strain A4 is not harmful to *C. elegans* and that *L. acidophilus* strain A4 is remarkably capable to colonize the *C. elegans* intestine in both plate counting assay and TEM assay compared with normal feeding bacterium *Escherichia coli* OP50. Moreover, persistent *L. acidophilus* strain A4 in the nematode intestine strongly enhanced the resistance of nematodes exposed to *Staphylococcus aureus* as well as significantly prolonged the lifespan of nematodes.

In addition, we employed DNA microarray, quantitative real time-polymerase chain reaction (qRT-PCR) and transgenic worms for exploring health-promoting pathways via probiotic bacteria in *C. elegans*. Based on DNA microarray results, conditioning of *L. acidophilus* strain A4 stimulated the specific gene regulation of multiple receptors including nuclear hormone receptors (NHR) family. Importantly, *L. acidophilus* strain A4 activates key signaling pathways involved in *C. elegans* immunity, including the p38 mitogen-activated protein kinase pathway and the β-catenin signaling pathway [3].

In conclusion, we describe that probiotic conditioning with *L. acidophilus* strain A4 may positively stimulate the longevity and resistance to foodborne Pathogen infections in *C. elegans in vivo* host.

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S6-2

Indigenous Yeasts Isolated from Traditional Fermented Soy-Sauce Can Prevent Pathogenic Bacteria Occurred at Low-Salt Fermentation Process

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Lately, concern on overindulgence of salt intake has been increasing steadily all over the world as it can increase the risk of hypertension and be directly related to the development of cardiovascular disease. Although great demands to reduce salt content of soy-sauce has being increased recently, simple reducing the amount of added-salt can bring about their conservation problems by restraining the proliferation of putrefactive salt-resistant fermenting microbes, and influence of their texture and flavor profile of final products and then it may consequentially cause a falling-off in quality.

Until now, most outstanding attempts undertaken to reduce the salt content in soy sauce were by using osmotically equivalent substitutes to replace salt such as ethanol, sugars, and polyols. However, although proliferation of putrefactive microbes was prevented, it can restrain the growth of some beneficial microbes such as yeast and bring about changing its taste and flavor by osmotically equivalent substitutes addition directly. Thereby is unsuitable for commercially acceptable soy sauce. Thus, an alternative method for reducing the salt content of fermented soy sauce is desirable. It was known that most pathogenic and spoilage microorganisms are not ethanol tolerant, even at low concentrations, and particularly it demonstrated that using ethanol in combination with NaCl can effectively inhibit spoilage microorganisms. However, those will be also change original character of soy products even if addressing preservative problem by restraining the growth of some beneficial microbes such as yeast and some bacteria. Moreover it also can produce bad impression by chemical material and thus, an alternative method for reducing the salt content of fermented soy sauce is desirable.

Microbial control of the pathogenic bacteria at fermented foods usually has been done usually by addition of preservatives such as salt, sugar and other spices or physiological control of waters. Recently, however, in the fermentation process, adding competitive starter cultures is considered to be most appropriate tools for inhibiting and/or controlling the growth of food-borne pathogens and spoilage microbial, preventing the formation of undesirable end-products or achieving the desired fermentation parameters. In particular, recently many researchers are more interested in selecting autochthonous microorganisms as a starter formulation can effectively survive well and preserve the indigenous characters of fermented products. It has been previously reported that use of autochthonous mixed starters in carrots and French beans fermentation were more competitive than those with allochthonous starters from other source, attributing remarkably sensory feature especially, appreciated for fragrance as well as fermentability. In a traditional dry-fermented sausage, preserving the typical sensory characteristics of traditional sausages with improving safety was achieved by adding autochthonous starter. Therefore, adaptation of indigenous starter could be an effective strategy by having many advantages for control of fermentation process containing reduced salt content. However, no studies have employed autochthonous microbial approaches to handle reduction of the salt content in soy sauce fermentation

up to this date.

In soy sauce fermentation, salt is very important ingredient to preserve the food by restraining the proliferation of putrefactive microbes, control the growth of salt-resistant fermenting microbes, and then improves its organoleptic quality, nutritional value, food safety and shelf-life (Chiou et al., 1999; Liem et al., 2011). Thus, pinpointing a negative effect caused by reducing salt content in soy-sauce is crucial to resolve the problem as well as to select and exploit indigenous starter cultures for development of low salt soy sauce without altering the typicality of this fermented product.

Herein, change of physiological and microbiological properties in soy-sauce fermentation by different salt concentration was examined. With decreased salt concentration of the prepared soy-sauce, pH, acidity and ethanol content was slightly decreased, but residual sugar increased. Moreover, alcohols and various volatile compounds were noticeably lower and not detected compared to high salt concentrations. The bacterial analysis based on DGGE analysis showed *Bacillus* species was the most predominant bacterial group all through soy sauce fermentation without affecting salt concentration. However compared to *Bacillus* species, *Staphylococcus* and *Enterococcus* sp. were detected only during soy-sauce fermentation with low salt concentration of 8%. Two indigenous yeast strains, producing high alcohol and flavor, *Torulaspora delbrueckii* JBCC 623 and *Pichia guilliermondii* JBCC 848 were selected respectively and applied to control pathogenic bacteria and physicochemical change occurred in low salt process. When these strains were used as starter, the putrefactive microbe *Staphylococcus* was undetected and *Enterococcus* sp. considerably decreased despite low-salt concentration without affecting flavor profile patterns obtained from the soy-sauce fermented with high-salt concentration. Hence, this treatment offers a technological option to manufacture salt-reducing soy sauce, not giving rise to microbial and physiochemical changes via treating functional yeast culture.

S6-3

Changes in Transcriptional Level of Subtilisin-like Proteases of *Bacillus licheniformis* during Fermentation of Fast-fermented Soybean Paste

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Sunchang County has been well known as the main area for the production of traditionally fermented red pepper paste. To uphold its reputation on premier quality and taste of red pepper paste nationwide, we have tried to improve fermentation by screening *Bacillus* strains that can exhibit the antimicrobial activities against pathogens, degradation activities of harmful biogenic amines, and growth inhibition of film-forming yeasts grown in low-salted soybean products.

Recently we isolated a *Bacillus licheniformis* strain, which was named as SCD B34 and was selected for manufacturing premier red pepper pastes due to the excellent fermentation capability described above. For characteristic comparison of genes that can play crucial roles in fermentation of red pepper paste between the selected strains, we sequenced the genome of the strain SCD B34 and then annotated it. The draft genome sequence of the strain SCD B34 consists of 4.42 megabases, organized in chromosome with an average GC content of 45.65 %, which was similar to the reference strain *B. licheniformis* DSM13 (=ATCC 14580) or *B. licheniformis* SCK B11 isolated by our lab. A total of 4,789 genes have been identified including 69 tRNAs and 5 rRNA operons. As *Bacillus licheniformis* is an important producer of exoenzymes, we were interested in the types of peptidase and glycosidase, of which activities could play a central role in degrading most of soybean proteins and glutinous rice starch during fermentation. Genome of the strain SCD B34 contained 32 types of peptidase and 23 types of glycosidase, while total genes coding for peptidase and glycosidase in the same genome were 89 and 48, respectively. Among genes coding for peptidase, genes for subtilisin-like serine protease (AprE) were major one, which consisted of 11% of total peptidase genes. By analyzing sequence homology between these serine proteases, we classified AprEs into 11 types. Subtilisins (EC 3.4.21.62) including nattokinase belong to the second large family of serine proteases. We conducted RT-PCR for quantification of transcribed mRNAs of 5 different AprEs containing signal sequences for extracellular excretion. The quantification of resulting cDNA was normalized using two house-keeping genes. The transcriptional level of mRNA of each AprE at time interval during fermentation of fast-fermented soybean paste was different, suggesting that each AprE has different function during growth.

In this work, we took the first step into genomic and transcriptional analysis to evaluate the usefulness of a *Bacillus* strain for fermentation and to elucidate the transcriptional changes of proteases involved in fermentation of soybean products. We expect to find easy ways for selection of the commercially valuable strains *in silico* by using cumulative information obtained from molecular works during fermentation.

S6-4

Antimicrobial Activity of *Bacillus licheniformis* Isolated From Korean Traditional Food Sources against Porcine Enteropathogenic Bacteria

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Probiotics are living microorganisms that benefit their host by providing intestinal flora balance through higher levels of favorable activities. In particular, fermented soybean products with *Bacillus* spp. indigenous to Asian and African countries have long been considered traditional and nutritious foods. *Bacillus licheniformis* has for many years been used in the industrial production of enzymes, antibiotics and detergents. Even though various probiotics are used in the field of animal feed, some research was carried out characterization and antibacterial activity test against porcine enteropathogenic bacteria. Therefore, the aim of this study was to analyze and evaluate the antimicrobial activity of 63 *Bacillus licheniformis* isolated from Korean traditional food sources against porcine enteric pathogenic bacteria (*Escherichia coli K88, Salmonella cholerasuis* and *Clostridium perfringens* type C). A multi-locus sequence typing (MLST) analysis, based on the sequence of six house-keeping genes (*adk, ccpA, recF, rpoB, spo0A* and *sucC*) of 63 *B. licheniformis* strains was performed. The result of the MLST analysis supported previous findings of two different subgroups (lineages) within this species, named "A" and "B" Statistical analysis of the MLST data indicated a higher rate of recombination within group "A". Among the tested strains, 50 strains were defined as STs [ST26 (19 strains), ST3 (12 strains), ST2 (6 strains), ST14 (5 strains), ST9 (4 strains), ST24 (2 strains), ST 12 (1 strain) and ST 13 (1 strain)] and 13 strains were defined as new STs. The results of antibacterial activity, one strain of named SRCRM100160 demonstrated a high antimicrobial potency. Especially, *B. licheniformis* (ST14) were showed high antibacterial activity against porcine enteropathogenic bacteria. Our findings suggest that pre-conditioning with probiotic *B. licheniformis* isolated from Korean traditional food sources may protect against porcine enteric bacteria.

Keywords: *Bacillus licheniformis*, Korean traditional foods, pig, antimicrobial activity

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Symposium [S7]

Host-Fungal Pathogen Interactions

2014 International Meeting of
the Microbiological Society of Korea

S7-1

Development of System-wide Functional Analysis Platform for Pathogenicity Genes in The Rice Blast Fungus

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Null mutants generated by targeted gene replacement are frequently used to reveal function of the genes in fungi. However, targeted gene deletions may be difficult to obtain or it may not be applicable, such as in the case of redundant or lethal genes. Constitutive expression system could be an alternative to avoid these difficulties and to provide new platform in fungal functional genomics research. Here we developed a novel platform for functional analysis genes in *Magnaporthe oryzae* by constitutive expression under a strong promoter. Employing a binary vector (pGOF1), carrying *EF1β* promoter, we generated a total of 4,432 transformants by *Agrobacterium tumefaciens*-mediated transformation. We have analyzed a subset of 54 transformants that have the vector inserted in the promoter region of individual genes, at distances ranging from 44 to 1,479 bp. These transformants showed increased transcript levels of the genes that are found immediately adjacent to the vector, compared to those of wild type. Ten transformants showed higher levels of expression relative to the wild type not only in mycelial stage but also during infection-related development. Two transformants that T-DNA was inserted in the promotor regions of putative lethal genes, *MoRPT4* and *MoDBP5*, showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes encoding alpha-glucosidase and alpha-mannosidase. These transformants also showed decreased mycelial growth and pathogenicity, implying successful application of this platform in functional analysis of the genes. Our data also demonstrated that comparative phenotypic analysis under over-expression and suppression of gene expression could prove a highly efficient system for functional analysis of the genes. Our over-expressed transformants library would be a valuable resource for functional characterization of the redundant or lethal genes in *M. oryzae* and this system may be applicable in other fungi.

S7-2

Population Structure of the Plant Pathogenic Fungus *Fusarium graminearum* in Korea

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Fusarium graminearum (Teleomorph *Gibberella zeae*) is an important fungal pathogen of cereal crops and produces mycotoxins, such as the trichothecenes nivalenol and deoxynivalenol. This species may be subdivided into a series of genetic lineages or phylogenetic species. We identified strains of *F. graminearum* from rice and maize fields in Korea to lineage, tested their ability to produce nivalenol and deoxynivalenol, and determined the genetic composition and structure of the populations from which they were recovered. Based on amplified fragment length polymorphism (AFLP), PCR genotyping, and chemical analyses of trichothecenes, all 249 isolates from rice fields in southern provinces belonged to lineage 6, with 241 having the nivalenol genotype and eight having the deoxynivalenol genotype. In rice fields of the eastern Korea province, we recovered 84 lineage 6 isolates with the nivalenol genotype and 23 lineage 7 isolates with the deoxynivalenol genotype. Amongst 333 lineage 6 isolates, 36% of the AFLP bands were polymorphic, and there were 270 multilocus haplotypes. Genetic identity among populations was high (> 0.972) and genotype diversity was low (30 to 58%). Out of 568 isolates of *F. graminearum* collected from maize at eight locations in Korea, lineage 7 was the most common (75%), followed by lineage 6 (12%), lineage 3 (12%) and lineage 2 (1%). The genetic identity among populations was high (> 0.98) and the effective migration rate between locations was higher than that between lineages. Female fertility varied by lineage: all lineage 7 isolates were fertile while 70%, 26%, and 14% of the isolates in lineages 6, 3 and 2, respectively, were fertile. All lineage 3 and lineage 7 isolates produced deoxynivalenol, whereas most lineage 2 and 6 isolates produced nivalenol. Genotypic diversity in lineage 3 and lineage 6 populations is similar to that found in rice populations, but genotypic diversity in lineage 7 is much lower even though similar levels of gene flow occur between lineage 7 populations, suggesting that lineage 7 is a relatively recently introduction to Korea, perhaps accompanying imported maize seeds. To test the adaptation of lineage 6 to rice, conidial mixtures of strains from lineages 3, 6 and 7 were inoculated on rice plants and then recovered from the rice grains produced. Strains representing lineages 6 and 7 were recovered from inoculated spikelets at similar frequencies that were much higher than those for the strain representing lineage 3. Abundant perithecia were produced on rice straw, and 247 single-ascospore isolates were recovered from 247 perithecia. Perithecia representing lineage 6 (87%) were the most common followed by those representing lineage 7 (13%), with perithecia representing lineage 3 not detected. These results suggest that *F. graminearum* lineage 6 may have a host preference for rice and that it may be more fit in a rice agroecosystem than are the other lineages present in Korea.

S7-3

Copper Homeostasis as a Virulence Factor in Systemic Infection by The Human Fungal Pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is a human fungal pathogen that is the causative agent of lethal meningitis in immunocompromised hosts as a consequence of HIV/AIDS, cancer chemotherapy, diabetes, and maintenance on immunosuppressants due to organ transplants or other pathologies. Copper (Cu) is an important metal to *C. neoformans*, as it is involved in processes that include, among others, respiration, iron acquisition, melanin formation, mating and superoxide dismutase activity. Previous reports suggest that the *C. neoformans* Cu-responsive transcription factor, Cuf1, contributes to virulence but the precise mechanisms for this are unclear. Cuf1 homologues in other fungi are known to activate expression of genes involved in Cu acquisition. We identified all of the genes induced either by Cu deficiency or excess in *C. neoformans*, including the previously known Ctr4 Cu importer, a new Cu importer, Ctr1, two metallothionein genes (MT1, MT2) and others. Surprisingly, both Cu inducible and Cu repressible genes are dependent on Cuf1 for their metalloregulation. To decipher which Cuf1 target genes contribute to virulence we generated Cuf1 target gene deletions and assayed survival to intra-nasal administration in mouse models of infection. Our studies will elucidate the contributions of the *C. neoformans* Cu homeostasis machinery to virulence and will provide a set of tools to investigate how *C. neoformans* and mammalian hosts do battle over Cu.

S7-4

Candida Infection and Antifungal Drug Resistance

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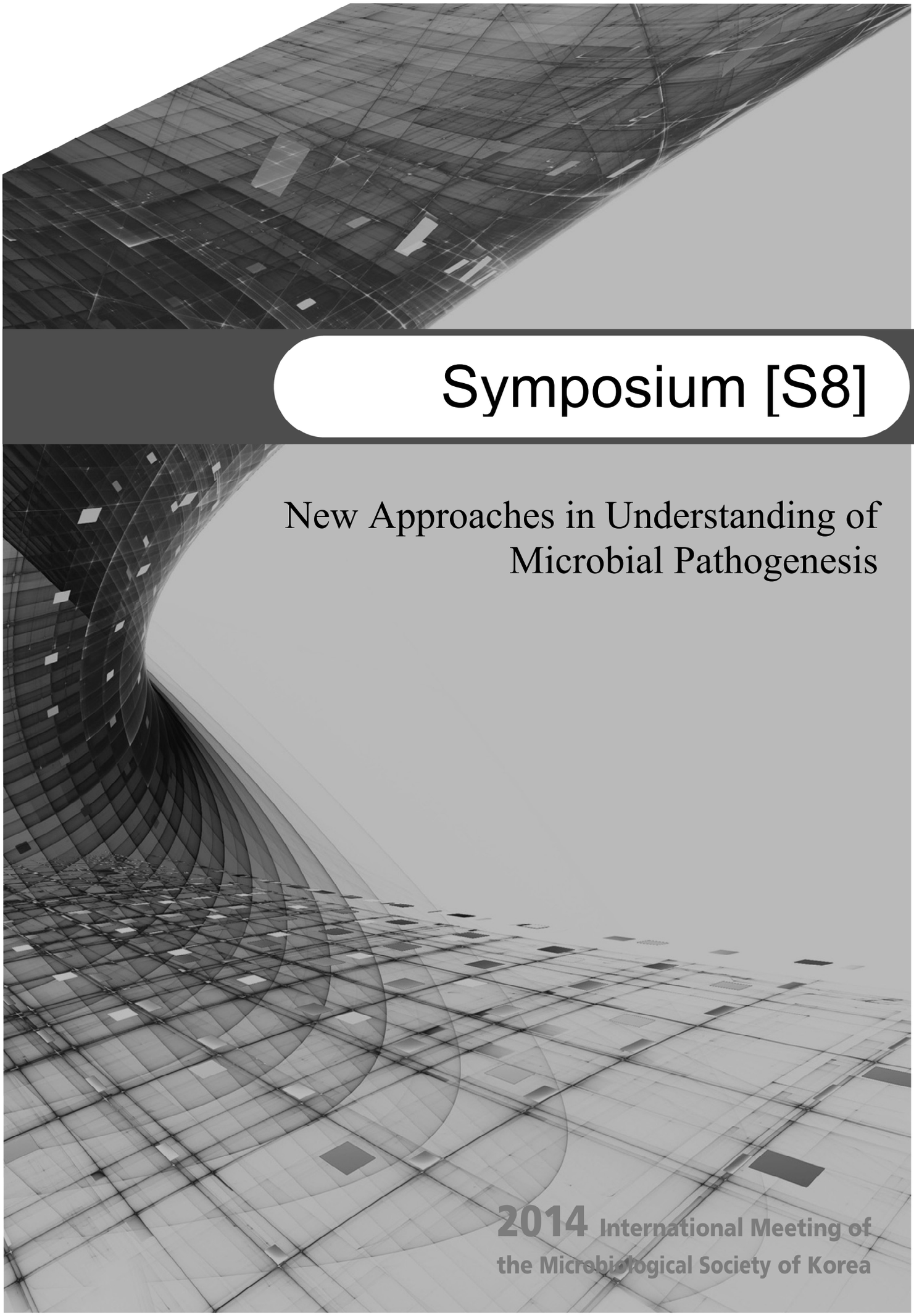
For the past two decades, hospitals have been experiencing increasing problems with nosocomial fungal infections. *Candida* species, the most common human fungal pathogens, ranks as the fourth-greatest cause of nosocomial bloodstream infections, with up to 40% mortality in epidemiological studies. *Candida* species colonize asymptomatically in around 30 to 50% of individuals in a population at any given time, but under conditions when the host defense of the individuals is weakened, they can cause both mucosal and systemic infections.

Although more than 100 species of *Candida* have been identified, fewer than 20 species have been implicated in nosocomial infections. *C. albicans* is the species most commonly isolated from clinical specimen and accounts for 40-70% of cases of invasive candidiasis. The second and third most frequently isolated species of *Candida* causing nosocomial candidiasis are dependent upon the age of patient and the geographic location of the hospital.

Both the frequency of invasive fungal infections and resistance to antifungal therapy continue to increase despite the introduction of new antifungal agents. In vitro antifungal susceptibility testing is now standardized internationally and is becoming essential in patient management and resistance surveillance. Although in vitro susceptibility testing is often used to select antimicrobial agents likely to be clinically active for a given infection, perhaps its most important function is the detection of resistance.

Antifungal resistance can be defined as microbiologic or clinical resistance. Microbiological resistance refers to nonsusceptibility of a fungus to an antifungal agent by in vitro susceptibility testing, in which the minimal inhibitory concentration (MIC) of the drug exceeds the susceptibility breakpoint for that organism. Microbiological resistance can be primary (intrinsic) or secondary (acquired). Primary resistance is found naturally among certain fungi without prior exposure to the drug and emphasizes the importance of identification of fungal species from clinical specimens. Secondary resistance develops among previously susceptible strains after exposure to the antifungal agent and is usually dependent on altered gene expression. Clinical resistance is defined as the failure to eradicate a fungal infection despite the administration of an antifungal agent with in vitro activity against the organism. Such failures can be attributed to a combination of factors related to the host, the antifungal agent, or the pathogen.

Antifungal drug resistance is a prominent feature in the management of invasive candidiasis. Fortunately, unlike bacteria, there are no described drug resistance plasmids or transposons to amplify antifungal resistance.



Symposium [S8]

New Approaches in Understanding of Microbial Pathogenesis

2014 International Meeting of the Microbiological Society of Korea

S8-1

Investigating *Salmonella* Pathogenesis for The Development of Targeted Intervention Strategies

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Antibiotics are important for both human and animal health but occasionally the use of antibiotics can have unintended consequences. For example, our research group demonstrated that *in vitro* exposure of multidrug-resistant *Salmonella enterica* serovar Typhimurium DT193 to the antibiotic tetracycline induces premature cellular invasion during logarithmic growth phase. Furthermore, the agricultural antibiotic carbadox was recently shown by our research group to induce phage-mediated gene transfer in multidrug-resistant *Salmonella* Typhimurium DT104 and DT120, including generalized transduction of multiple antibiotic resistance genes. The potential collateral effects of antibiotic usage illustrate that alternatives to antibiotics for animal production management should be developed and employed when possible.

Microbial interventions to reduce *Salmonella* colonization and disease in food-producing animals are needed to enhance animal health and improve food safety while alleviating the demand for antibiotic usage during animal production. *Salmonella* colonization and pathogenesis in the animal host is a complex interaction between multiple factors including host genetics and immunity, the competitive microbial flora, and virulence and survival mechanisms of the pathogen. Therefore, an effective reduction of *Salmonella* colonization and pathogenesis in food-producing animals will likely require the simultaneous use of several intervention strategies. Our *Salmonella* research program has taken a multi-faceted approach with investigations of the swine gastrointestinal microbiota, a porcine immune modulator, and a live, attenuated *Salmonella* vaccine.

Analysis of the porcine microbiota in swine prior to and following experimental inoculation of *Salmonella* Typhimurium indicates that the microbial community of the swine gastrointestinal tract influences pathogen colonization and fecal shedding. Prior to *S.* Typhimurium inoculation a significant difference was seen comparing the microbiota of swine that "will" shed low levels of *S.* Typhimurium [Low-shedder (LS)] compared to swine that "will" shed high levels of the pathogen [High-shedder (HS)] following inoculation. Two-days following inoculation, the presence of *S.* Typhimurium induced significant changes in the microbiota of HS pigs but not LS pigs. These results suggest that the composition of the gastrointestinal microbiota may impact the colonization potential of *Salmonella*, either antagonistically or synergistically.

Administration of the immunomodulatory cytokine Granulocyte-colony stimulating factor (G-CSF) enhances the porcine immune response and reduces *S.* Typhimurium colonization and shedding in swine. The G-CSF cytokine is involved in the production, differentiation and function of granulocytes that fight infectious disease agents such as viruses and bacteria. Swine injected with a replication-defective adenovirus (Ad5) encoding porcine G-CSF, four days prior to *S.* Typhimurium inoculation, had significantly reduced fecal shedding of *S.* Typhimurium at 2 and 3 days post-challenge compared to pigs that received the adenovirus vector alone. This study reveals that modulation of the immune response with biotherapeutic proteins, such as G-CSF, may hold potential to enhance animal health and improve food safety, especially during typical periods of stress and pathogen exposure (weaning, transportation).

Development and evaluation of a live, attenuated *S.* Typhimurium vaccine strain induces cross-protection against *Salmonella* serovars and can be used to differentiate infected from vaccinated animals (DIVA). Vaccination of swine with the rationally designed *S.* Typhimurium strain significantly reduced disease severity, fecal shedding, and tissue colonization following virulent *S.* Typhimurium challenge. Furthermore, vaccine administration was cross-protective against virulent *S.* Choleraesuis as disease severity, recovery of bacteria from systemic sites, and tissue colonization was significantly reduced. These experiments demonstrate that the *S.* Typhimurium vaccine can be given to food-producing animals to decrease *Salmonella* colonization, transmission, and clinical disease.

Our research program investigates the complex relationship between the host, the pathogen, and the microbial flora for the development of pathogen interventions. Our ultimate goal is to develop a suite of complementary intervention strategies as alternatives to antibiotics that will decrease the incidence of *Salmonella* in food-producing animals and prevent *Salmonella* outbreaks in humans.

S8-2

Structural and Biophysical Insights into Flagellin-mediated Activation of TLR5 Signaling

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When pathogenic flagellated bacteria infect the host, the bacterial flagellin protein is recognized as a pathogen-associated molecular pattern by an innate immune receptor, Toll-like receptor 5 (TLR5), which activates potent immune responses against pathogens. Flagellin contains at least two conserved domains (D0 and D1) that are required for formation of the flagellar filament in bacteria and for TLR5 activation in a host. To enhance our understanding of the flagellin-mediated TLR5 activation, we have analyzed the TLR5-flagellin interaction and signaling using diverse flagellins through biophysical binding assays, X-ray crystallography, and cell-based assays. Our structural studies on TLR5 and *Salmonella* and *Bacillus* flagellins revealed that TLR5 makes contacts with three long α-helices of the flagellin D1 domain through 'primary binding' and 'secondary homodimerization' interfaces. In addition, the D1 domain, irrespective of bacterial species, exhibited high binding affinity for TLR5 and exerted similar TLR5 signaling activities. Interestingly, although the flagellin D0 domain did not contribute to the TLR5 binding energy, the D0 domain substantially enhanced TLR5 signaling activity. Our extensive studies on the TLR5-flagellin interaction would provide deeper insights into the design of flagellin vaccines and anti-radiation therapeutics.

S8-3

FoxP3+ Tregs, PD-1 and CTLA4: Negative Immune Regulatory Pathways in Patients with Chronic HIV and/or HCV Infection

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FoxP3+ CD4+ regulatory T cells (FoxP3+ Tregs) play a key role in mediating immune tolerance to self and non-self antigens, including viral pathogens. Programmed death-1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4) are receptors in CD28 family of costimulatory molecules, induced by T cell activation but also providing inhibitory signals to T cells. There are further cross-talks between Tregs and T cell costimulatory pathways.

HCV persists with increased circulating CD4+FoxP3+ Tregs and increased PD-1 and/or CTLA-4 expression on intrahepatic virus-specific T cells. HIV persists with dysfunctional antiviral effector T cells with increased PD-1 and/or CTLA-4 expression. Importantly, HCV/HIV coinfected patients display increased liver disease progression and mortality, suggesting that HIV-associated immune dysregulation with the loss of CD4 T-cells may disturb the balance between immune regulatory and effector mechanisms. FoxP3+ Tregs from HCV/HIV coinfected patients has recently reported to have increased level of expression comparted to FoxP3+ Tregs from HCV monoinfected patients. However, the nature of FoxP3+ Tregs from HCV/HIV coinfected patients has not been characterized in terms of phenotype, function and relationship with other inhibitory receptors (PD-1, CTLA-4). In this study, we hypothesized that HIV-associated loss of CD4 T cells may differentially affect FoxP3+ Tregs and T cell expression of various costimulatory receptors that may further influence HIV and/or HCV pathogenesis.

Peripheral blood mononuclear cells (PBMC) from HCV monoinfected (HCVmono), HIV monoinfected (HIVmono) and HCV/HIV co-infected (HCV/HIVco) patients were examined for FoxP3+ Treg frequency and expression of co-inhibitory receptor Programmed Death-1(PD1) and cytotoxic T-lymphocyte antigen 4 (CTLA4) on T-cell subsets by flow cytometry. MHC/peptide tetramers were used to detect virus-specific effector CD8 T-cells.

Here, we report that CD4 T cells from HCV/HIV coinfected patients are significantly enriched for FoxP3 expression compared to CD4 T cells from HIV or HCV monoinfected patients. FoxP3 expression on CD4 T cells also correlate with their PD-1 and CTLA-4 expression and CD4 T cells from HCV/HIV-coinfected patients displayed significantly increased PD-1 and CTLA-4 expression than other groups. Induction of FoxP3, PD-1 and CTLA-4 expression on CD4 T cells showed a significant inverse association with the overall circulating CD4 T cell frequency. Collectively, our study suggests that FoxP3+ T regs in HCV/HIV coinfected patients contributes to effector T cell inhibition. Contrary to our hypothesis, our findings show that Tregs are in fact preserved in HCV/HIV-coinfection in direct association with PD-1 and CTLA-4 pathways but not with clinical parameters of liver disease or the level of viremia. We also find that these immune regulatory pathways are tightly linked to the overall CD4 T cell frequency, suggesting a global homeostatic mechanism towards immune regulation with absolute CD4 T cell loss.

S8-4

Tumor Cell Modulation by Mucosa-associated *Escherichia coli* as an Internal Exposome via Macrophage Inhibitory Cytokine 1

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Commensal bacterial community shifts in the pathogenic colonic environment and chronic colonization of mucosa-associated *Escherichia coli* (MAEC) has been linked to colonic tumourigenesis. Enteropathogenic *Escherichia coli* (EPEC) is one of commonly identified MAEC in colorectal cancer patients. The aim of this study is to address the contribution of MAEC colonization to human carcinogenesis. EPEC infection of cancer cell caused alterations in affect locomotion-related behaviors of cancer cell including detachment, migration, cytoskeleton rearrangement, dissemination and survival via induction of macrophage inhibitory cytokine 1 (MIC-1). Mechanistically, MIC-1 promoted NF-kappaB, or RhoA GTPase which mediated survival and activated inflammatory stimulation in the cancer cells. In terms of signaling pathway, MIC-1 triggered TGF-beta-activated kinase 1, which enhanced expression of RhoA GTPase. Conclusively, mucosal EPEC enhanced MIC-1 gene expression in the human intestinal cancer cells, which was associated with enhanced tumor cell resistance to anoikis and subsequent survival via enhanced TAK-1 and RhoA GTPase. [This work was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by Ministry of Education, Science, and Technology Grant 2012R1A1A2005837].

Symposium [S9]

Novel Concepts in Gene Expression System

2014 International Meeting of
the Microbiological Society of Korea

S9-1

Proof of Concept Trials for Functional Overexpression and High Throughput Assay of Proteins

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Cells innately control gene expression using a variety of cis- and trans-acting elements such as DNA, RNA, protein and DNA-modifying regulators. It was also recognized well that interactions between these elements were logically programed. Thus, a lot of attempts have tried to understand the principle and logic of the genetically installed program on genome for gene expression. Any of these findings could have a revolutionary impact on biotechnology, such as designing module to express the proteins of interest in a controllable manner, programing cells to sense and respond to signals from internal or external environment for gene expression, or engineering factory cells that can overproduce the proteins of interest as functional state in soluble fractions. However, building synthetic module, based on the innate principle and logic of cells for gene expression, remains one of the greatest challenges in the field, where even simple module is labor intensive to build and lacks the performance of its natural counterparts.

As described above, the research field in gene expression has experienced many challenges in discovering and developing new tools for functional overexpression of proteins (especially medical and diagnostic proteins), including efficient activity analysis methods with target-oriented approaches in a high-throughput manner. In response, a lot of novel concepts and trials are reported to be working to pave a novel route for the development of practical application process. In line with this, we present here two novel approaches that describe and provide evidences for functional overexpression of proteins in soluble fraction by synthesized ramp-tag, and one approach that has been described a rapid and sensitive assay method for the detection of a cofactor NADPH using a dose-dependent fluorescent protein mBFP. The principle of strategy for the later approach can be applied to screen NADP(H)-dependent enzymes with promise but weak activity *in-vitro* and also to detect the level of NADPH or metabolic conversion of cells or *in-vivo*. The validation processes are currently being undertaken to evaluate the performance of our approaches for practical application.

S9-2

Incorporation of Unnatural Amino Acids into Proteins in *Escherichia coli* and Their Applications

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The expansion and engineering of genetic code is an emerging field in chemical biology. The ability to genetically encode unnatural amino acids beyond the common 20 into proteins has allowed unprecedented control over the chemical structures of recombinantly expressed proteins and new dimensions to protein engineering. Now days, unnatural amino acid incorporation into proteins has become an indispensible tool for biologists, for generating proteins with novel functionalities. Moreover, this method is greatly beneficial to understand the structure–function relationship of proteins. In this seminar, I will present our recent efforts to enhance the biophysical properties of functional proteins through *in vivo* incorporation of unnatural amino acids.

S9-3

New Strategy for Enhancing Heterologous Protein Expression through the Alternating N-terminal Codons

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Many recombinant proteins/enzymes have been successfully produced using *Escherichia coli*. However, in many cases, target proteins/enzymes are not functionally overexpressed, and form inactive inclusion bodies in *E. coli*. Thus, complex and expensive processes of denaturation and refolding or altering promoter are required to obtain biologically active overexpressed soluble form. In this study, we developed a novel strategy for enhancing functional expression of heterologous proteins through replacing the first ten variable codons with synonymous codons. The alternation of synonymous codons was induced by PCR with forward primers with wobble letters at third position in codon and a reverse primer. Resulting codon adjustment mutants were directly inserted upstream mcherry without linkers. Following transformation and cultivation, colonies with the red fluorescence of mcherry were selected and assayed. With exocellulase screened from metagenome as a model protein, we increased the functional expression level by 155 fold or 407 fold with or without fusion of mcherry at C-terminal region, respectively. Surprisingly, the stability of mRNA secondary structure of 5' untranslated region and the first 33 nucleotides in codon variants was not correlated with functional expression level. This strategy, requiring small library in size without using complex bioinformatics tools, is effective in enhancing functional expression without changing primary structure of a target protein.

S9-4

Strategy to Overexpress a Large Biosynthetic Gene Cluster in *Streptomyces* Species

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Functional expression of an entire secondary metabolite biosynthetic pathway gene cluster is an attractive alternative to facilitate production improvement and biosynthetic modification of a potentially-valuable natural product derived from various genetically-recalcitrant *Streptomyces* species. Previously, a versatile *Escherichia coli-Streptomyces* shuttle Bacterial Artificial Chromosomal (BAC) conjugation vector, pSBAC was successfully used for heterologous expression of a meridamycin biosynthesis gene cluster in *S. lividans*.

Using pSBAC-driven genome engineering techniques as well as PCR-targeted gene manipulation, here we show the both homologous and heterologous expressions of an entire biosynthetic pathway gene cluster of tautomycetin (TMC), a protein phosphatase PP1/PP2A inhibitor and T-cell-specific immunosuppressant.

The recombinant pSBAC construct containing the entire TMC cluster in *E. coli* was conjugated into the model *Streptomyces* strains such as *S. lividans* or *S. coelicolor*, resulting in the fast and enhanced TMC production. Moreover, re-introduction of the TMC cluster-containing pSBAC into the wild-type *Streptomyces* sp. CK4412 resulted in a tandem repeat of an entire TMC cluster in the chromosome with 50-fold increased TMC productivities. The strategy described here may set the stage for the custom-optimized over-expression scheme for the metabolite pathway cluster present in the actinomycetes.

Symposium [S10]

Structural Microbiology and Pathogenesis

Co-organized by Systems & Synthetic Agrobiotech Center

2014 International Meeting of
the Microbiological Society of Korea

S10-1

Crystal Structures of Bifunctional Penicillin-Binding Protein 4 from *Listeria monocytogenes*

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Penicillin-binding proteins (PBPs), which catalyze the biosynthesis of the peptidoglycan chain of the bacterial cell wall, are the major molecular target of bacterial antibiotics. Here, we present the crystal structures of the bifunctional peptidoglycan glycosyltransferase (GT)/transpeptidase (TP) PBP4 from Listeria monocytogenes in the apo-form and covalently linked to two β-lactam antibiotics, ampicillin and carbenicillin. The orientation of the TP domain with respect to the GT domain is distinct from that observed in the previously reported structures of bifunctional PBPs, suggesting interdomain flexibility. In this structure, the active site of the GT domain is occluded by the close apposition of the linker domain, which supports the hypothesis that interdomain flexibility is related to the regulation of GT activity. The acylated structures reveal the mode of action of β-lactam antibiotics toward the class A PBP4 from the human pathogen L. monocytogenes. Ampicillin and carbenicillin can access the active site and be acylated without requiring a structural rearrangement. In addition, the active site of the TP domain in the apo-form is occupied by the tartrate molecule via extensive hydrogen bond interactions with the catalytically important residues; thus, derivatives of the tartrate molecule may be useful in the search for new antibiotics to inhibit PBPs.

S10-2

Structural Basis for The Recognition of Peptidoglycan Tripeptide by *Helicobacter pylori* Csd4, a D,L-carboxypeptidase Controlling The Helical Cell Shape

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Helicobacter pylori infection causes a variety of gastrointestinal diseases including peptic ulcers and gastric cancer. The colonization of this bacterium in the gastric mucosa is required for the survival in the stomach. Its colonization of the gastric mucosa of human stomach depends on its motility, which is facilitated by the helical cell shape. In *H. pylori*, peptidoglycan crosslinking relaxation promotes the helical shape. Among several cell shape-determining peptidoglycan hydrolases identified in *H. pylori*, Csd4 is a Zn^{2+}-dependent D,L-carboxypeptidase that cleaves the bond between the γ-D-Glu and mDAP of the uncrosslinked tripeptide of peptidoglycan (L-Ala-γ-D-Glu-mDAP) to produce L-Ala-γ-D-Glu dipeptide and mDAP, affecting the helical cell shape. Inhibition of D,L-carboxypeptidase activity of Csd4 may represent a novel therapeutic approach. We report here the crystal structures of *H. pylori* Csd4 (HP1075) in three different states: the ligand-free form, the substrate-bound form, and the product-bound form. *H. pylori* Csd4 consists of three domains: an N-terminal D,L-carboxypeptidase domain, a novel β-barrel domain, and a C-terminal immunoglobulin-like domain. The D,L-carboxypeptidase domain exhibited typical carboxypeptidase folds and provided structural basis of peptidoglycan recognition by D,L-carboxypeptidase. *H. pylori* Csd4 recognizes primarily the terminal mDAP of the tripeptide substrate and undergoes a significant structural change upon binding either mDAP or mDAP-containing tripeptide.

S10-3

Structure and Function of Fungal Zn Finger Transcription Factor in Sterol Homeostasis and Antifungal Resistance

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Transcriptional regulation of ergosterol biosynthesis in fungi is crucial for sterol homeostasis and for resistance to azole drugs. Due to the essential role of ergosterol in fungal membranes, azole drugs that inhibit ergosterol biosynthesis are widely used for the treatment of fungal infections. In *Saccharomyces cerevisiae*, the Upc2 transcription factor activates the expression of related genes in response to sterol depletion by poorly understood mechanisms. Upc2 contains a N-terminal DNA-binding domain characterized by a conserved zinc finger motif and a C-terminal conserved domain of unknown function. In this study, 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. The C-terminal activation loop is essential for ligand binding and for transcriptional regulation. Upc2 displays a distinct structural fold of ligand binding domain but shares a common regulatory mechanism with metazoan nuclear receptors.

Our findings highlight Upc2 as a novel target for the developments of anti-fungal therapeutics. The deletion of Upc2 leads to anaerobic inviability and high susceptibility to azole antifungals in *S. cerevisiae* and in *Candida albicans.* Therefore, 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. Upc2 LBD displays a novel fold of ligand binding domain and a deep hydrophobic pocket that could serve as an excellent pharmacopore for the design of small molecule inhibitors. In addition, Upc2 is specific to fungal sterols and has no affinities to other types of sterols providing key information for ligand based drug design. Thus, the discovery of the Upc2 transcription factor as a fungal nuclear receptor opens the way for the development of new anti-microbial agents.

S10-4

Dxo1, a Novel Eukaryotic Enzyme with Both Decapping and 5'-3' Exoribonuclease Activity

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The 5'-ends of messenger RNA precursors are rapidly capped during transcription in eukaryotes, and it was generally believed that 5'-end capping always proceeds to completion. However, recent studies showed that yeast protein Rai1 functions in a quality control mechanism to clear cells of incompletely 5' end-capped messenger RNAs (mRNAs). Rai1 possesses a novel decapping activity that can remove the entire cap structure dinucleotide from an mRNA. This activity is targeted preferentially towards mRNAs with unmethylated caps in contrast to the canonical decapping enzyme, Dcp2, which targets mRNAs with a methylated cap. In addition, Rai1 also has RNA pyrophosphohydrolase (PPH) activity that turns a 5'-end triphosphate to monophosphate RNA. Therefore, Rai1 is involved in a novel RNA 5'-end capping quality checkpoint, and is required for the degradation of RNAs with defective caps, especially under nutritional stress.

S. cerevisiae and several other fungal species contain a weak sequence homolog of Rai1, with the systematic name Ydr370C, although little was known about this protein. Here we report the crystal structure of fungal species *Kluyveromyces lactis* Ydr370c at 2.4 Å resolution. The overall structure of Ydr370c is similar to Rai1, but Ydr370C also contains unique features in the active site. It is shown that Ydr370C has a structural similarity to D-(D/E)XK nucleases such as EBV alkaline nuclease, RecE exonucleases and λ exonuclease. Based on the three dimensional structure, we perform biochemical and functional studies on this protein. Unlike Rai1, Ydr370C has robust decapping activity on RNAs with both methylated and unmethylated caps, but it has no detectable PPH activity. Unexpectedly, our assays also demonstrate 5'-3' exonuclease activity for Ydr370C, distinct from Rai1. We propose the name Dxo1 for this novel eukaryotic enzyme with both decapping and exonuclease activities. Studies of yeast in which both Dxo1 and Rai1 are disrupted reveal that mRNAs with incomplete caps are produced even under normal growth conditions, in sharp contrast to current understanding of the capping process.

Symposium [S11]

Molecular Epidemiology and Control of Enteric Virus

2014 International Meeting of the Microbiological Society of Korea

S11-1

Management Strategy of Norovirus in South Korea

In-Sun Joo

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Since the mass outbreak of food poisoning among school children caused by norovirus in 2006, Ministry of Food and Drug Safety(MFDS) has established and operated various management measures through the cooperation of different governmental agencies to prevent food poisoning outbreak beforehand.

Through this presentation, I will introduce some of the prevention strategies that can be used to reduce norovirus food poisoning in South Korea. I will also talk about the occurrence cases of the latest food poisoning outbreak due to norovirus, and future research directions.

In order to monitor and to investigate the cause of norovirus food poisoning, MFDS has been conducting research & development projects such as research on detection method of norovirus in shellfish, vegetables, fruits and drinking water(underground water). The established methods get included in the guideline for the investigation of food poisoning and utilized to determine the causative agent.

Furthermore, since 2009, MFDS has been trying to prevent food poisoning caused by norovirus-contaminated ground water. To achieve this, we have extended the scope of food service institutions to include schools, youth training facilities, social welfare facilities, military bases where underground water is used for drinking and cooking and conducted inspection of norovirus contamination in these facilities. The facilities where the norovirus is detected from the underground water were subjected to eduction for food poisoning prevention, disinfection and sanitation of the facilities. The first year of conducting this norovirus safety management project in 2009, the detection rate of norovirus was 3.1% while it was decreased to 0.8% after 5 years continuously conducting the survey and it can be considered that this is resulted from the effectiveness of the prevention management. Since there has been major norovirus food poisoning outbreaks due to products made using underground water being distributed to food service institutions in the past 2 years. Unheated products produced by using underground water has become a main focus of the management in 2013.

Once the food poisoning outbreak occurs, it is important to prevent the outbreak from spreading through rapid investigation of causative agent. The final causative agent can be determined by investigating the consumed food products based on the causative pathogen identified in patients and once the product in concern is singled out, underground water, food producing facility and surrounding environment are investigated. The food poisoning outbreaks occurred in the past 2 years were caused by contaminated underground water or seawater and it has been confirmed that the identical norovirus genotype was found in underground water and seawater used for food production, food product and in patients.

MFDS organized a project group for food poisoning prevention research to investigate the causative agent. The main research areas are to prevent by causative agent such as seawater surrounding shellfish farm, inflow from inland fish farm, underground water and agricultural water. We can also develop on-site detection kits which are easily available to improve the sanitary control ability of the companies and conduct research on complex technology for reduction by assessing and developing disinfectants. Furthermore, MFDS will conduct infectious disease-animal model research as a challenging base study to achieve not only domestic food poisoning safety management, but to strengthen its global positioning through international cooperative researches.

S11-2

Public Health Impact of Human Noroviruses

Jan Vinjé

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Noroviruses are the leading cause of outbreaks and sporadic cases of acute gastroenteritis in humans worldwide. In a recent study in the United States, approximately 16% of all norovirus outbreaks are reported to be caused by contaminated foods. These highly infectious viruses usually cause self-limited disease in persons of all ages but the young, elderly and sick may suffer more severe consequences of illness such as dehydration requiring medical attention. In closed settings such as nursing homes, cruise ships, schools, and the military, norovirus often is transmitted person-to-person resulting in high attack rates and large outbreaks. Food may become contaminated with norovirus at the source (i.e., irrigation, shellfish) or at point of service (e.g. infected foodhandler). Over the past two decades, this appreciation of the major and expanded burden of norovirus and sapovirus disease has been brought about by laboratory advances in the molecular detection of these viruses and their genetic characterization. Harmonization of typing methods for norovirus allows for more standardized surveillance through electronic networks such as CaliciNet and NoroNet). Worldwide, the majority of norovirus outbreaks are caused by genogroup (G) II, genotype 4 (GII.4) viruses, which were first recognized as a pandemic strain in the mid-1990's, followed by several new emerging GII.4 variants over the past 15 years. However, recent data indicate that non-GII.4 viruses are more likely to be associated with foodborne exposure whereas GI viruses are frequently detected in waterborne outbreaks, supporting the need for rapid typing as person to person transmission and foodborne exposures require different intervention measures. Recent data from human volunteer studies as well as from natural outbreaks show that host genetics, such as secretor status, plays an important role in susceptibility of individuals to norovirus infection. Histoblood group antigens act as attachment factors required for infection by noroviruses but their polymorphism contributes to restriction of the transmission of any given strain. Control efforts focus on hand hygiene, isolation of ill people, and environmental disinfection. An effective norovirus vaccine in under development and could play a pivotal role to control norovirus disease burden in certain target groups such as the elderly, healthcare workers and the military although several significant challenges remain to be solved. Therefore, surveillance systems such as CaliciNet will be critical to monitor how the virus will change over time. This presentation will cover the most current knowledge on virus taxonomy, detection, surveillance, and prevention of human noroviruses.

S11-3

Norovirus: The Main Target for Food Safety and Control

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Recently, the research on the norovirus (NoV) has been receiving great attention due to the importance of public health worldwide. NoV is highly infectious, resistant to various environmental stresses and has low infectious dose. However, NoV has been greatly understudied worldwide due to inability to cultivate in conventional cell culture techniques. Recently, Korean Food and Drug Administration (KFDA) developed more than 5 million US dollars initiative program, so called NoroTECL (TEam for ControL of Noroviral Foodborne Outbreaks), for preventing the outbreaks caused by norovirus in South Korea. This initiative program includes the surveillance of seafood and agricultural foods, the identification of source of viral contamination in major food producing area, development of novel diagnostic and control techniques, and development of tools for noroviral research. In this presentation, some examples of prompt diagnosis and control of NoV would be presented.

S11-4

Antiviral Activity and Its Mechanism of Ginsenoisdes against Norovirus Surrogates

Changsun Choi

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Korean red ginseng has been studied various biological activities such as immune, anti-oxidative, anti-microbial, and anti-cancer activities but antiviral activity and its mechanism were not elucidated. This study aimed to investigate the antiviral effects and its mechanism of Korea red ginseng extract and ginsenosides on norovirus surrogate, including murine norovirus (MNV) and feline calicivirus (FCV). The pre-, co-, and post-treatment effects of Korean red ginseng (KRG), ginsenosides Rb1 and Rg1 was measured. To measure the antiviral effect and cytotoxicity of Korean red ginseng extract, and ginsenosides Rb1 and Rg1, we treated Crandell-Reese Feline Kidney (CRFK) for FCV or RAW264.7 cells for MNV with concentrations of 0, 5, 6.7, 10, 20 μg/ml total saponin. There was cytotoxic effect in the highest concentration 20 μg/ml of KRG extract so this concentration was excluded in this study. The FCV titer was significantly reduced to 0.23-0.83 log 50% tissue culture infectious dose ($TCID_{50}$)/mL in groups pre-treated with red ginseng extract or ginsenosides. The titer of MNV was significantly reduced to 0.37-1.48 log $TCID_{50}$/mL in groups pre-treated with red ginseng extract or ginsenosides. However, there was no observed antiviral effect in groups co-treated or post-treated with KRG and its constituents. CRFK cells that were pretreated for 48 h with 10 μg/mL of KRG extract or purified ginsenoside Rb1 or Rg1, were inoculated with FCV. RNA extracted from each treated group was examined for the expression of antiviral cytokines, including interferon-α (IFN-α), interferon-β (IFN-β), interferon-ω (IFN-ω), Mx, and zinc finger antiviral protein shorter isoform (ZAPS), by relative real-time reverse transcription-polymerase chain reaction. mRNA expression of IFN-α, IFN-β, IFN-ω, Mx, and ZAPS was significantly induced in the FCV-challenged group pretreated with the KRG extract or ginsenosides, and it was higher than the group treated with FCV alone. Mx protein expression was confirmed by western blotting of CRFK cells pretreated with the ginsenoside Rb1 or with Rg1. Induction of antiviral cytokines contributes to the reduction of the viral titer in CRFK cells pretreated with the KRG extract and purified ginsenosides. Our data suggest that Korean red ginseng extract has an antiviral effect against norovirus surrogates and that protein and mRNA level of antiviral cytokines was significantly induced on cells pretreated with ginsenosides In further study, antiviral activity of natural compounds extracted from oriental herbs or phytochemicals should be invested in the animal models for human norovirus.

Symposium [S12]

Microbial and Toxicological Burdens in Biomaterials : How to Measure and Remove?

2014 International Meeting of the Microbiological Society of Korea

S12-1

Nonclinical Safety Research and Related Matters for Drug Development

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There are various kinds of relations between drugs and microorganisms. Here, in my presentation, we see microorganism as drug seeds. This is the report about non-clinical safety research and its related matters that should be considered in the process of drug development.

New drug development is roughly divided into 3 steps; drug discovery research step, drug development research step and market expansion study step. In the first drug discovery research step, we look for seeds and select drug candidate compounds. Microorganism is included in one of these seeds. The products of microorganism work as leading compounds, and the target of the developing candidate compounds is selected after the products go through screening studies. In the second step of drug development research, non-clinical research using animal, and clinical research using human are conducted. Pharmacological efficacy research, pharmacokinetics research, and safety research are carried out in non-clinical research. After the safety and efficacy of the compound are finally confirmed in non- clinical research, it is allowed to go forward to human clinical research. The clinical research is divided into three phases. Every phases are proceeded cautiously and through this process, the safety and efficacy are confirmed. Safety research of non-clinical research should be conducted according to GLP guidelines and clinical research should be complied with the GCP guidelines. With non-clinical and clinical research, a medical chemistry research establishes the way to extract and synthesize the compound. The drug formulation research maximizes the outcome of therapeutic efficacy of the compounds. The research is designed to maintain the stability and to form the compound for homogeneous and convenient dosing. The function and the role of safety research are to assess the usefulness of substance and extrapolation to human from toxicity data. Safety research of drugs are roughly divided into three fields; general toxicity studies, special toxicity studies and safety pharmacology studies. These study methods are approved by ICH(International Conference on Harmonization of Technical Requirement for Registration of Pharmaceutical for Human Use)and the studies should be complied with its guidelines. In general toxicity studies, there are single dose and repeated-dose toxicity studies using rodents and non-rodents. After single dosing, dosing frequency is gradually increased, and the influence of repeated dosing is observed. The purpose of single dose toxicity studies is to estimate the influence of over-dosing, the one of repeated-dose toxicity studies is to understand the interaction between dosing and dosing duration for toxic changes. Administration periods depend on periods of clinical use. Special toxicity studies that examine targeted toxicity include reproduction studies, genotoxicity studies, carcinogenicity studies, local irritability studies, immunotoxicity studies and drug dependency studies. Safety pharmacology studies examine the effects of test compound on the vital function such as cardiac, respiratory, CNS and so on. After the second step of drug development research, the compounds approved to be manufactured move on to the third step. In this step, the product is improved and manufactured to be suitable to the market; which is so called “market expansion research”. It is said that it takes 15-17 years and costs 25-30

billion yen to formulate a new drug.

An experimental animal has an important role in non-clinical safety research for drug development. In animal experiment, a genetic, microbiological, environmental and experimental factor influence the way animal react to the test article and this has impact on the study results. Therefore, an adequate control of these factors can enhance the reproducible animal experiment result.

GLP is abbreviation of Good Laboratory Practice. It is the regulation providing practice standards for the conduct of non-clinical research of pharmaceutical. It is established to ensure the reliability of non-clinical safety data attached to NDA documents. GLP is originated by FDA in the US. Before establishing the regulation, the defects FDA pointed out include the loss of animals, tissue specimens and original data, and transcription error, defective study protocol, insufficiency in personnel education and training, inadequate animal care procedures, confusion of animal identification, and arbitrary data selection. GLP studies are required to comply with study protocols and SOPs. The study protocol is prepared by a study director, and SOP is established under the responsibility of a facility director. Study operation and raw data collection are performed by study staffs. Final report is prepared by a study director. GLP places importance on the studies under adequate environment, emphasis on storage of records (raw data), clarified responsibilities and standardized operating procedures.

S12-2

Virological Safety Aspects of Biopharmaceuticals Produced in Mammalian Cell Cultures

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Biopharmaceuticals produced in mammalian cell cultures (MCC) become increasingly important for public health and the pharmaceutical industry. Microbial contamination is a major safety concern for biopharmaceuticals produced in MCC. In contrast to contamination of bacteria, fungi, and mycoplasma, which can be relatively easily detected, viral contamination presents a serious threat because of the difficulty in detecting some viruses and the lack of effective methods of treating infected cell cultures. Viral contamination can originate from contaminated cell lines, contaminated raw materials, or from a GMP breakdown in the production and purification process. To ensure safety, government regulations require manufacturers to demonstrate that biopharmaceuticals produced in MCC are free of adventitious agents. The overall safety assurance is accomplished by the combination of raw material control/testing including cell line validation, in-process control/testing, and virus clearance studies. In this talk, I'd like to introduce virolgical safety aspects of biopharmaceuticals produced in MCC.

Cell bank characterization: Cell bank (CB) testing is an essential part of the overall safety strategy for biopharmaceuticals produced in MCC. Characterization of the CB allows the manufacturer to assess this source with regard to presence of cells from other lines, adventitious agents, endogenous agents, and molecular contaminants (e.g., toxins or antibiotics from the host organism). The objective of this testing is to confirm the identity, purity, and suitability of the cell substrate for manufacturing use. For the testing of adventitious viruses, the most common assay is *in vitro* assay for the presence of viral contamination in which the test material is inoculated into susceptible cell lines such as the African green monkey kidney cell line Vero and the human diploid fibroblast cell line MRC-5, and the readout is a visible cytopathic effect. The inoculated cultures should be tested for haemadsorbing viruses using guinea pig, chicken, and human type O red blood cells at the end of the observation period. *In vivo* assay for viral contamination also should be conducted to detect adventitious viral agents in the test article by inoculation of embryonated eggs, suckling mice, adult mice, and guinea pigs. Embryonated eggs are inoculated by 3 routes; the allantoic, the yolk sac, and the amniotic cavity. *Orthomyxoviruses* and *Paramyxoviruse*s can be detected by by inoculation of embryonated eggs. Suckling mice are susceptible to a wide range of viruses including *Tagaviruses*, *Bunyaviruses*, *Flaviviruses*, *Picornaviruses*, and *Herpesviruses*. Adult mice are susceptible host for a number of viral agents, including the *Coxsackieviruses* and members of the *Flavivirus* group. Guinea pigs are susceptible host for a number of viral agents, including *Paramyxoviruses* and *Reoviruses*. The presence of retrovirus should be tested using transmission electron microscopy analysis, infectivity test by co-cultivation with the permissive cells for retroviruses, and reverse transcriptase assay using the PERT assay. The contamination of bovine viruses such as *Bluetongue virus*, *Bovine adenoviruses*, *Bovine parvovirus*, *Bovine respiratory syncytial virus*, *Reovirus*, *Rabies virus*, *Bovine herpes virus*, and *Bovine virus diarrhoea* virus should be checked using molecular detection methods. Also the presence of

porcine viruses such as *Porcine adenovirus*, *Porcine parvovirus*, *Transmissible gastroenteritis virus*, *Porcine circovirus*, and *Porcine hemagglutinating encephalitis virus* should be tested using molecular detection methods. Human viruses such as HAV, HBV, HCV, EBV, CMV, HIV 1&2, HTLV I&II, and PB19 also should be tested.

In-process control/testing: In-process controls (**IPC**) are checks that are carried out before the manufacturing process is completed. The function of in-process controls is monitoring and – if necessary – adaptation of the manufacturing process in order to comply with the specifications. This may include control of equipment and environment, too. Adventitious viral contamination should be checked by in-process controls using appropriate testing methods.

Viral clearance validation: The manufacturing processes of biopharmaceuticals must include virus inactivation and/or removal processes to ensure viral safety. Specific viral inactivation processes such as solvent/detergent (S/D) treatment, pasteurization, dry-heat treatment, and low pH incubation are the cornerstone in ensuring a sufficient margin of safety of biopharmaceuticals. Protein purification by chromatography contributes to the removal of viruses by partitioning. Nanofiltration is a specific approach to eliminate viruses. Validation of the process for viral inactivation and/or removal plays an essential and important role in establishing the safety of biopharmaceutical. Viral clearance studies are designed to assess the effectiveness of individual steps in a manufacturing process to reduce (removing or inactivating) potential contaminant viruses. The data is used from a study to provide a quantitative estimate of the overall level of virus clearance obtained during the manufacturing process. For viral clearance studies, infectivity assays are the preferred method to determine the effectiveness of a process step in removing and/or inactivating infectious agents. Valuable additional data on the partitioning of viruses can be obtained by the use of a quantitative polymerase chain reaction (Q-PCR) assay.

Effective risk management for virus safety should be a transparent process that enables appropriate decisions regarding risk control for the product to be made and effectively communicated to all concerned. The application of risk based management to the virus safety of biopharmaceutical products has been in operation for many years, but successful implementation requires an in-depth knowledge of the sources of potential risk, available measures for reducing and controlling the baseline risk, as well as an understanding of the regulatory history upon which the testing requirements have been built.

S12-3

What Testings Should be Done to Confirm The Safety of Biologics such as Gene Medicines?

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Biological medicines (biologics) are produced inherently through biological processes in contrast that the small molecules are chemically synthesized. In order to secure both efficacy and safety of biologics, a variety of biological methods should be done before the administration of them into patients; the classical microbiological culturing methods are used to investigate the presence of aerobic or anaerobic microbes in the products. The state-of-the art molecular biological analyses can be applied to examine the contamination of infectious agents. It is very challenging to determine what methods should be selected to prove the intended purpose, because biological medicines can be derived from very different biological materials such as recombinant proteins, monoclonal antibodies, living cells and viruses. Thus analytical methods should be selected and developed in the consideration of the product-specific characteristics. The specific cases can be helpful to understand it and for researchers to design the analytical methods for their products. The cases obtained from the development of gene medicines will be shared, presented, and discussed through the presentation.

S12-4

Toxicology Tests of Microbial and Agrochemical Pesticides

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Agrochemical agents such as chemically synthesized agrochemical pesticides and soil conditioners involve many problems including the risk of residual toxicity, the destruction of the ecosystem, the mutagenicity on pests, and the water and soil pollutions. In order to reduce the above problems of the chemically synthesized agrochemical agents, various biopesticides using microorganisms have been developed. According to Agricultural Chemicals Regulation Law, various test results such as a physicochemical analysis report, an efficacy and toxicity test report, a mammalian toxicity test report, an environmental and biological toxicity assessment report, and a persistence test report(it can be omitted when a test material is approved as having no risk in the mammalian toxicity test and environmental and biological toxicity assessment) should be submitted for the registration of an agrochemical pesticide and an active ingredient thereof. The mammalian toxicity test of a natural plant protection agent, which is produced using microorganisms as active ingredients, is conducted in three steps: tests for registration are conducted for active ingredients and products, respectively, and the agent can be registered when it shows toxicity under grade 3 (normal toxicity) in acute oral and acute dermal toxicity tests.

In this study, we examined acute oral toxicity/pathogenicity, acute dermal toxicity, eye mucous membrane irritation and skin irritation of three microbial formulations comprising *Bacillus* sp. (GM027), *Bacillus licheniformis* KJ-9 and *Trichoderma harzianum*, respectively, in accordance with '[Separate Table 12] Standard and Method for the Mammalian Toxicity Test' in the Section "Standard for the registration of an agrochemical pesticide and an active ingredient thereof" of the Rural Development Administration Notice No. 2012-37 (November 12, 2012). In an acute oral toxicity/pathogenicity test conducted with SD rats, GM027 was administered to the rats in a single dose of 1.0×10^8 CFU/ml and the rats were examined for the excretion of microorganisms in feces at 1, 3, 7 and 14 days after the administration. Also, test animals were sacrificed at 3, 7, 14 and 21 days and observed for the microorganism infection of their organs. In addition, general symptom, body weight gains and mortality were observed during the 21-day period. In an acute dermal toxicity test using SD rats, the rats were exposed to GM027 at a concentration of 1.0×10^8 CFU/ml for 24 hours, and then, general symptom, body weight gains and mortality were observed for 14 days. In an eye mucosa membrane irritation test, GM027 was administered to the conjunctiva sac of the New Zealand white (NZW) rabbits and then, general symptom, mortality and irritation to cornea, iris and conjunctiva were observed for 7 days. In a skin irritation test using NZW rabbits, the excoriated and non-excoriated sites of the skin of rabbits were exposed to GM027 at a concentration of 1.0×10^8 CFU/0.5 ml for 4 hours, and then, general symptom, mortality and irritation symptoms such as erythema, incrustation and edema formation were observed.

Bacillus sp. (GM027) showed no toxicity findings with regard to mortality, body weight changes and autopsy findings during the test period in the acute oral toxicity/pathogenicity test. Further, in a study of microbial population change in rat's excretion, the microorganisms in feces were observed at 1, 3, 7 and 14 days after the

oral administration, but no microorganisms were detected in feces thereafter. The infection of microorganisms was observed in the stomach at 3, 7 and 14 days after the oral administration, but no microorganisms were observed in all organs at 21 days after the administration. In the acute dermal toxicity test, no toxicity was observed with regard to mortality, body weight changes and autopsy findings. In addition, no abnormal findings were observed in the applied site after the termination of application of GM027. In the eye mucosa membrane irritation test, no abnormal findings and mortality caused by the application of GM027 were observed, and no abnormal findings were observed in the applied site. In the skin irritation test, no abnormal findings and mortality caused by the application of GM027 were observed, and no abnormal findings were observed in the applied site. Accordingly, it is concluded that GM027 does not have significant toxicity and irritant property.

B. licheniformis KJ-9 was subjected to an acute dermal toxicity test and a local irritation toxicity test. In the acute dermal toxicity test, *B. licheniformis* KJ-9 showed no abnormal findings and mortality at a concentration of 1.0×10^8 CFU/ml. In the eye mucosa membrane irritation test, *B. licheniformis* KJ-9 produced no abnormal findings and mortality. *Trichoderma harzianum* was subjected to acute oral toxicity/pathogenicity tests and acute dermal toxicity tests. Consequently, no abnormal findings and mortality were observed at a concentration of 1.0×10^8 CFU/ml.

From the above test results, it is concluded that the three microbial pesticides do not have a significant toxicity. It is considered that more continuous and long-term experiments are further required for the test of efficacy, toxicity and organ pathogenicity/persistence of microorganisms, along with the improvement of a new microorganism species for the development of various formulations using microorganisms and registration of the organic agrochemical materials.

Symposium [S13]

Animal Gut Microbiome

2014 International Meeting of
the Microbiological Society of Korea

S13-1

Gut Microbiota of *Tenebrio molitor* and Their Responses to Environmental Changes

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Insect guts provide distinctive characteristics for microbial habitats. Bacteria in the insect gut potentially play many beneficial roles to their hosts. Insects exhibited a wide range of dependence on gut bacteria for basic biological functions such as aiding digestion of low nutrient food, protecting them from disease or predators, mating and reproductive system. *Tenebrio molitor* is a species of darkling beetles and its larva is called mealworm. As generally decomposer, they feed on decaying plant materials dead insects. They are used for biological research due to easy handling, however, interaction with microorganisms are not well understood. To enhance the understanding of insects gut microbiota, we investigated the bacterial community of *T. molitor*, which has never been explored. Culture-independent community analysis using pyrosequencing showed that gut of *T. molitor* contained a relatively simple bacterial community consisting of *Tenericutes*, *Proteobacteria*, and *Firmicutes* at phylum level and *Spiroplasma*, *Lactobacillus*, unclassified *Enterobacteriaceae*, and unclassified *Lachnospiraceae* at genus level. Large portion of sequences was unclassified to genus level, indicating the possible presence of novel species. Ampicillin was direct injected or supplemented to the diet and the response of bacterial community was evaluated. Negative correlation between antibiotics concentration and bacterial diversity was identified. Culture-dependent community analysis showed no growth on ampicillin-added media, indicating gut microbiota of *T. molitor* is very sensitive to antibiotics. Interestingly, *Spiroplasma* was the only genus survived from ampicillin treatment regardless its concentration. We reasoned that *Spiroplasma* could avoid the cell-wall biosynthesis-inhibiting activity of ampicillin because it is a non-cell-wall bacterium. The responses of gut bacterial community to the input of highly diverse exogenous community will be discussed by incubating *T. molitor* in soil microcosm. This study is the first report of gut bacterial community of *T. molitor*, promoting the understanding of the microbes-insects interaction.

S13-2

Change of Gut Bacterial Communities Based on Evolution of Animal Host Species

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It is now well known that the animal gut is densely populated by microbial symbionts. Symbiotic gut microbiota help host to absorb nutrients through the fermentation of dietary fiber and provide protection from invading pathogens. They also help to develop and regulate the immune system of the hosts. However, current studies on ecological significance of gut bacteria have been dedicated into mostly the mammals. Therefore, gut microbiota in various invertebrate and vertebrate animals are now not well understood. Here, we report gut bacterial communities in insects and fishes caught in Korea, although these are only parts of overall schemes in change of gut microbiota based on evolution of animal host species.

The gut bacterial communities of insects and fishes have been analyzed host taxon-specifically via culture-dependent methods. However, culture-dependent methods often produce biased results depending upon the conditions and techniques used. Culture-independent molecular ecological approaches based on the analysis of the 16S rRNA gene have resulted in a dramatic improvement in our understanding of those microbes living within the guts of insects and fishes. Recent advances in molecular biology and the application of high-throughput next-generation sequencing technologies to microbial ecology show that the diversity of microbial populations is significantly higher than previously estimated by traditional culture-based and conventional molecular methods, and that 'rare biospheres' may be masked by dominant microorganisms. Comprehensive analysis of bacterial diversity within host species is a prerequisite in both insect and fish physiology and microbial ecology to allow better understanding of the ecological roles of gut symbionts and interactions with their insect hosts. However, most studies on gut bacterial diversity have been taxon-specific in insects such as termites, ants, fire bugs, fruit flies, beetles and bees, leaving a need for broader and systematic characterization and comparison across all insects and fishes.

The present study used high-throughput 454 pyrosequencing of 16S rRNA genes to examine comprehensively the gut microbiota of 305 individual insects, representing 218 species and belonging to 21 taxonomic orders of Insecta. The specific insect taxa from different geographical locations or at different developmental stages were also analyzed. The results provide detailed information about the bacterial profiles, including the diverse bacterial composition and distribution within the insect gut at different phylogenetic levels, the distribution of suspected heritable symbionts, and the relationships between microbial gut composition and the environmental niche, diet, developmental stage and phylogenetic position of insect hosts.

In insects gut analysis, 174,374 sequence reads were obtained, identifying 9,301 bacterial operational taxonomic units (OTUs) at the 3% distance level, with an average of 84.3 (±97.7) OTUs per sample. A total of 18 bacterial phyla and unclassified bacteria were detected across 21 orders. The majority of sequences were those of the Proteobacteria (62.1% of the classified sequences) and Firmicutes (20.7%), followed by Bacteroidetes (6.4%), Actinobacteria (4.8%), Tenericutes (1.9%) and unclassified bacteria (3.0%). At the bacterial class level, 34.1%,

7.5%, and 19.6% of the total sequences represented the Alpha-, Beta-, and Gammaproteobacteria, respectively. Bacilli and Clostridia (belonging to the phylum Firmicutes) represented 18.0% and 2.3% of the sequences, respectively, followed by 4.8% Actinobacteria, 3.1% Bacteroidia, 2.1% Flavobacteria (Bacteroidetes), and 1.9% Mollicutes (Tenericutes). At the family level, the Anaplasmataceae (14.1%) and Enterobacteriaceae (12.0%) were the most dominant. At the genus level, the Wolbachia group (14.1%) was most prevalent (Fig. 2b). To determine the novelty of the bacterial communities in the insect guts, the 16S rRNA gene sequences reported here were compared with those in the CAMERA database. The mean value for the percentage sequence identity ranged from 93.1% to 99.2% (average: 97.1% ± 0.10%) (Fig. 3). Relatively low sequence similarity values were obtained from the orders Megaloptera (mean value 92.1%), Mecoptera (93.5%), Blattaria (94.7%), Archaeognatha (96.1%) and Plecoptera (96.1%). This indicates that a large number of novel candidate bacterial groups are present in insect guts.

Significant differences were found in the relative abundance of anaerobes in insects classified according to the criteria of host environmental niche, diet, developmental stage and phylogeny. Gut bacterial diversity was significantly higher in omnivorous insects than in stenophagous (carnivorous and herbivorous) insects. This insect order-spanning investigation of gut microbiota provides insights into the relationships between their biology and gut bacterial communities.

We have also directly caught more than 100 species of fishes from East, West and South Sea of Korea and compared their gut bacterial communities of freshwater ones via 454 pyrosequencing of 16S rRNA genes. These comprehensively characterized fish-associated gut bacteria will be discussed in the presentation.

S13-3

Genetic Basis for Intestinal Colonization by Gut Microbes Revealed by a Metagenomic Screen

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Evidence suggests that gut microbes colonize the mammalian intestine through propagation as an adhesive microbial community. A bacterial artificial chromosome (BAC) library of murine bowel microbiota DNA in the surrogate host *Escherichia coli* DH10B was screened for enhanced adherence capability. Two out of 5,472 DH10B clones, 10G6 and 25G1, exhibited enhanced capabilities to adhere to inanimate surfaces in functional screens. DNA segments inserted into the 10G6 and 25G1 clones were 52 and 41 kb and included 47 and 41 protein-coding open reading frames (ORFs), respectively. DNA sequence alignments, tetranucleotide frequency, and codon usage analysis strongly suggest that these two DNA fragments are derived from species belonging to the genus *Bacteroides*. Consistent with this finding, a large portion of the predicted gene products were highly homologous to those of *Bacteroides* spp. Transposon mutagenesis and subsequent experiments that involved heterologous expression identified two operons associated with enhanced adherence. *E. coli* strains transformed with the 10a or 25b operon adhered to the surface of intestinal epithelium and colonized the mouse intestine more vigorously than did the control strain. This study has revealed the genetic determinants of unknown commensals (probably resembling *Bacteroides* species) that enhance the ability of the bacteria to colonize the murine bowel.

S13-4

The Biological Functions of Novel Symbiotic Factors in *Riptortus–Burkholderia* Symbiotic System

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The *Riptortus–Burkholderia* symbiotic system represents a promising experimental model to study the molecular mechanisms involved in insect–bacterium symbiosis due to the availability of genetically manipulated Burkholderia symbiont. This symbiont belongs to a member of the betaproteobacterial genus *Burkholderia*, and is acquired orally by host nymphs from the environment every generation. Further, *Burkholderia* can be easily cultivable and genetically manipulatable. This insect harbors a beneficial and specific symbiont *Burkholderia* in a specialized region of the posterior midgut. The symbiont is orally acquired by host nymphs from the environment every generation. Using this model system, we recently identified several novel symbiotic factors.

Firstly, we found a symbiosis-deficient mutant that was able to colonize the host insect but failed to induce normal development of host's symbiotic organ. The disrupted gene was identified as purL involved in purine biosynthesis. In vitro growth impairment of the purL mutant and its growth dependency on adenine and adenosine confirmed the functional disruption of the purine synthesis gene. The purl mutant also showed defects in biofilm formation, and this defect was not rescued by supplementation of purine derivatives. When inoculated to host insects, the purL mutant was initially able to colonize the symbiotic organ but failed to attain a normal infection density. The low level of infection density of the purL mutant attenuated the development of the host's symbiotic organ at early instar stages and reduced the host's fitness throughout the nymphal stages. Another symbiont mutantdeficient in a purine biosynthesis gene, purM, showed phenotypes similar to those of the purL mutant both *in vitro* and *in vivo*, confirming that the purL phenotypes are due to disrupted purine biosynthesis. These results demonstrate that the purine biosynthesis genes of the *Burkholderia* symbiont are critical for the successful accommodation of symbiont within the host, thereby facilitating the development of the host's symbiotic organ and enhancing the host's fitness values.

Secondly, when we compared biochemical and cytological comparisons between symbiotic and cultured Burkholderia, we observed that symbiotic *Burkholderia* showed more PHA granules consisting of poly-3-hydroxybutyrate and associated phasin (PhaP) protein. Among major PHA synthesis genes, phaB and phaC were disrupted by homologous recombination together with the phaP gene, whereby ΔphaB, ΔphaC, and ΔphaP mutants were generated. Both in culture and in symbiosis, accumulation of PHA granules was strongly suppressed in ΔphaB and ΔphaC, but only moderately in ΔphaP. In symbiosis, the host insects infected with ΔphaB and ΔphaC exhibited significantly lower symbiont densities and smaller body sizes. These deficient phenotypes associated with ΔphaB and ΔphaC were restored by complementation of the mutants with plasmids encoding a functional phaB/phaC gene. Retention analysis of the plasmids revealed positive selection acting on the functional phaB/phaC in symbiosis. These results indicate that the PHA synthesis genes of the *Burkholderia* symbiont are required for normal symbiotic association with the Riptortus host. *In vitro* culturing analyses confirmed vulnerability of the PHA gene mutants to environmental stresses, suggesting that PHA may play a role in resisting stress under symbiotic conditions.

Based on these data, I will present the molecular cross-talks between symbiotic *Burkholderia* and host bean-bug Riptortus insect in this symposium.

Symposium [S14]

Molecular and Cellular Biology of Yeast

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the Microbiological Society of Korea

S14-1

Nst1 Functions as an Adapting Protein to Mediates a Crosstalk of Cell Wall Integrity and HOG MAPK Pathways in Response to Heat Stress in Budding Yeast *Saccharomyces cerevisiae*

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In budding yeast, various environment stresses cause diverse cellular responses through activating limited numbers of mitogen-activated protein kinase (MAPK) pathways. The coordination and crosstalks among different MAPK pathways enhance the capacity and specificity of response to numerous signals. Cell wall integrity (CWI) MAPK pathway, comprised of Pkc1, Bck1, Mkk1/Mkk2 and Slt2, is activated by heat stress. However, the MAP kinase Slt2 is still activated without upstream Pkc1 or Bck1 in response to heat stress, suggesting an alternative pathway to activate Slt2. In this study, we showed that *NST1* knock-out mutant increases heat sensitivity and delays heat-induced Slt2 activation. Δ*nst1*Δ*bck1* double mutant displayed more severe growth defect than Δ*bck1* in response to heat stress and totally blocked heat-induced Slt2 activation. We also observed that Δ*sho1* and Δ*ste11* mutants in the upstream of HOG MAPK pathway show increased heat sensitivity and Δ*nst1*Δ*sho1* has more severe heat sensitivity than Δ*sho1*. These results suggest Nst1 functions in the downstream of both Bck1 and Sho1 for Slt2 activation by heat. Nst1 physically interacts with Ste11, Mkk1, and Slt2 by co-immunoprecipitations. Strikingly, we also detected the co-precipitation of Ste11 and Mkk1, which is not observed in Δ*nst1*. Nst1 is necessary for the interaction of Ste11-Mkk1 and Mkk1-Slt2. Taken together, these evidences demonstrate that Nst1 mediates the interaction of Ste11-Mkk1-Slt2 that connects Sho1 branch of HOG pathway to CWI pathway and provides a more comprehensive heat stress mechanism in budding yeast.

S14-2

Control of Gene Induction Kinetics by Set3 HDAC and Overlapping Non-coding RNA Transcription

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The Set3 histone deacetylase complex (Set3C) binds histone H3 dimethylated at lysine 4 (H3K4me2) to mediate deacetylation of histones in 5' transcribed regions. To discern how Set3C affects gene expression, genome-wide transcription was analyzed in yeast undergoing a series of carbon source shifts. Deleting *SET3* primarily caused changes during transition periods, as genes were induced or repressed. Surprisingly, a majority of Set3-affected genes are overlapped by noncoding RNA (ncRNA) transcription. Many Set3-repressed genes have H3K4me2 instead of me3 over promoter regions, due to either reduced H3K4me3 or ncRNA transcription from distal or antisense promoters. Set3C also represses internal cryptic promoters, but in different regions of genes than the Set2/Rpd3S pathway. Finally, Set3C stimulates some genes by repressing an overlapping antagonistic antisense transcript. These results show that overlapping noncoding transcription can fine-tune gene expression, not via the ncRNA but by depositing H3K4me2 to recruit the Set3C deacetylase.

S14-3

The Sec62/Sec63 Translocon Mediates Topogenesis of Membrane Proteins

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Proteins destined to the secretory pathway in the eukaryotic cell are first targeted to the endoplasmic reticulum (ER) membrane either by an signal recognition particle (SRP) dependent co-translational or an SRP independent post-translational translocation. Majority of membrane proteins are co-translationally translocated in the ER, thus the Sec62/Sec63 complex which mediates post-translational translocation of a subset of primarily secretory proteins into the ER, has been thought uninvolved in targeting and translocation of membrane proteins. By systematic analysis of single and multi-spanning membrane proteins with broad sequence context; varying hydrophobicity, flanking charged residues and orientation of transmembrane (TM) segments, in a set of Sec62 mutant yeast strains, we show that mutations in the N-terminal cytosolic domain of Sec62 impair interaction with Sec63 and lead to defects in membrane insertion and the C-terminal translocation of membrane proteins. These results suggest an unappreciated function of the Sec62/Sec63 translocon as a general membrane chaperone that regulates topogenesis of membrane proteins in the eukaryotic cell.

S14-4

Multistep Functions of Dna2 Nuclease in DNA Double-strand Break Repair by Homologous Recombination

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Dna2 is a well-conserved essential nuclease/helicase required for primary DNA metabolisms such as DNA replication, recombination, and stabilization of telomeric ends, all of which are mechanisms inevitable ultimately for the maintenance of genome integrity. While the helicase activity of Dna2 is dispensible for viability in budding yeast, its RPA-dependent endonuclease activity specific for long single-stranded DNA flaps is crucial for Okazaki fragment processing during the lagging strand DNA synthesis. Dna2 nuclease/helicase is recruited to double-strand break (DSB) ends in RPA-dependent manner and promotes 5' strand resection functionally paired with Sgs1 helicase, and this mechanism is very well conserved from bacteria (RecQ/RecJ) to higher eukaryotes such as Xenopus (WRN/Dna2) and human (BLM/Dna2). Here we investigate the DSB repair in strains lacking Dna2 and show that Dna2 is required for break-induced replication (BIR), camptothecin resistance, and recombination-dependent telomere maintenance. In addition to *pif1* mutant that previously showed partial defect in BIR as manifested by increased half-crossover and chromosome loss, Dna2 deficient cells show complete BIR defect and fail to initiate new DNA synthesis during DSB repair suggesting an early role of Dna2 in BIR. Surprisingly, additional deletion of *POL32*, a gene encoding nonessential component of Polδ, in *pif1 dna2* mutant partially restores BIR and telomere recombination, however not 5' strand resection during DSB repair, suggesting that interaction of Pif1, Dna2, and Pol32 at the invaded DSB end is crucial for the initial primer extension step of BIR.

Symposium [S15]

Lactic Acid Bacteria and Human Health

Sponsored by Korea Yakult

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the Microbiological Society of Korea

S15-1

Lactic Acid Bacteria: An Overview of Beneficial Effects

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Lactic acid bacteria (LAB) and their fermented food products have long been used for their proposed health promoting properties. LAB has been frequently used for lactose intolerance, vitamins supplement, constipation, diarrhea and immune potentiation. The most commonly used strains are lactobacilli, enterococci and bifidobacteria. The choice what microbe to use as probiotic is determined by many different factors, (1) resistance to digestive enzymes, stomachic acid and bile, (2) adhesion to the intestinal mucosa, (3) safety, (4) stability, (5) oxygen tolerance and (6) beneficial effects. Of them, I will focus beneficial biological effects of LAB.

The gastrointestinal tract of human body is a complex microenvironment where myeloid and lymphoid cells (organs) interface with a myriad of endogenous and exogenous stimuli. The gut mucosa constantly protects antigens of foods and intestinal microbiota via antigen degradation, defense immune elimination (helper/cytotoxic T cells, neutrophils, and macrophages) and IgA antibody production. Orally administered LAB inhibits proliferation of pathogens (bacteria or viruses) infected into the intestine by competing adhesion site and nutrients or/and antimicrobial substances.

Gut microbiota regulates systemic and local immune responsiveness, including hyporesponsiveness to antigen derived from microorganisms and foods. However, several gut-related inflammations, such as colitis, disturbed healthy host-microbe interaction. However, probiotics therapy improved gut-related inflammatory diseases via regulating T cells, proinflammatory and anti-inflammatory cytokines.

LAB also regulates allergic disease via suppressing lymphocyte proliferation and IL-4 generation in vitro and in vivo. Furthermore, clinical effects have been seen as a significant improvement in the coursed of atopic eczema in infants given LAB-supplemented elimination diets.

In addition, the composition of gut microbiota, which is related to host diseases including obesity, autism, and colitis, is influenced by diets, environment, host genetics, etc. Therefore, strategies to manipulate the gut microbiota may be able to be applied to cure some diseases, such colitis, obesity and allergies. While it is widely accepted that obesity is associated with low-grade systemic inflammation, the molecular origin of the inflammation remains unknown. Recently, we found that high fat diet (HFD) increased both plasma and fecal endotoxin levels and resulted in dysregulation of the gut microbiota by increasing the Firmicutes to Bacteriodetes ratio. HFD induced the growth of Enterobacteriaceae and the production of endotoxin in vitro. Furthermore, HFD induced colonic inflammation, including the increased expression of proinflammatory cytokines, the induction of Toll-like receptor 4 (TLR4), iNOS, COX-2, and the activation of NF-κB in the colon. HFD reduced the expression of tight junction-associated proteins claudin-1 and occludin in the colon. While the body weight of HFD-fed mice was significantly increased in both TLR4-deficient and wild type mice, the epididymal fat weight of HFD-fed TLR4-deficient mice were 69% of HFD-fed wild type mice. Furthermore, HFD did not significantly increase proinflammatory cytokine levels in TLR4-deficient mice. However, LAB treatment reduced body weight, ameliorated scopolamine-induced and D-galactose-induced memory impairment, and improved bacterial and candida vaginitis in mice. Finally, the potential use of LAB inside and outside gastrointestinal tract merits to be explored further. The evidence-based clinical studies will expand the acceptance of LAB for the treatment and prevention of selected diseases including intractable diseases.

S15-2

Analysis of Human Milk Oligosaccharides and Their Utilization by *Bifidobacterium*

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Human milk oligosaccharides (HMOs) exhibited numerous biological functions including a prebiotics to stimulate the growth of beneficial intestinal bacteria, a receptor analogue to inhibit binding of pathogens, and a substance that promote postnatal brain development. Due to the strong correlation of the structure of oligosaccharides with their absorption, catabolism, and biological functions the elucidation and characterization of structures are key to understand the molecular underpinnings in-between the gastrointestinal components. HMOs are composed of hexoses (Hex) and N-Acetylhexosamines (HexNAc) connected through b1-3 or b1-4-glycosidic linkage with additional decoration of fucose (Fuc) and N-Acetylneuraminic acid (NeuAc), which structurally similar to the O-glycan in human body. Because oligosaccharides are produced by competing enzymes that endow the large structural diversity and heterogeneity, rapid identification of oligosaccharide structure has been hindered. Recent advance of mass spectrometric analysis coupled with the nanoflow chromatography enabled to provide the vast information of oligosaccharides with high reproducibility and accuracy. A library of HMOs has been built with respect to the isomers. To increase the quantitative and qualitative information of oligosaccharides, MALDI-TOF/TOF has been used as well to deduce the compositional distribution of HMO. Reduction of oligosaccharides from aldose to alditiol form could eliminate the confusion between alpha and beta-rotation of reducing ends of HMOs. Based on the specific and rapid identification, the consumption of HMOs by *Bifidobacterium* sp. has been evaluated liking the structural commonalities of HMOs to the bacterial consumption preferences. From the deduced information, minimal structural requirements of HMOs that makes the HMO as a bifidogenic agents.

S15-3

Comparative and Functional Genomic Analysis of Bifidobacteria Reveals Its Genomic Adaptation into Human Intestinal Habitat

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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 IS*30* 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.

S15-4

Probiotics as an Immune Modulator for Hyper-immune Disorders

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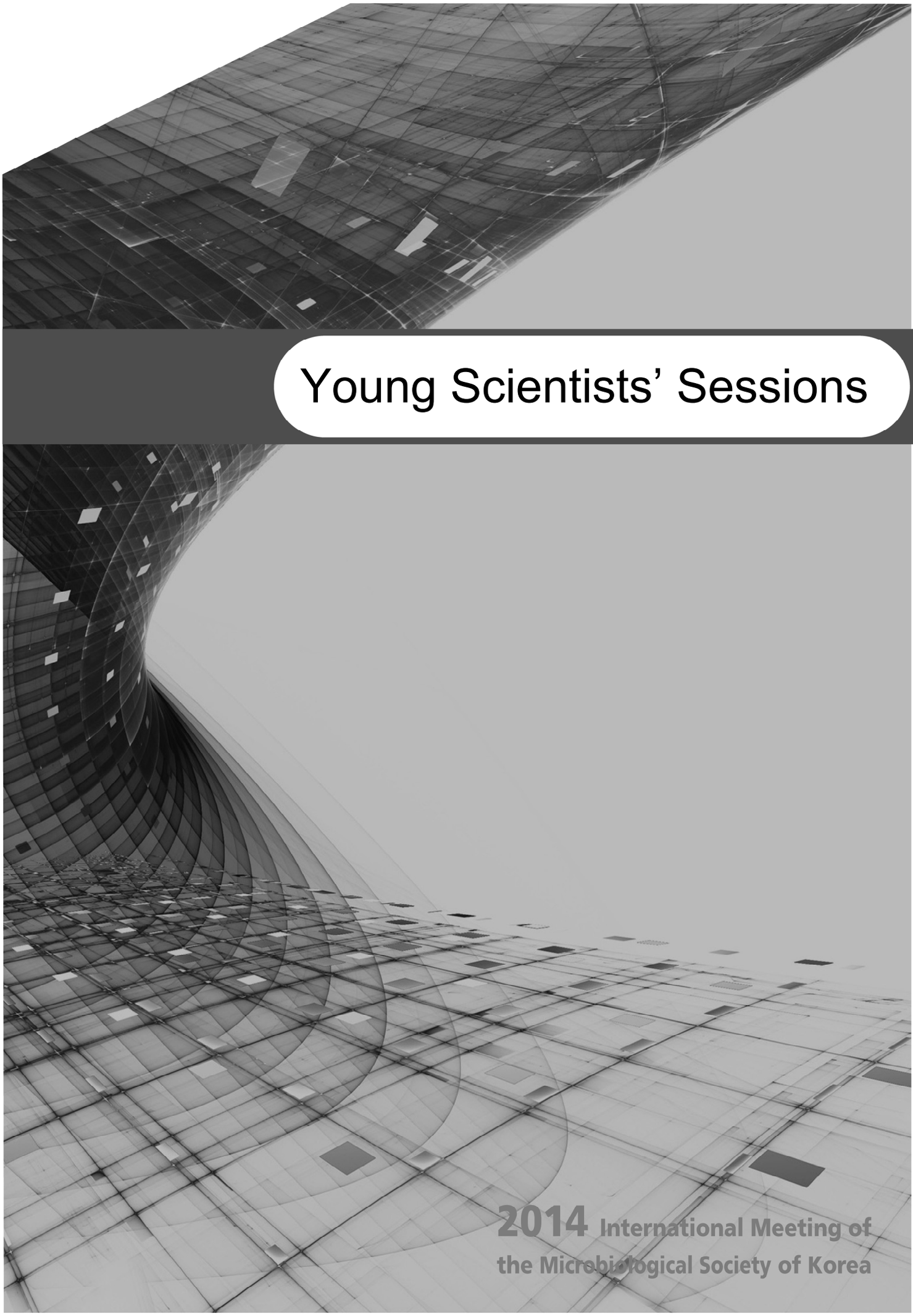
Probiotics can provide beneficial effects on host's health in many diseases, although the mechanism by which they modulate the immune system is poorly understood. In addition, therapeutic or prophylactic effects of probiotics on various diseases depend on the strains, administered routes, doses and the disease states. We have previously shown that a mixture of probiotics (named as IRT5; Proc Natl Acad Sci U S A. 2010 Feb 2;107(5):2159-64) upregulates $CD4^+Foxp3^+$ regulatory T cells (Tregs) through generation of regulatory dendritic cells (rDCs). In this study, we have tested the immunomodulatory effect of IRT5 probiotics on neural autoimmune disorders such as experimental autoimmune myasthenia gravis (EAMG) and experimental autoimmune encephalomyelitis (EAE). Pretreatment of IRT5 probiotics before disease onset significantly suppressed progression of EAMG and EAE. In EAMG study, treatment with IRT5 probiotics to the ongoing EAE delayed the disease onset while little effect was observed in EAMG. Administration of IRT5 probiotics decreased lymphocyte proliferation, anti-AChR reactive IgG levels and inflammatory cytokine levels such as IFN-γ, TNF-α, IL-6 and IL-17 through generation of regulatory dendritic cells (rDCs) that express increased levels of IL-10, TGF-β, arginase 1 and aldh1a2. Furthermore, DCs isolated from IRT5 probiotics-fed group effectively converted $CD4^+$ T cells into $CD4^+Foxp3^+$ regulatory T cells compared with control DCs. In EAE, administration of IRT5 probiotics inhibited the pro-inflammatory Th1/Th17 polarization, while inducing $IL\text{-}10^+$ or/and $Foxp3^+$ regulatory T cells, both in the peripheral immune system and at the site of inflammation. Our data suggest that IRT5 probiotics could be applicable to modulate neuronal autoimmune diseases.



Young Scientists' Sessions

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YS1-1

The Role of a Specific Hemagglutinin Residue as an Indicator of The Evolution Dynamics of Human Influenza A H1N1 Viruses

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Influenza A virus has evolved and thrived in human populations. Since the 1918 influenza A pandemic, human H1N1 viruses had acquired additional N-linked glycosylation (NLG) sites within the globular head region of hemagglutinin (HA) until the NLG-free HA head pattern of the 1918 H1N1 virus was renewed with the swine-derived 2009 pandemic H1N1 virus. Moreover, the HA of the 2009 H1N1 virus appeared to be antigenically related to that of the 1918 H1N1 virus. Hence it is possible that descendants of the 2009 H1N1 virus might recapitulate the acquisition of HA head glycoslylation sites through their evolutionary drift as a means to evade pre-existing immunity. Here we evaluate the evolution signature of glycosylations found in the globular head region of H1 HA in order to determine their impact in virulence and transmission of H1N1 viruses. We identified a polymorphism at HA residue 147 associated with the acquisition of glycosylation at residues 144 and 172. By *in vitro* and *in vivo* analyses using mutant viruses, we also found that the polymorphism at HA residue 147 compensated for the loss of replication, virulence and transmissibility associated with the presence of the N-linked glycans. Our findings suggest that the polymorphism in H1 HA at position 147 modulate viral fitness by buffering the constraints caused by N-linked glycans, and provide insights into the evolution dynamics of influenza viruses with implications in vaccine immunogenicity.

YS1-2

Inverse Regulation of Fe- and Ni-containing SOD Genes by a Fur Family Regulator Nur Through Small RNA Processed from 3'UTR of The *sodF* mRNA

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Superoxide dismutases (SODs) are widely distributed enzymes that convert superoxides to hydrogen peroxide and molecular oxygen, using various metals as cofactors. Many actinobacteria contain genes for both Ni-containing (*sodN*) and Fe-containing (*sodF*) SODs. In *Streptomyces coelicolor,* expression of the *sodF* and *sodN* genes is inversely regulated by nickel-specific Nur, a Fur-family regulator. With sufficient nickel, Nur directly represses *sodF* transcription, while inducing *sodN* indirectly. Bioinformatic search revealed that a conserved 19 nt stretch upstream of *sodN* matches perfectly with the *sodF* downstream sequence. We found that the *sodF* gene produced a stable small-sized RNA species (s-SodF) of ~90 nt that harbors the anti-*sodN* sequence complementary to *sodN* mRNA from the 5' end up to the ribosome binding site. Absence of nearby promoters and sensitivity to 5'-phosphate-specific exonuclease indicated that the s-SodF RNA is a likely processed product of *sodF* mRNA. The s-SodF RNA caused a significant decrease in the half-life of the *sodN* mRNA. Therefore, Nur activates *sodN* expression through inhibiting the synthesis of *sodF* mRNA, from which inhibitory s-SodF RNA is generated. This reveals a novel mechanism by which antagonistic regulation of one gene is achieved by small RNA processed from the 3'UTR of another gene's mRNA.

YS1-3

Characterization of Catalytic Functions of Bacterial CYP191A1

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Bacterial P450s are easier to handle and more stable than other eukaryotic P450s. In addition, their high catalytic activity and extensive genetic diversity are increasing the interest in using bacterial P450s as biocatalysts for the drug discovery and development process.

It is well known that *Mycobacterium tuberculosis* P450s including CYP51 are therapeutic targets for azole antifungal drugs. Non-pathogenic mycobacteria, *Mycobacterium smegmatis* is an attractive model organism to study the physiological function of mycobacterial pathogen like *M. tuberculosis*. Previous study showed extremely tight binding of several azoles to CYP191A1 from *M. smegmatis*, which highlights their therapeutic potential. However, biochemical properties and crystal structure of CYP191A1 are not known yet. In this study, diverse catalytic functions of CYP191A1 from, *M. smegmatis* were investigated.

Here, CYP191A1 from *M. smegmatis* was expressed in *Escherichia coli* and purified with high yield. CYP191A1 catalytic activities and binding affinities toward saturated fatty acids (C10~C16) were examined using spinach ferredoxin (Fdx) and ferredoxin reductase (FdR) as an electron transfer system. As carbon chain lengths of fatty acids were increased, K_d values decreased. The results obtained from GC-MS analysis have shown that CYP191A1 catalyzes the subterminal hydroxylation (ω-1 to ω-3) of saturated fatty acids with a chain length of 10~13 carbons. Surprisingly, the preference of hydroxylation reaction toward long fatty acids changed from ω-1 to the ω-3 position. The results suggest that CYP191A1 has a thin and long substrate binding pocket allowing the longer fatty acid to slip deeper. CYP191A1 also showed apparent oxidation activities toward typical human P450 substrates including chlorzoxazone and 7-ethoxycoumarin. Possible applications of CYP191A1 for developing a new target for azole drug therapy have been discussed.

YS1-4

Identification of Colistin Resistance Mechanism Using Transcriptome Anlaysis in *Acinetobacter baumannii*

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Acinetobacter baumannii is a particularly problematic Gram-negative pathogen, due to the worldwide emergence of multidrug resistant (MDR) strains. The emergence of colistin-resistant strains is especially concerning, since colistin is often regarded as the last option for treating MDR *A. baumannii* infections. Using mRNA sequencing, we compared whole genome transcriptomes of colistin-susceptible and colistin-resistant *A. baumannii* strains, with the aim of identifying genes involved in colistin resistance. A wild-type colistin-susceptible strain (06AC-179) and a colistin-resistant strain (07AC-052) were analyzed in this study. In addition, a colistin-resistant mutant (06AC-179-R1) was derived from the susceptible parent strain (06AC-179), and was also included in this study. High throughput mRNA sequencing was performed with an Illumina HiSeq TM 2000 sequencer. Among 17 genes showing >5-fold increased expression in the wild-type and *in vitro*-derived resistant strains compared with the susceptible strain, 11 genes were validated by qRT-PCR. Of these, nine candidates were deleted in the wild-type colistin-resistant strain (07AC-111) by allelic replacement, yielding eight knockout mutants. All deletion mutants but two (Δ01518::Km and Δ02907::Km) became significantly more sensitive to colistin, compared with the wild-type resistant strain. In addition, the reduced survival rates of the mutant strains in the presence of colistin were recovered by complementation with the appropriate gene, with the exception of only one complemented strain (111Δ02895::Km +pJN105/02895). In total, six genes were identified as associated with colistin resistance in *A. baumannii*. These six genes encode PmrAB two-component regulatory enzymes, PmrC (a lipid A phosphoethanolamine transferase), a 4-amino-4-deoxy-L-arabinose (Ara4N) transferase, a glycosyltransferase, and a poly-β-1,6-*N*-acetylglucosamine deacetylase. Since these genes are all associated with either lipopolysaccharide biosynthesis or electrostatic changes in the bacterial cell membrane, this study indicates that lipopolysaccharide modifications are the principal mode of acquisition of colistin resistance in *A. baumannii*.

YS1-5

HPr Antagonizes the Anti- σ^{70} Activity of Rsd in *Escherichia coli*

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The bacterial phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) transports and phosphorylates the PTS sugars. In addition to sugar uptake, PTS proteins have been shown to regulate many proteins through protein-protein interactions. Especially, $EIIA^{Glc}$, the glucose-specific enzyme II, interacts with and regulates several proteins, such as FrsA, adenylate cyclase, and lactose permease. However, in the case of the histidine phosphocarrier protein (HPr), one of the general PTS components, only one HPr-binding protein, glycogen phosphorylase, has been reported. Since HPr is known to be more abundant than $EIIA^{Glc}$ in enteric bacteria, we assumed that there might be more regulatory mechanisms connected with HPr. The ligand-fishing experiment in this study identified Rsd, an anti-sigma factor known to make a complex with σ^{70} in stationary-phase cells, as a new HPr-binding protein in *E. coli*. Only the dephosphorylated form of HPr made a tight complex with Rsd and thus inhibited complex formation between Rsd and σ^{70}. Dephosphorylated HPr, but not its phosphorylated form, antagonized the inhibitory effect of Rsd on σ^{70}-dependent transcriptions both *in vivo* and *in vitro* and also influenced the competition between σ^{70} and σ^{S} for core RNA polymerase in the presence of Rsd. Based on these data, we propose that HPr plays a role as an anti-anti-σ factor for σ^{70} and thus it is involved in transcriptional switching in *E. coli*.

In the previous study, it was shown that Rsd could affect the expression of subset of σ^{S}-dependent genes needed for the survival of *E. coli* in low-pH condition. However, an *rsd*-deficient mutant shows no apparent differences in its growth and viability in various media, compared to wild type. In this study, we found new phenotypes of the *rsd* mutant. Deletion of *rsd* affected the cell surface hydrophobicity and also mutant cells sank much faster than wild type. Rapid settling of mutant cells results from cell-cell autoaggregation. It is assumed that Rsd influences the expression of cell surface proteins through the regulation of σ^{70} activity.

YS1-6

Fitness of Plasmid Bearing *bla*$_{CTX-M-15}$ Gene in *Klebsiella pneumoniae*

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Objectives: To compare the molecular and virulence characteristics between CTX-M-producing and non-ESBL-producing *Klebsiella pneumoniae* isolates from Korea.

Methods: Thirty-three CTX-M-type extended-spectrum β-lactamase (ESBL)-producing and 65 non-ESBL-producing *K. pneumoniae* bloodstream infection isolates, which were collected in 2008 from Korean hospitals, were included in this study. *In vitro* antimicrobial susceptibility testing, multilocus sequence typing, and virulence assays, such as the detection of virulence genes, hypermucoviscosity, α-hemolysin production, and human serum sensitivity tests were performed. In addition, a plasmid harboring the *bla*$_{CTX-M-15}$ gene was transconjugated into five non-ESBL-producing sequence type (ST) 11 isolates, and the change in human serum sensitivity was monitored. Furthermore, the plasmid was sequenced using the 454 Genome Sequencer FLX system.

Results: Nineteen and 36 STs were identified among CTX-M-producing and non-ESBL-producing *K. pneumoniae* isolates, respectively. As virulence factors, the *cfa29a* and *allS* genes were more frequently found in non-ESBL-producing isolates. Non-ESBL-producing isolates showed a significantly higher survival rate against human serum than CTX-M-producing isolates, and hyperviscosity was also frequently identified in non-ESBL-producing isolates (29.2% vs. 9.1%; p, 0.038). Four out of five transconjugants receiving a plasmid with the *bla*$_{CTX-M-15}$ gene showed increased serum resistance levels compared with their non-ESBL-producing ST11 hosts. Analysis of the plasmid harbouring *bla*$_{CTX-M-15}$ gene revealed that the plasmid was composed of subregions separated by insertion sequences including IS*26* and IS*Ecp1*.

Conclusion: Diverse genotypes in both CTX-M-producing and non-ESBL-producing *K. pneumoniae* isolates from Korea, with no overlap of genotype between the two groups, suggest that most CTX-M-producing *K. pneumoniae* isolates in Korea did not occur by transfer of the *bla*$_{CTX-M}$ gene into susceptible isolates. The findings that the plasmid with the *bla*$_{CTX-M-15}$ gene confers virulence as well as antimicrobial resistance suggest that a CTX-M-15-producing *K. pneumoniae* clone such as ST11 may have a selective advantage even in an environment without antibiotic pressure, which would be of great concern to public health.

YS1-7

Altered Gut Microbiota Composition Affects Mouse Susceptibility to *Vibrio cholerae* Infection

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Gut microbiota prevent pathogen infection through colonization resistance and by promoting the development of mucosal immune system. In many animal models of enteritis, antibiotics are employed to eliminate the indigenous microbiota to establish consistent enteric infections by various pathogens including *Salmonella typhimurium* , *Vibrio cholerae*, and Enterohaemorrhagic *Escherichia coli*. Disruption of the indigenous microbiota by treating with broad-spectrum antibiotics can lead to a substantial increase in the susceptibility to *Clostridium difficile* infections. Although evidence suggests that the gut microbiota plays important roles in defense to enteric infections, some enteric pathogens could utilize the gut commensals to promote their colonization and survival. To obtain evidence for pivotal commensal microbe on improvement of host resistance to enteric infection, we hypothesized that the disruption of specific indigenous microbe can facilitate colonization of *V. cholerae* within mouse gut. We treated BALB/c mice with each of five different antibiotics to disrupt normal microbiota composition and evaluated their effect on colonization by several predominant bacterial groups. We used a quantitative RT-PCR with primers targeting the bacterial small-subunit (16S) rRNA region combined with viable cell counting to determine the community structure of the gut microbiota. Next, mice were inoculated with *V. cholerae* to investigate the role of antibiotic-induced microbiota disruption in host susceptibility to the infection. We found that antibiotics varied in their abilities to alter bacterial compositions in the gut, and none of the antibiotics completely eliminated the intestinal microbiota. Most of the antibiotic treatments altered gut microbial compositions. The level of bificobacterium decreased in ampicillin-treated mice, whereas those of Enterobacteriaceae and Enterococci increased in mice treated with vancomycin and clindamycin, reflecting the modified resistance activity of the altered gut microbiota. Notably, vancomycin treatment showed dramatic changes, including an enhanced population of Enterobacteriaceae and Enterococci. The different microbiota compositions, as a result of perturbations in the microbial community, affected the mouse susceptibility to *Vibrio cholerae* infections. Especially, the overgrowth of *Enterococcus* was sufficient to make mice more susceptible to *Vibrio cholerae.* These results suggest that complete colonization resistance depends on the compositional balance of bacterial diversity, rather than total numbers of bacteria. Our results demonstrate that antibiotic-mediated alteration of the gut microbiota convert the host's resistance ability on enteric infection of *V. cholerae* by disrupting intestinal homeostasis. The overgrowth of the intact microbiota could stimulate *V. cholerae* invasion by effectively helping with *V. cholerae* for nutrition. The imbalance of microbial community through may not only lead to physical and biological changes but also result in changed metabolic activity in the gut circumstance.

YS1-8

Genomic Variations Between Colistin-susceptible and -resistant *Pseudomonas aeruginosa* Clinical Isolates and Their Effects on Colistin Resistance

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As a consequence of increased reliance on colistin for treating multidrug-resistant (MDR) *Pseudomonas aeruginosa* infections, the emergence of colistin-resistant *P. aeruginosa* is becoming a serious concern worldwide. We investigated genetic variations involved in acquisition and loss of colistin resistance in three clinical isogenic *P. aeruginosa* isolates (GKK-1, GKK-2, and GKK-3) recovered from a single patient and assessed their impacts on colistin resistance. Whole genome sequencing technology was applied to identify single nucleotide polymorphisms (SNPs) and insertion or deletion (indels) in two colistin-resistant isolates compared with a susceptible isolate.

Thirty-seven nonsynonymous mutations in 33 coding sequences were detected in the colistin-resistant isolates, GKK-1 and GKK-3. Only one gene (PA1375) was significantly downregulated in both colistin-resistant isolates; this gene encodes erythronate-4-phosphate dehydrogenase. Among the 8 genes that were upregulated in the colistin-resistant isolates, 5 except 3 hypothetical genes (PA1938, PA2928 and PA4541) predicted to be involved in core biological functions, which are a cell wall-associated hydrolase (PA1199), a response regulator EraR (PA1980), a sensor/response regulator hybrid (PA2583), a glycosyltransferase (PA5447), and an arabinose efflux permease (PA5548). All mutants with allelic replacement of these candidate genes but one (PA1375) exhibited increases in colistin susceptibility, ranging from 2- to 16-fold. Colistin susceptibility decreased in complemented strains compared with the mutants; however, in 3 cases, resistance did not reach wild-type level.

This study demonstrates genetic differences between *P. aeruginosa* isogenic isolates, and identifies novel determinants which may be associated with acquisition of colistin resistance. These findings will lay the foundation for a complete understanding of the molecular mechanisms of colistin resistance in *P. aeruginosa*.

YS1-9

Tn7 Transposition: Importance of Protein-Protein Interactions Between TnsABCD

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The excision of transposon *Tn7* from a donor site and its insertion into its preferred target site *attTn7* is mediated by four *Tn7*-encoded proteins, TnsA, TnsB, TnsC and TnsD. Transposition requires the assembly of a nucleoprotein complex containing all four Tns proteins and the DNA substrates, the donor site containing *Tn7* and the preferred target site *attTn7*.

TnsA and TnsB together form the heteromeric *Tn7* transposase and TnsD is a target-selecting protein that binds specifically to *attTn7*. TnsC is the key regulator of transposition, interacting with both the TnsAB transposase and TnsD-*attTn7*. TnsC may also be recruited to *attTn7* as part of a TnsA-TnsC complex as TnsA and TnsC can co-purify as a $TnsA_2C_2$ heterotetramer.

In this work, we show that TnsA and TnsB interact directly and identify several TnsA and TnsB amino acids involved in this interaction. We also show that TnsA can stimulate two key activities of TnsB, specific binding to the ends and pairing of the *Tn7* ends.

We demonstrate here that TnsC interacts directly with TnsB and identify the specific region of TnsC involved in TnsB-TnsC interaction during transposition. *Tn7* displays *cis*-acting target immunity, which blocks *Tn7* insertion into a target DNA that already contains *Tn7*. We provide evidence that the direct TnsB-TnsC interaction also mediates *cis*-acting Tn7 target immunity. We also show that TnsC interacts directly with the target selector protein TnsD. All these protein-protein interactions is a fundamental key for Tn7 transposition.

YS2-1

Characterization and *In Vitro* Inhibition Studies of *Bacillus anthracis* FtsZ: A Potential Antibacterial Target

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FtsZ is an essential bacterial cell division protein that is an attractive target for the development of antibacterial agents. FtsZ is a homologue of eukaryotic tubulin, has GTPase activity and forms a ring-type structure to initiate cell division. In this study, the FtsZ of Bacillus anthracis was cloned into a bacterial expression vector and overexpressed into *E. coli* BL21 (DE3) cells. The over-expressed *B. anthracis* FtsZ was soluble and purified to homogeneity using Ni-His-tag affinity chromatography. Like other known FtsZs, the recombinant *B. anthracis* FtsZ also showed GTP-dependent polymerization, which was analyzed using both spectrophotometric and Transmission Electronic Microscope (TEM) analysis.

Using the purified FtsZ, we screened a naturally extracted chemical library to identify potent and novel inhibitors. The screening yielded three chemicals, SA-011, SA-059, and SA-069, that inhibited the in vitro polymerization activity of FtsZ in the micromolar range (IC50 of 55–168 μM). The inhibition potency was significantly comparable with that of berberine, a known potential inhibitor of FtsZ. Understanding the biochemical basis of the effect of these inhibitors on *B. anthracis* growth would provide a promising path for the development of new anti-anthrax drugs.

YS2-2

Induction of Apoptosis by a *Vibrio vulnificus* Metalloprotease

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Vibrio vulnificus, a causative agent of septicemia and gastroenteritis, secretes various proteins, which are assumed to be involved in various functional types of interactions with hosts during infection process. Protease-zymogram analyses revealed the presence of multiple extracellular proteases in the supernatant cultured by *V. vulnificus*. One of them is a putative metalloprotease containing a Zn-binding motif, whose proteolytic activity was abolished by specific inhibitors against metalloproteases. Thus, it was named by VvpM to distinguish from VvpE that has been considered as a representative extracellular protease of *V. vulnificus*. To investigate whether this newly identified protease, VvpM has pathogenic role in host interaction in addition to proteolytic role, several human cell lines were incubated in the presence of rVvpM. VvpM-challenged cells showed typical apoptosis characterized by cell shrinkage, morphological changes in nucleus, and appearance of vacuoles in cytoplasm. Apoptotic cell death was further evidenced by estimating the Annexin V-stained cells, whose proportions were dependent upon the concentrations of rVvpM treated to human cells. To elucidate the signaling pathway for VvpM-induced apoptosis, three MAPKs were tested if their activation were mediated by rVvpM, and found that ERK1/2 was phosphorylated by rVvpM and rVvpM-induced cell death was blocked by a specific inhibitor against ERK1/2. Since mitochondrion is one of the components in amplifying apoptosis signaled via ERK activation, release of cytochrome c from mitochondria in rVvpM-treated cells was examined. It was found that the levels of cytochrome c in cytosol were increased as a VvpM concentration-dependent manner, while the levels of cytochrome c in mitochondria were decreased. These human cell deaths were further accompanied by apparent cleavages of procaspases-9 and -3 to the active caspases-9 and -3, respectively. Therefore, this study demonstrates that VvpM induces apoptosis of human cells via a pathway consisting of ERK activation, cytochrome c release, and then activation of caspases-9 and -3.

YS2-3

Translation of Leaderless Transcripts are Regulated by Non-coding RNAs in *Thermococcus onnurineus* NA1

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Thermococcus onnurineus NA1 isolated from a deep-sea hydrothermal vent area is a hydrogen-producing archaea. In spite of industrial potential for producing H_2, it is still limited the information about the genomic features related with cellular events in *T. onnurineus* NA1. Here, we provide comprehensive transcriptome landscapes of *T. onnurineus* NA1 with 5'-end of individual RNA transcripts at single nucleotide resolution using directional and differential RNA sequencing methods. From the landscape, we could detect 874 transcription start sites (TSS), leading to the discovery of large portion of 'leaderless' genes (~31% of 874 TSS). Leaderless genes are lack of 5'-end untranslated region (UTR) which is the regulatory region of translation. Hence, it has been reported that leaderless genes can be translated by direct binding of mRNAs, the translational machinery composed of the translation initiation factor IF-2 (IF2) with Met-$tRNA_i^{Met}$, and 30S ribosomal subunit. The important issue is how leaderless genes control the translation process efficiently. Interestingly, our analysis discovered that two of non-coding RNAs control the transcriptional level of IF2 depending on the experimental conditions. In conclusion, we could obtain the clues that explain the fine-tuning regulatory mechanism of leaderless genes by regulatory non-coding RNAs.

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YS2-4

Inhibition of HCV Replication with HCV NS5B Specific RNA Aptamer

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Almost 170 million people are chronically infected worldwide by HCV, and ~27% of all cases of liver cirrhosis and ~25% of hepatocellular carcinoma might be related to HCV infection, efficient, specific, and safe antiviral therapy has not yet been developed. In this study, we developed two anti-HCV approaches based on RNA aptamers targeting HCV NS5B replicase that is essential for HCV replication. (I) Aptamers were used as intracellular decoys against the target protein. We isolated RNA aptamers consisting of 2'-hydroxyl or 2'-fluoro pyrimidines, which bind tightly and competitively to NS5B. Cytoplasmic expression of 2'-hydroxyl aptamer or direct administration of chemically modified and liver-cell permeable ligand-conjugated 2'-fluoro aptamer inhibited various HCV genotypes through sequestering the target protein in cells. Importantly, the aptamers suppressed HCV replication without escape mutant appearance, neither causing toxicity, nor inducing innate immune response. Moreover, the aptamer showed good pharmacokinetic properties, efficient bioavailability and safety profile. Of note, therapeutically feasible quantities of the aptamer were delivered to liver tissue in mice. Therefore, cytoplasmic expression of 2'-hydroxyl aptamer or direct administration of chemically synthesized and ligand-conjugated 2'-fluoro aptamer against HCV NS5B could be a potent anti-HCV approach.

YS2-5

Novel Na^+-Dependent Respiration in Hyperthermophilic Archaeon, *Thermococcus onnurineus* NA1

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Chemiosmotic energy transduction to ATP synthesis is the most basic process in life form. Throughout the prokaryotic membranes or mitochondrial inner membranes, energy-transducing protein complexes help transform the chemical energy from the cell's metabolic intake into various useful forms of energy for the cell. Some of these complexes couple reduction-oxidation (redox) reactions to transporting and establishing chemiosmotic gradient across the membrane to drive otherwise energetically unfavorable process such as ATP synthesis and assorted transporters of other ions and/or molecules. Complex I is the most well known energy transducing enzyme that consists of 14 enzymes in bacteria, transferring electrons from NADH through a complex chain to quinones which process pumps protons across membrane and then generate proton coupled electrical membrane potential. Complex I is an example of the modular structure composed of electron donor/transfer module, connecting module, and intrinsic membrane module in *Escherichia coli*, has been also thought to have close relationship with Group 4 hydrogenases. The group 4 hydrogenases that widely distributed among bacteria and archaea, have been recognized as a energy converting enzyme composed in modular structure as well as the key enzyme in hydrogen production. Of particular, Mbh hydrogenase from *Pyrococcus furiosus* which catalyzes the reduction of H^+ with electrons derived from reduced ferredoxins has been demonstrated to conserve energy using proton gradient to synthesize ATP. However, the identity of osmotic ion in the respiratory chain of the group 4 hydrogenase, especially in archaea, has been often questioned. Sodium bioenergetics using a ΔpNa is considered an early step in the evolution of cellular bioenergetics. However, the energy conservation through sodium gradient has never been demonstrated in any biological system.

Previously, we reported that several hyperthermophilic archaeal strains belonging to Thermoccocales including *T. onnurineus* NA1 were able to grow on formate and produce a substantial amount of H_2. *fdh2-mfh2-mnh2* gene cluster was reported to be responsible for formate-oxidizing, hydrogen-producing growth, and multisubunit monovalent cation/proton antiporter encoded by *mnh2* has been thought to participate in formate-dependent energy conservation for the synthesis of ATPs. The hydrogenase gene cluster was unique in retaining a putative cation/proton antiporter. The tripartite modular organization of group 4 hydrogenase could be found in many archaeal genomes, however, there is no report to unveil the physiological role of multisubunit monovalent cation/proton antiporter participating in mediating the electron relay of the respiratory chain to date. The multisubunit monovalent cation/proton antiporter (Mrp homologues) has been mainly studied in bacteria at a physiological function of alkaline pH homeostasis and Na^+ resistance, cell sporulation, symbiotic nitrogen fixation, arsenite resistance and bile salt resistance.

Here, we try to address the identity of chemiosmotic ion to couple formate oxidation to ATP synthesis in the anaerobic respiratory mechanism of formate oxidation. Additionally, the physiological role of the multisubunit monovalent cation/proton antiporter related with ATP synthesis was considered.

YS2-6

Application of a New Cultivation Technology, I-tip, for Studying Microbial Diversity in Freshwater Sponges of Lake Baikal, Russia

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One of the fundamental methods for cultivating bacterial strains is conventional plating on solid media, but this method does not reveal the true diversity of the bacterial community. In this study we developed a new technique and introduce a new device. We term it I-tip (*in situ* cultivation by tip), because its main element is a standard micropipette tip. The tip is filled with microbeads and agar; for cultivation it is positioned such that its narrow end touches the surface of the animal. Microorganisms are expected to proliferate in agar using nutrients diffusing from the environment; microbeads act as a barrier to prevent larger organisms to invade the space. We used the new method to cultivate microorganisms from Bakalian sponges and compared the results with conventional plating as well as a pyrosequencing-based molecular survey. The I-tip method produced cultures of 34 species from 5 major phyla, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Firmicutes, and Gammaproteobacteria, or 71% of what was detected by pyrosequencing. Standard cultivation produced a smaller collection: 16 species from Betaproteobacteria, Firmicutes and Gammaproteobacteria, or 42% of major phyla detected by pyrosequencing. We conclude that the I-tip method can narrow the gap between cultivated and uncultivated species, at least for some of the more challenging microbial communities such as those associated with animal hosts.

YS2-7

Comparative Genomic and Transcriptomic Analyses of *Acinetobacter* and *Alishewanella* Species Adapted to Different Habitats

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To understand the adaptation of bacterial species dwelling in different habitats, which belong to the same genus, *Acinetobacter* and *Alishewanella* were chosen because they are expected to have high adaptability to diverse environments and relevant genetic evidences as evolutionary records.

Acinetobacter species was chosen for comparative genomic analysis because they have been isolated from a variety of habitats such as soils, activated sludge, seawater, and human clinical specimens, suggesting the high adaptability of this genus to various environments. Soil-borne *Acinetobacter oleivorans* DR1 was of special interest due to the scarcity of research on natural environment-originated *Acinetobacter* species. Genome sequencing results showed that *A. oleivorans* DR1 possesses the largest genome (4.15 Mb) within *Acinetobacter* genus. The multiple genome alignment revealed that large genomic regions did not have significant matches in other genomes. These regions contained phage-related genes such as phage integrase, primase, prophage transcriptional regulator, and phage DNA methylase. Regarding transposases, only two fragmented genes were identified whereas the genomes of *A. baylyi* and *A. baumannii* harbored 14 and 19 predicted transposase-related genes, respectively. Incorporation of phage genes and a low abundance of IS elements and transposases may be the reason for the relatively large size of the DR1 genome. Comparative genomic analyses confirmed that DR1 was the only species which possessed gentisate 1, 2-dioxygenase (*nagI*) among all the other *Acinetobacter*. Growth test with gentisate as a sole carbon source confirmed the exclusive ability of DR1 to utilize gentisate. Reverse-transcriptase PCR, and quantitative real-time PCR confirmed the polycistronic expression of *nagI* and downstream genes when gentisate was provided as a sole carbon source, indicating that gentisate metabolism could distinguish soil-borne *A. oleivorans* DR1 from other species.

Alishewanella genus provides another good opportunity to chase evolutionary history because *Alishewanella* contains only 5 type species and their isolation sources were all different. *A. jeotgali*, *A. aestuarii*, and *A. agri* have been isolated from fermented seafood, tidal flat sediment, and soil, respectively. Diverse habitats of *Alishewanella* imply that they have a broad range of niches and high adaptability. Phylogenetic analysis of 16S rRNA genes from *Alishewanella* and taxonomically neighboring species showed that *Alishewanella* species have evolved from an ancestor dwelling in the marine environment. Interestingly, reciprocal BLASTP comparison determined that *A.agri* acquired genes horizontally from diverse soil bacteria, indicating that *A. agri* moved its habitat from marine environment to soil and the accumulation of exogenously acquired genes may have resulted from the genomic evolution of *A. agri*. Gene content of genomic islands also suggest that horizontal gene transfer may have conferred important physiological features with relevance to the environmental conditions and contributed to speciation from a common ancestor. Pectin utilization is an important characteristic of *Alishewanella* species. Genomic analysis showed genes coding for pectinolytic enzymes were closely associated on a genomic locus. Transcriptional analyses of *Alishewanella* species grown on pectin confirmed the expression

of pectin metabolism-related genes. Most remarkable result from transcriptomic analysis was that glyoxylate bypass was highly expressed from 3 *Alishewanella* species whereas acetyl-CoA was not a direct metabolite. Flow cytometry analysis validated that pectin metabolism induced oxidative stress in *Alishewanella* species and oxidative stress could induce the glyoxylate bypass, suggesting that the up-regulation of glyoxylate bypass would be important in oxidative stress defense during pectin metabolism.

In conclusion, this study suggested that unraveling of genetic information and its experimental verification are important to understand bacterial adaptation because genome is considered as an archive of past adaptation processes. Comparative genomics and transcriptomic analyses performed with *Acinetobacter* and *Alishewanella* species isolated from different habitats will provide an essential clue to understand bacterial adaptation to different environments.

YS2-8

Genome-scale Probing of *In Vivo* Organization of Bacterial Transcription Initiation Complexes

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The DNA dependent RNA polymerase (RNAP) is the decisive enzyme for the transcription of the genetic information in all organisms, which is conserved from bacteria to human in its sequence, structure, and function. Numerous studies to investigate the mechanism of transcription have been performed through various approaches such as structural study, biochemical assay, and single molecule fluorescence resonance energy transfer (FRET) monitoring. Nevertheless, there are still restrictions to interpret the whole dynamic behaviors occurred by massive RNAP holoenzyme *in vivo*, since most studied have shown only frozen snapshots using specific genes or synthetic genes *in vitro*. Recently, a remarkable advance of high-throughput technology and data processing enabled us to observe the transcription event *in vivo* under genomic scale studies. Until now, some researchers tried to explain the transcription mode as tracking core proteins necessary for transcription. However it still remained unclear to interpret the dynamics of transcription microscopically owing to the low resolution of existing genome-scale experiments such as ChIP-chip or ChIP-seq data.

Here we first disclosed the dynamics of the transcription occurred in bacteria using ChIP-exo method with extremely high resolution of single base, together with ChIP-seq and RNA-seq. Individual promoters recognized by σ70 and RNAP were discovered through differential RNA-seq method over the whole genome. Further the dynamic behavior of RNAP holoenzyme at transcription initiation was elucidated through the integral observation of active RNAP and sigma factor functioning *in vivo*. Through genome-scale scanning of RNAP and sigma factor, the transcription initiation is caused by the scrunching of DNA, not by the translocation of the RNAP, is proved experimentally. We could explain how sigma factor participates in subsequent transcription processing such as abortive RNA transcription as well as promoter recognition. Our research supports the reliable window to observe and interpret intricate transcription events in perspective of a genomic scale.

YS2-9

RNA-mediated Regulation of Photosynthesis in *Synechocystis* sp. PCC6803

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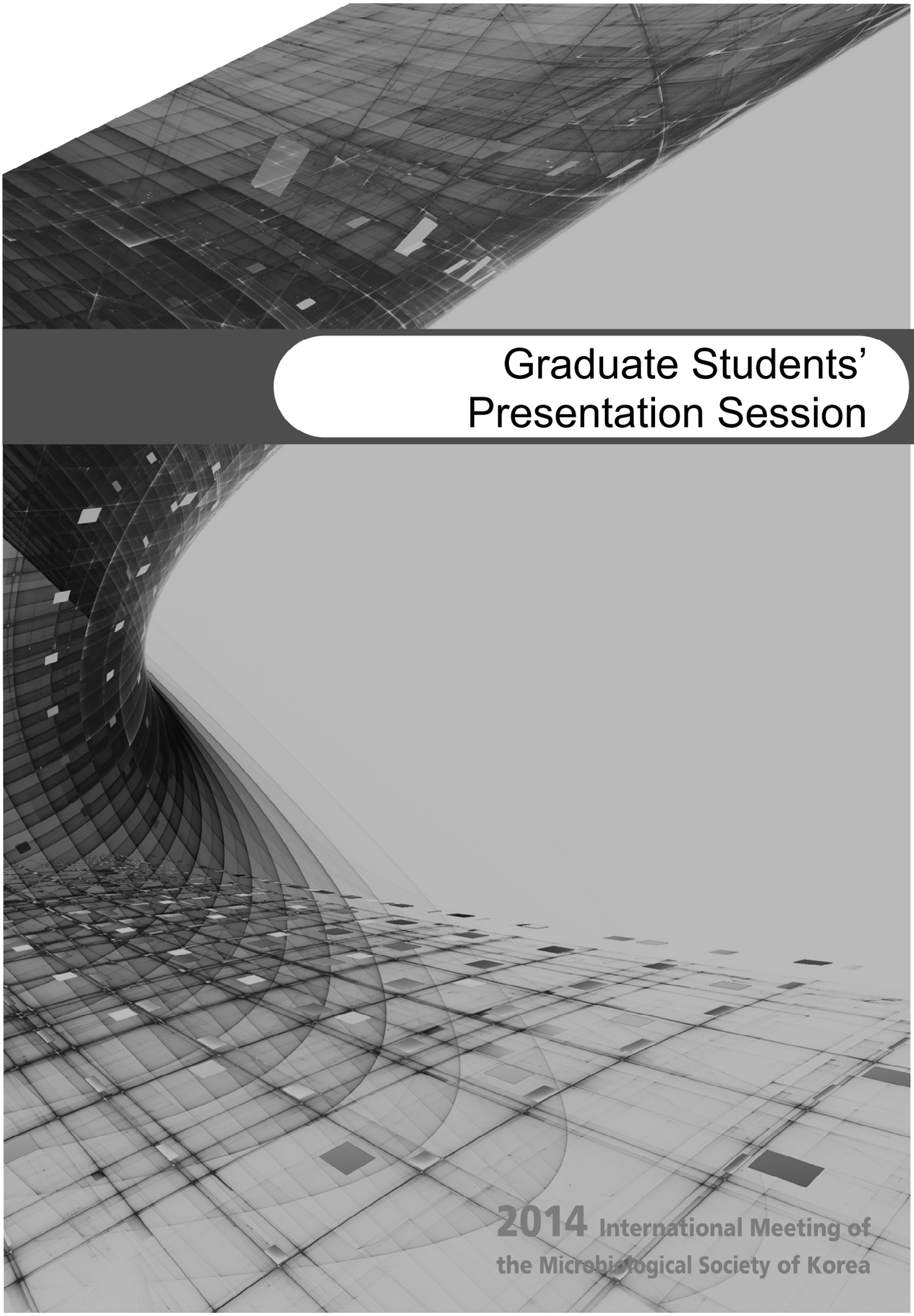
Non-coding RNAs (ncRNAs) have important roles on gene regulation in bacteria. In cyanobacteria, ncRNA consist of similar or larger fraction of the protein coding genes. However, their modes of regulation and functional relationship to photosynthetic processes are unclear. Here we use directional RNA-seq to identify ncRNAs to understand functional relationship with photosynthesis at the genome scale. With the support of proteogenomics and bioinformatics, we identified differentially expressed ncRNAs upon high light stress and low temperature stress and both conditions. Our result shows that ncRNAs mediate regulation of photosynthetic genes for temperatures stress as well as light stress.



Graduate Students' Presentation Session

2014 International Meeting of the Microbiological Society of Korea

GS-1

Multiple Resistance Mechanisms of High-level Fluoroquinolone Resistant *Aeromonas* sp. Strain C3 Isolated from Waste Water Treatment Plant

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Aeromonas sp. strain C3 was isolated from a waste water treatment plant and found to be highly resistant to fluoroquinolones, suggesting a combination of various resistance mechanisms in this strain. We investigated multiple resistance mechanisms of the isolate to understand the high-level fluoroquinolone resistance. Analysis of quinolone resistance-determining regions (QRDRs) revealed point mutations on *gyrA* and *parC* genes, which confer fluoroquinolone resistance. The resistance of *Aeromonas* sp. strain C3 against fluoroquinolones was reduced in the presence of efflux pump inhibitors, indicating that efflux pumps were involved in the fluoroquinolone resistance. Production of *N*-acetylfluoroquinolone by the strain was found to be responsible for the *aac(6')-Ib-cr* gene located in a natural plasmid. Sequencing of the plasmid (pAC3) revealed that it was an IncC-type plasmid (15,872 bp) and contained two fluoroquinolone resistance genes, *aac(6')-Ib-cr* and *qnrS.* Increased MIC and acquired *N*-acetylation activity of *E. coli* DH5α containing pAC3 indicated plasmid-mediated quinolone resistance (PMQR) and transferability of the fluoroquinolone resistance. The expression of *qnrS* revealed by proteomic analysis could explain the resistance against enrofloxacin and perfloxacin which cannot be substrates for *N*-acetylation. This is the first report that a high-level fluoroquinolone resistant *Aeromonas* sp. possessed four different resistance mechanisms some of which were conferred by a transferable plasmid.

GS-2

Metabolic Pathway Analysis for Efficient Succinic Acid Production

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Mannheimia succiniciproducens, a capnophilic gram-negative rumen bacterium, has intensively been studied due to its native capabilities to produce a substantial amount of succinic acid. Although a metabolic network of *M. succiniciproducens* was previously studied in a systematic manner, it still has more to be explored. In order to gain further insights in its metabolic network for biotechnological implications, we used elementary mode (EM) analysis, one of the well-established metabolic pathway analysis approaches, and conducted comparative analysis of *M. succiniciproducens* and *Escherichia coli* metabolisms. In this study, we reconstructed a small scale model of *M. succiniciproducens* that consists of central metabolic reactions including biomass equation according to demand ratio of major precursor as described previously. Among the thousands of EMs, we designed a efficient approach, pathway clustering analysis, by clustering optimal EMs that have the higher succinic acid production without loss of growth rates to systemically identify metabolic network. It was shown that phosphoenolpyruvate carboxykinase (*pckA*) which converts phosphoenolpyruvate to oxaloacetate with simultaneously generating ATP is the major factor of efficient succinic acid production. Also, pathway clustering analysis could present linear relationships with biomass or succinic acid in both *M. succiniciproducens* and *E. coli* metabolic network. In this proof-of-concept study, the biochemical network of *M. succiniciproducens* was rewired to improve succinate production by overexpressing pentose phosphate pathway in LPK7 strain with *ldhA* (lactate dehydrogenase), *pta-ack* (phosphotransacetylase and acetate kinase) and *pflB* (pyruvate formate lyase) genes disrupted. In conclusion, the newly devised approach, pathway clustering analysis, has applied to *M. succiniciproducens* and *E. coli* to grasp the notable differences between two organisms. Furthermore, this analysis successfully redesigned the biochemical network for higher production of succinic acid.

GS-3

Quorum Sensing for Biofilm Formation and Oil Degradation in *Acinetobacter oleivorans* DR1

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The complete genome of *Acinetobacter oleivorans* DR1 contains AqsR and AqsI genes, which are LuxR and LuxI homologues, respectively. In a previous study, we demonstrated that quorum sensing (QS) signals play an important role in biofilm formation and hexadecane biodegradation. However, the regulation of genes controlled by the QS system in DR1 remains unexplored. We constructed an *aqsR* mutant and performed RNA sequencing analysis to understand the QS system. A total of 353 genes were differentially expressed during the stationary phase of wild-type cells compared to that of the *aqsR* mutant. AqsR appears to be an exceptionally important regulator, because knockout of *aqsR* affected global gene expression. Genes involved in posttranslational modification, chaperones, cell wall structure, secondary metabolites biosynthesis, and stress defense were highly up-regulated only in the wild type. Among up-regulated genes, both the AOLE_03905 (putative surface adhesion protein) and the AOLE_11355 (L-asparaginase) genes have putative LuxR binding sites at their promoter regions. Soluble AqsR proteins were successfully purified in *Escherichia coli* harboring both *aqsR* and *aqsI*. Comparison of QS signals in an AqsI-AqsR co-overexpression strain with N-acyl homoserine lactone standards showed that the cognate N-acyl homoserine lactone binding to AqsR might be 3OH C12HSL. Our electrophoretic mobility shift assays with purified AqsR revealed direct binding of AqsR to those promoter regions. Our data showed that AqsR functions as an important regulator and is associated with several phenotypes, such as hexadecane utilization, biofilm formation, and sensitivity to cumene hydroperoxide. Interestingly, QS-controlled phenotypes appeared to be inhibited by indole, and the *aqsR* mutant had the same phenotypes. We confirmed that the turnover rate of AqsR became more rapid without the AHL signal and that indole could increase the expression of many protease and chaperone proteins. The addition of exogenous indole decreased the expression of two AqsR-targeted genes, which were AOLE_03905 (putative surface adhesion protein) and AOLE_11355 (L-asparaginase). The overexpression of AqsR in *Escherichia coli* was impossible with the indole treatment. Surprisingly, [^{35}S]-methionine pulse labeling data demonstrated that the stability and folding of the AqsR protein decreased in the presence of indole without changing the *aqsR* mRNA expression in *E. coli*. Here, we provided evidence for the first time showing that the indole effect on QS-controlled bacterial phenotypes is due to inhibited QS regulator folding and not a reduced QS signal.

GS-4

Community Structure Analysis and Characterization of Soil Humic Substances-Degrading Bacteria from Cold Environments

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Soil humic substances (HS), composed of mainly humic acids (HA) and fulvic acids (FA), are widely distributed in cold natural environments and known as an important fraction of soil organic carbon. Although bacteria dominate cold environments, there have been few studies on the HS utilization by individual bacterial strains. This study investigates the correlation between cold-adapted bacteria and their HS degradation. Under microcosm systems with subarctic HS-rich tundra soil (soil temperature in thawing period, ~5.6°C) of Council in Alaska, HA content significantly decreased to 48% after 99 day-incubation at 5°C by biologically mediated process, while FA, putative HA degradation products, consistently increased to 172% during the identical period. Culture-independent community analysis showed that, during the microcosm experiments, the relative abundance of phyla *Proteobacteria* largely increased, indicating their involvement in HS degradation. When the indigenous bacteria in the soil were enriched in an artificial mineral medium spiked with HA, the changes in relative abundance were most conspicuous in *Proteobacteria* (from 60.2% to 79.0%) and class *Betaproteobacteria*-related bacteria were highly enriched.

In order to examine the bacterial functions as HS-degraders in cold soil environments, a total of 122 bacterial strains were isolated on minimal agar plates containing HA from 66 different soil samples in Alaska. They were identified based on their 16S rRNA gene similarity using EzTaxon program, with *Bacilli* (79.5%) and *Gammaproteobacteria* (17.1%) comprising the largest portion. These isolates showed steady increase in HA degradation rates with temperature rise in a range of 0-20°C, with even more drastic increase at 8°C compared to 6°C. These results indicate that bacterial HS degradation is in progress at temperature as low as 5°C in cold tundra ecosystem. A detailed analysis of the sequences of 45 good HA-degraders showed that they are affiliated to several taxa: *Paenibacillus* spp., 27 strains; *Pseudomonas* spp., 15 strains; *Rhodococcus* spp., 2 strains; *Serratia* sp., 1 strain. The 45 strains were in detail tested on their degradability for HA and various monocyclic aromatics which are putative degradative metabolites of HS. Finally, two bacterial strains (*Pseudomonas* sp. PAMC 26793 and *Paenibacillus* sp. PAMC 26794) were selected as excellent HS-degraders and characterized to have different pathway(s) for HS degradation. When the initial and final structures of HA after incubation with PAMC 26793 or PAMC 26794 were compared, significant changes in the functional groups and molecular distribution were detected in the final structure by FT-IR and gel permeation chromatography analyses.

To search for essential initial enzymes for HS-degradation, the genomes of PAMC 26793 and 26794 were sequenced and analyzed. Interestingly, a laccase-coding gene was detected on PAMC 26793 genome. Because fungal laccases and other nonspecific oxidizing enzymes (i.e., lignin peroxidase and manganese peroxidase) have been known as the first actor for degradation of HS, the laccase gene is under the study for its function in HS-degradation through gene knockout. In addition, cold-adapted HA-utilizing bacterial strains were isolated also from the Antarctic King Sejong Station (53 strains) and the Arctic Dasan Station (20 strains) to examine the

distribution and function of HS degraders in bi-polar regions. The isolates had optimal degradability at 15-20°C, while they could not grow over 30°C. Based on 16S rRNA gene similarity, the 73 HA-degraders were affiliated to two main taxa: *Pseudomonas* spp., 69.9% and *Rhodococcus* spp., 13.7%. Among the bi-polar strains, 52 isolates (71%) showed approximately 142 bp-PCR products for laccase-like multicopper oxidases (LMCO) conserved region. In conclusion, the information on microbial degradative activities against HS would help us to predict the effects on polar ecosystems of climate change like global warming.

GS-5

Development of Rapid One-Step Inactivation Tool and Engineering of *E. coli* to Produce Fumaric Acid

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We developed an integration helper plasmid-based gene manipulation system for more efficient and rapid engineering of *E. coli* and this tool was directly used for developing fumaric acid producing strains. The integration helper plasmid, pCW611, contains two recombinases which are expressed in reverse direction by two independent inducible systems. The main advantage of this system is that the time and effort required can be significantly reduced because the iterative transformation of the helper plasmids 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 (*adhE*, *sfcA*, *frdABCD*, and *ackA*) and two genes simultaneously for two cases (*adhE-aspA* and *sfcA-aspA*). Also, sequential deletion of four target genes (*fumB*, *iclR, fumA*, and *fumC*) was successfully performed for the construction of fumaric acid producing strain. Additionally, strain performances were further improved by sequential deletion of the *arcA*, *ptsG*, *aspA* genes and replacement of native promoter with strong *trc* promoter. (Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A2026556) funded by the Ministry of Education, Science and Technology)

GS-6

Genetic Bases of Enhanced *Pseudomonas aeruginosa* Biofilm Development by Sub-Minimum Inhibitory Concentration Treatment of Antibiotics

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Pseudomonas aeruginosa is one of the most popular bacteria which studied by numerous microbiologist for many years. It is a nosocomial Gram negative opportunistic pathogen. The bacterium is readily isolated from environments around us and is not a serious threat to healthy individuals. *Pseudomonas aeruginosa*, however, can cause serious infections in immunocompromised individuals such as respiratory infections, urinary tract infections, otitis media, wound infections, gastrointestinal infections, and bacteremia. Most of these infections are associated with biofilms. *Pseudomonas aeruginosa* is known to be one of the powerful biofilm formers. Biofilm infections have been getting more attention recently because it is the mode of infection for chronic bacterial infections and capable of causing many serious problems. These problems include evasion of immune defense of hosts and increased resistance against antimicrobial agents.

The removal of biofilm from infected site was one of the biggest challenges to treat the biofilm infections. So far there are no effective ways to treat biofilm infections other than surgical removal of the infected sites. Few studies suggested that sub-inhibitory concentration of antimicrobial treatments can alter phenotypes of *Pseudomonas aeruginosa* to prevent the attachment of the bacteria on surfaces which results in preventing biofilm formation. However, this may not be the case because there are many other studies presenting that sub-Minimum inhibitory concentration (MIC) of antimicrobial treatments (carbapenems and aminoglycosides) enhances the development of biofilms. The phenomena had, also, been confirmed in our lab using two other types of antimicrobials (β-lamtams and polymyxin antibiotics). Even though the sub-MIC of antimicrobial treatments has shown to enhance biofilm developments, only few studies were conducted on the genetic and mechanism bases of the phenomenon. In this investigation, we are using forward-genetics techniques to identify the significant genes and possible mechanisms that influence the enhanced biofilm development. The identification of genetic factors for the biofilm enhancement under sub-MIC treatments of antimicrobials can be applied to preventing and reducing the chance of biofilm infections caused by residual antibiotics from previous treatments.

GS-7

Comparison of CO-dependent H_2 Production with Strong Promoters in *Thermococcus onnurineus* NA1

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To overproduce biotechnologically valuable products, the expression level of target genes has been modulated by using strong promoters. In a hyperthermophilic archaeon *Thermococcus onnurineus* NA1, two promoters, P_{TN0413} and P_{TN0157}, which drive expression of the genes encoding the Slayer protein and glutamate dehydrogenase were inserted in front of a gene cluster encoding a carbon monoxide dehydrogenase, a hydrogenase and a Na^+/H^+ antiporter. Two promoters exhibited strong activity by increasing the transcription and translation levels of the gene cluster in the mutant strains by 2.5- to 49-folds and 1.4- to 3.3-folds, respectively, than the native promoter in the wild-type strain. While KS0413 with P_{TN0413} promoter exhibited 2.7 to 4.7 times higher transcript level than KS0157 with P_{TN0157} promoter, the levels of proteins were a little different between them. The biomass concentrations and H_2 production rates of two mutants were 2- to 3-fold higher than those of the wild-type strain in a bioreactor where CO was supplied at a flow rate of 120 ml/min. Two mutants showed differential response to the higher CO flow rate, 240 ml/min, in terms of growth pattern and product formation, indicating two promoters were regulated by culture conditions. The results demonstrate that not only promoter strength but also product-forming conditions should be considered in promoter engineering.

GS-8

Characterization of Plasmid pEMB1 Harboring a β-Lactamase Gene and a Toxin-antitoxin System

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Antibiotic resistance genes have become a major problem for human, animal and plant health. These genes are not only detected in clinical environments, including sewage, river, canal and wastewater, but also have been found in various natural (non-clinical) environments, such as remote Alaskan soil, Antarctic marine waters, ancient sediment sample and Glacier ice core. In present study, unique plasmid encoded ampicillin resistance gene with Toxin-Antitoxin system was isolated from clinical (sewage treatment plant from Tanchoen in Korea) and natural environment (Mt. Jeombong in Korea) by directly transformation into *E. coli*, named pEMB1. The plasmid pEMB1 was determined 8,744 bp in length and six putative ORFs (Open Reading Frames), including putative Tn3 transposon, consist of transposase (*tnpA*), DNA invertase (*tnpR*) and β-lactamase (*bla*), putative plasmid replication protein (*repB*), *orf5* and putative toxin (*parE*). BLAST homology searches indicated that *orf5* gene likely encoded a CopG family transcriptional regulator. Here, we report physiological and genetic evidence that *orf5* gene encodes antitoxin. Accordingly, a name for *orf5*, *parD*, was proposed. Monitoring of the pEMB1-like plasmids was attempted from various clinical and natural environments by nested touchdown-PCR. As a result, pEMB1-like plasmids was identified from other human activity-related samples and remote mountain soil samples. This study will shed light on understanding the development of antibiotic resistant bacteria and the dissemination of antibiotic resistance genes in environments.

GS-9

Identification and Role of the DNA-Damage Response Two Component System, DrtR/S, in *Deinococcus radiodurans*

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Bacteria are able to adapt to changes in the environment using two-component signal transduction systems (TCSs) composed of a histidine kinase (HK) and a response regulator (RR). HKs are the first proteins to sense and relay the environmental signal to its partner protein, RR. *Deinococcus radiodurans*, one of the most resistant organisms to ionizing radiation, has 20 putative HKs and 25 putative RRs. In this study, we constructed 12 *D. radiodurans* mutant strains lacking a gene encoding a HK and surveyed their resistance to γ-radiation, UV-B radiation, mitomycin C (MMC), and H_2O_2. Among them, *dr2416* (HK) mutant is sensitive to γ-radiation, UV-B, MMC, and H_2O_2 compared with the wild-type strain. DR2415, which seems to be a cognate RR of DR2416, plays a role in the resistance to DNA-damaging agents. Thus, we named this TCS, composed of DR2415/DR2416 DrtR/S, the DNA damage Response TCS Regulator/Sensor. We investigated the expression of the *dr0053* gene known as a DNA damage-inducible (*din*) gene, in *dr2415* and *dr2416* mutant strains. The *dr0053* gene was highly induced upon gamma radiation in the presence of DrtR/S as well as RecA, suggesting that the DrtR/S is involved in *din* gene. Microarray analysis was performd in the absence of *drtR*. The function of up-regulated genes, *drB0007* and *drB0125*, belong to iron uptake system. Considering the capacity of iron to generate reactive oxygen species, this result suggests that DrtR/S can exert its protective effect by modulating iron homeostasis.

GS-10

Potassium Ion-Mediated Regulation of Biofilm Formation via Controlling Cellular Level of Sigma S

Yu-Sook Chung, Kyung-Jo Lee, and Kyu-Ho Lee*

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Biofilm-forming activity of *Vibrio vulnificus* is essentially required for eliciting the pathogenicity in host environments. Thus its activity is programmed to be regulated at multiple levels during the whole period of biofilm developmental stages. Upon maturing biofilms, quorum-sensing plays a regulatory role by inducing production of capsular polysaccharide (CPS) and enhancing dispersal of cells to enter the final stage of biofilm life-cycle. Since cells within a mature biofilm of high cell-density structure are presumed to be starved for some essential nutrients, a global stress regulator, RpoS was investigated in this study whether it functions as a key factor for another regulatory circuit controlling formation of biofilm. *rpoS*-deficient *V. vulnificus* formed significantly increased biofilms compared to wild type. Examination of the mutant phenotypes revealed that *rpoS* mutant exhibited reduced production of CPS due to decreased transcription of the gene-cluster for CPS biosynthesis. Reduced CPS production provided its cell surface with increased hydrophobicity, resulting in increased formation of biofilm. Regulation of RpoS expression is achieved at various levels including transcription, post-transcription, and post-translation. In addition, potassium ion has been shown to regulate the RpoS activity in *E. coli*. Thus, the effect of potassium ion on RpoS was investigated in *V. vulnificus* and found that cellular level of RpoS was increased under the limited concentrations of potassium ion, due to an increased stability of RpoS *in vivo*. Furthermore, the potassium ion-depleted cells showed increased CPS production and reduced biofilms. Therefore, this study suggests that biofilm cells under limited supply of essential nutrients, such as potassium ion, are facilitated to be dispersed to other niches via increased production of CPS, which is achieved by increased level of its transcription activator, RpoS.

GS-11

Metagenomic and Metatranscriptomic Analysis of Kimchi, a Traditional Korean Fermented Food

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Kimchi is a Korean traditional food made by fermentation of vegetables. Lactic acid bacteria are well known to perform significant roles in kimchi fermentation. In this study, metagenomic and metatranscriptomic analyses were performed to monitor changes in microbial community structure, metabolic potential, and gene expression during the kimchi fermentation. Metagenomic DNA and expressed mRNA were extracted from periodic kimchi samples and sequenced using 454 GS FLX Titanium and Illumina GA IIx, respectively. Taxonomic analysis based on 16S rRNA genes from the metagenome indicated that the kimchi microbiome was dominated by *Leuconostoc* (*Lc.*), *Lactobacillus* (*Lb.*), and *Weissella* (*W.*). Assignment of metagenomic sequences to SEED categories revealed the prevalence of carbohydrate metabolism and lactic acid fermentation. Interestingly, a large number of phage DNA sequences were identified, possibly indicating a high proportion of cells were infected by bacteriophages during fermentation. To investigate metatranscriptomic gene-expression profiles, the mRNA sequencing reads were mapped onto representative genome sequences of the predominated six LAB species (*Lc. mesenteroides*, *Lb. sakei*, *W. koreensis*, *Lc. gelidum*, *Lc. carnosum*, and *Lc. gasicomitatum*), which showed that *Lc. mesenteroides* was most active during the early-stage fermentation, whereas gene expression by *Lb. sakei* and *W. koreensis* was high during later stages. However, gene expression by *Lb. sakei* decreased rapidly at 25 days of fermentation, which was possibly caused by bacteriophage infection. Many genes related to carbohydrate transport and hydrolysis and lactate fermentation were actively expressed, which indicated typical heterolactic acid fermentation. These results provide insights into the kimchi microbial community and also contribute to knowledge of the active populations and gene expression in the LAB community responsible for an important fermentation process.

GS-12

Staphylococcus aureus Vesicles Modulate the Surface Hydrophobicity Which Inhibits Other ESKAPE Pathogens from Forming Biofilms

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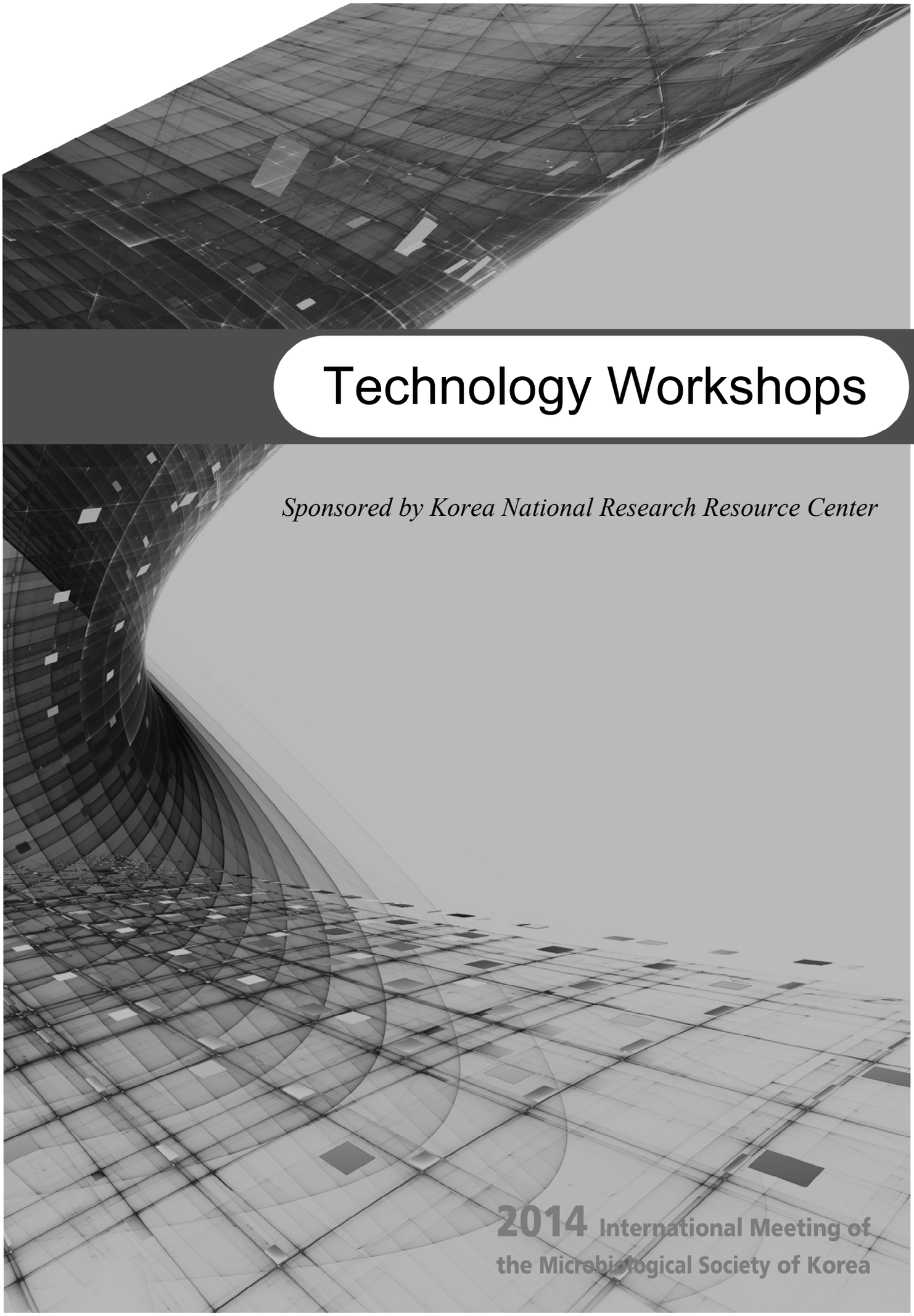

Technology Workshops

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TW1-1

Next-generation Sequencing 기술의 원리와 응용

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차세대 시퀀싱 기술은 기존의 Sanger 시퀀싱 방법에 비해 훨씬 저렴하면서도 막대한 양의 데이터를 얻을 수 있는 장점이 있지만, 다양한 시퀀싱 플랫폼 및 그로부터 유래되는 방대한 양의 데이터는 분석에 있어서 오히려 어려움을 가져오는 요소가 되고 있습니다. 현재 Illumina와 Roche 454 시퀀싱 기술은 미생물 게놈 분석에 가장 널리 사용되는 기술로 각각 생산되는 데이터 대비 비용이 저렴하고 시퀀스 길이가 긴 장점이 있으며 이외에도 Pacific Biosciences의 SMRT 시퀀싱 기술 및 Life Technologies의 Ion torrent 등의 여러 다른 기술들이 개발되어 있으나, 연구자의 목적에 부합하는 시퀀싱을 위해서는 각 NGS 시퀀싱 플랫폼의 특징을 알고 용도에 맞게 적용할 필요가 있겠습니다. 이에 각 NGS 시퀀싱 플랫폼의 원리와 그 결과 데이터의 특성, 적합한 용도 등에 대하여 알아보고자 합니다.

TW1-2

Next-generation Sequencing 기술을 이용한 세균의 분류동정

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Genetic information has been used as one of the key elements in circumscribing bacterial species since the molecular method was introduced to the field of microbiology. With the recent revolution of DNA sequencing technologies, the number of sequenced bacterial genomes has been increased dramatically at an unflagging rate. With the combination of low-cost genome sequencing methods and suitable bioinformatics tools, the use of whole genome information has a great potential in bacterial taxonomy, especially in ways that bacterial species is defined. Traditional taxonomic traits could be replaced or refined by more precise and accurate genome-driven traits. DNA G+C content can be determined more accurately by simply counting the proportion of guanine and cytosine from genome sequences. Laborious DNA-DNA hybridization (DDH) can be superseded by 'digital' DDH (e.g. average nucleotide identity) by direct comparison between two genome sequences. Bacterial taxonomy can also be improved by additional genome information. Phenotypic traits can be further supported by the presence or absence of diagnostic genes. Phylogenomic approach based on whole genome data is able to provide a more comprehensive view in inferring the evolutionary relationship between organisms. As genome sequencing becomes more common, genomics will be more rapidly incorporated into the current taxonomic framework of bacteria.

TW1-3

Genome Resequencing을 이용한 세균 적응진화연구

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Over the past decade or so, dramatic developments in our ability to experimentally determine the contents and functions of genomes have taken place. In particular, high-throughput technologies are now inspiring a new understanding of the bacterial genome on a global scale. Recently, the development of next-generation DNA sequencing (NGS) techniques provide the ability to overcome the limitations of microarrays and now allow for the investigation of transcription at single nucleotide resolution with better signal-to-noise ratio. In particular, NGS techniques offer thousands of times faster, deeper and cheaper reading tools than traditional methods for obtaining genome-wide profiles of mRNAs, regulatory RNAs, transcription factor binding regions, structure of chromatin and DNA modification patterns in addition to the genome sequencing. NGS tools are being used to study laboratory evolution by providing complete determination of the genetic basis of adaptation. Here, we introduce how to apply genome resequencing tool to elucidate the genetic basis of adaptation and the dynamics with which laboratory populations evolve.

TW1-4

Transcriptome Analysis and Its Applications by Using Stranded/Differential RNA-sequencing Technologies

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Unlike the previous microarray experiments, RNA-sequencing technology, which directly sequence RNA, does not use the hybridization probes for the experiments. Thus, the RNA-seq reduces noise signals level that occur on the hybridization step. In addition, RNA-seq has much more dynamic range than microarray; RNA-seq utilizes number of reads to determine expression level.

The procedure of RNA-seq is as followed: i) total RNAs are collected from cells, and fragmented into small pieces around the specific size, ii) adaptors are ligated at both ends of RNA, iii) cDNA gets synthesized by the adaptors, iv) finally, cDNAs are amplified by PCR, then DNA library is completed. This library contains information on the whole transcriptome. When cDNA is synthesized by using only primary transcripts, which constructed by an enzyme treatment of total transcripts (differential RNA-sequencing), more accurate genome architectures can be identified. At last, transcriptome sequencing prepared by the strand-specific RNA (stranded RNA-sequencing) produces more reliable expression level and unique mapping regions than microarray data.

In this talk, procedures of stranded RNA-seq and differential RNA-seq and various applications will be presented in detail.

TW1-5

Principle and Application of ChIP-seq to Understand Transcriptional Regulation

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ChIP-seq is the primary method for the discovery of the regions where a DNA-binding protein binds the genome *in vivo* on a genomic scale. The most widely investigated DNA binding protein is transcription factors but any proteins that have DNA-binding ability such as DNA-binding enzymes, chaperones, or nucleosomes can be investigated. ChIP-seq is conducted by crosslinking bounded proteins to the DNA fragments and selectively captures the DNA-proteins complexes by using an antibody specific to the protein. Captured DNA fragments are rendered to massive sequencing by using next-generation sequencing (NGS). Through the computational analysis of the sequenced DNA identifies the genomic locations and thereby show the role and regulation of the DNA-binding protein. In this talk, the principle of ChIP-seq experiment and their computational analysis will be presented.

TW1-6

Translatomic Analysis by Using Ribo-seq Technique

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Development in sequencing technologies have allowed huge advances in genomics and transcriptomics. However research for translatomics has not benefit yet until the recent development of the Ribo-seq method. Ribo-seq allows the sequencing of mRNA buried inside the active ribosomes for translation. By analyzing these mRNAs, we can obtain data on actual mRNA sequence that are being translated, identity of the reading frames, and ribosomal density at position within the mRNAs. These data allows the calculation of translation efficiency (TE) and determine different regulation of translation in comparison to mRNA level. In this workshop, we present actual application of Ribo-seq for the *Stretomyces coelicolor*.

TW2-1

Introduction to Metabolomics: Methods, Protocols and Applications

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Metabolomics, as integral part of the whole '-Omics', is "comprehensive analysis of the whole metabolome under a given set of conditions" (Fiehn, O., et. al Nat. Biotechnol. 2000;18:1157-1161). In the near future, metabolomics might play central role in '-Omics' science since the metabolome is outcome of up-stream regulatory events of genome, transcriptome, and proteome, and its level change can be regarded as the ultimate response of biological systems to genetic or environmental changes, and is the most predictive of phenotype.

This presentation introduces nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy (MS) methods and protocols for metabolomics; (i) instrumentations and principles, (ii) sample and preparation, (iii) data acquisition and processing, (iv) metabolite identification and quantification, (v) and multivariate analysis, such as principle component analysis (PCA), partial least squares discriminant analysis (PLS-DA).

Finally, based on published papers, exemplary applications of metabolomics in clinical cancer research, plant biology, and microbial research are presented.

TW2-2

Glycosylation 분석을 위한 질량분석기 원리와 응용

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당화(Glycosylation)는 박테리아와 같은 미생물에서 암세포 등의 동물세포까지 거의 모든 세포표면에 나타나 있으며, 다당체(Polysaccharides), 당단백질(Glycoproteins), 당지질체(Glycolipids) 등의 당복합체(Glycoconjugates) 형태로 존재한다. 주로 세포표면에 발현되어 있는 이러한 당복합체는 외부자극과 가까운 곳에 있기 때문에, 세포-세포 상호작용, 숙주-병원균 상호작용, 병원균의 항생제 내성 몇 병독성 등 생물학적으로 중요한 역할을 담당하고 있다. 그렇기 때문에 특정 당복합체의 생물학적 역할을 이해하기 위해서 그 분자구조를 분석하고 규명하는 것이 필요하다. 최근에는 극미량의 샘플로 부터 정확한 분석을 구현하는 고분해능 질량분석기 (High-resolution mass spectrometry)의 장점으로 인해 미량의 다양한 생체시료들로부터 특정 물질의 정성/정량 분석이 가능해지고 있다. 본 워크샵에서는 질량분석기를 이용해서 박테리아에서 보고되고 있는 당단백질 (*O*- and *N*-linked glycoproteins)을 분석하기 위해 필요한 과정에 대해 설명하고자 한다.

TW2-3

Principle and Application of Bimolecular Fluorescence Complementation Assay for Protein-protein Interaction Study

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Protein-protein interactions (PPIs) are crucial for all biological processes. Their identification and characterization are necessary in order to elucidate these processes and for the functional identification of unknown proteins. There are many methods currently in use for the detection of protein interactions with new methods continually being developed. Bimolecular fluorescence complementation (BiFC) is a suitable technique to investigate the formation of protein complexes and the localization of protein–protein interactions in the natural cellular context. Here, we introduce a BiFC technique to study PPI in a high-throughput manner in living cells. First, we describe the construction of a *Saccharomyces cerevisiae* fusion library in which each endogenous gene is C-terminally tagged with the N-terminal fragment of Venus (VN) for a genome-wide BiFC assay. Using the VN fusion library, we systematically analyzed the interactome of the small ubiquitin-related modifier (SUMO). Furthermore, we also report a high-throughput method for cloning human G-protein coupled receptor (GPCR) cDNAs into adenoviral bimolecular fluorescence complementation (AdBiFC) vectors, performing the β-arrestin BiFC assay and screening GPCR heterodimerization in mammalian cells. Collectively, the VN fusion library and the AdBiFC system provide a useful research tool that makes it feasible to systematically analyze PPIs in the natural cellular context.

TW2-4

Synthetic Regulatory Small RNA for Fine-tuning Gene Expression and Its Application

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Efficient and convenient control over gene expression in genome-wide scale is essential in fundamental and applied biological studies, but is still one of great challenges due to the difficulties in constructing libraries. Small regulatory RNAs (sRNAs) are short non-coding RNAs that can finely control the expression of target genes in *trans* at the translational level in prokaryotes. Recently, I reported the development of a new genome-wide gene expression control system based on synthetic sRNAs, which utilizes the abilities and characteristics of natural sRNAs. Synthetic sRNAs can be utilized for diverse experiments where gene expression regulation is needed. One of promising applications is high-throughput screening of the target genes to be manipulated and multiple strains simultaneously to enhance the production of chemicals of interest. Such simultaneous optimization of gene targets and strains has been one of the big challenges in metabolic engineering. Another application is to fine tune the expression of the screened genes for flux optimization, which would enhance chemical production further by balancing the flux between biomass formation and target chemical production. Synthetic sRNAs can also be applied to finely regulating genetic interactions in a circuit or network, which is essential in synthetic biology. This lecture will be useful for all researchers in the academia and industry who are interested in the use of synthetic sRNAs for fundamental and applied biological and biotechnological studies.

TW2-5

The Principle and Application of RNA-guided Nuclease Based Genome Editing

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Since the success of Human Genome Project, gene therapy tools like zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) became spotlighted as a solution for genetic disorders. With specific interaction of nucleotide and protein domains, these genome editing tools induce non-homologous end joining or homologous recombination by introducing double-stranded break to target genome locus. Although its advantage of site-specific genome engineering, it has great deficiency due to protein library construction which requires time consuming procedure. Clustered regularly interspaced short palindromic repeats (CRISPR) system, which functions as a defense mechanism against bacteriophages in bacteria, arisen as a novel genome editing tool. It works with CRISPR RNAs (crRNAs) that lead Cas9 protein to specific genome locus. Because the target diversity of the system is depend on crRNAs, it allows much more efficient genome editing system. Throughout the session, the principle and application of CRISPR/Cas based genome editing tools and its experimental procedure will be introduced.

TW2-6

Bio-Imaging: How to Make a Good Digital Image

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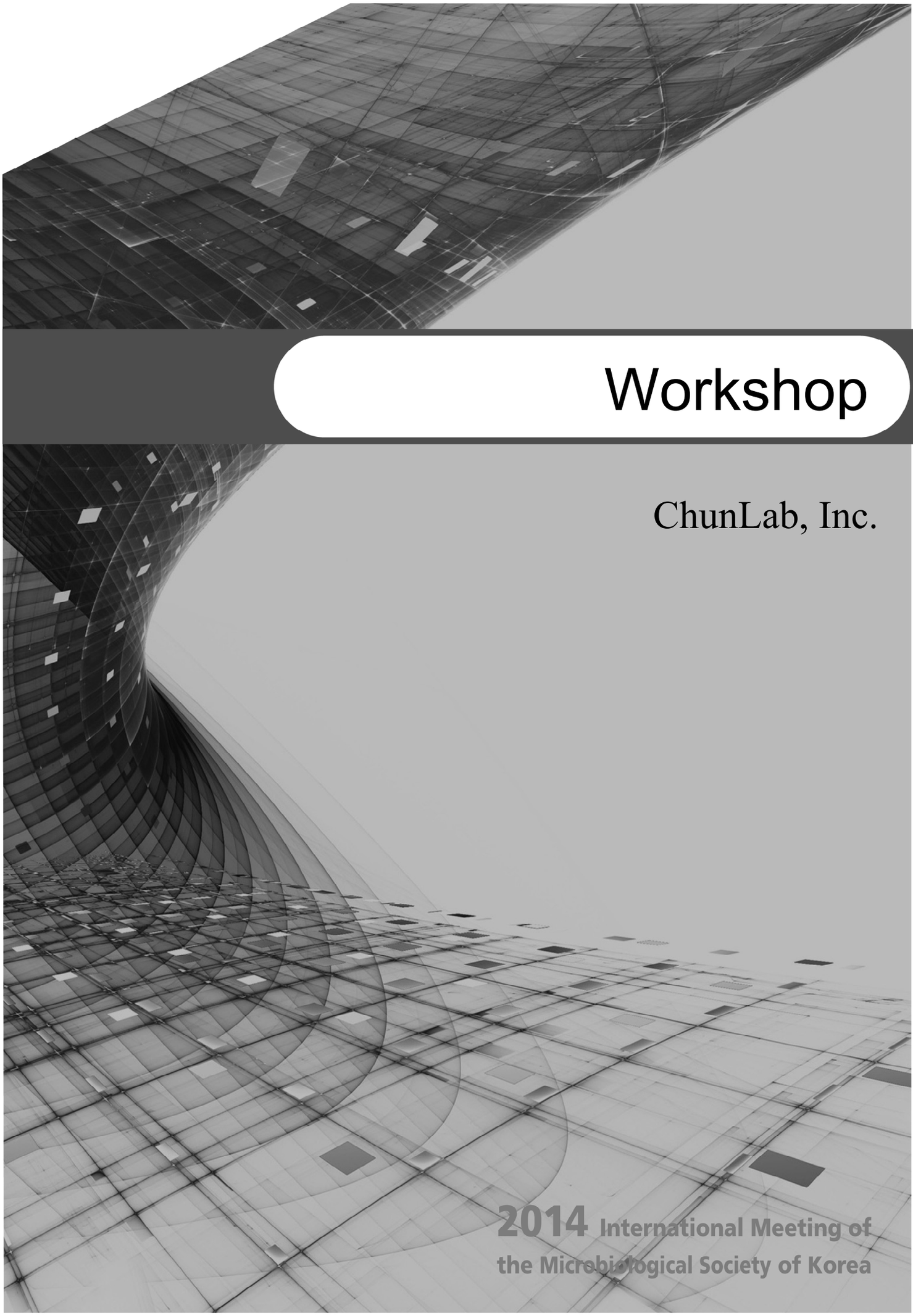
Image is an artificial record of visual information, usually captured by optical devices. There has been a tremendous improvement in tools for imaging, but the core rules that build an image have never changed since the development of the first camera by Joseph Nicéphore Niépce in 1826. In this talk, I would like to briefly introduce elements of an image, how the image is taken, and discuss about how to make a good image that can effectively depict the information. Contrast and Resolution are the two main keys, and components that build these two will be presented. As images of biological specimen in these days are obtained and stored in electronic devices, digital image and its processing will be mainly reviewed. Also, this talk will cover recent developments of bio-imaging, in the regard of super-resolution microscope for the breakthrough of resolution limit to see the unseen by traditional methods.



Workshop

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2014 International Meeting of
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W

RNA-Seq Analysis: Current Methods and Its Applications

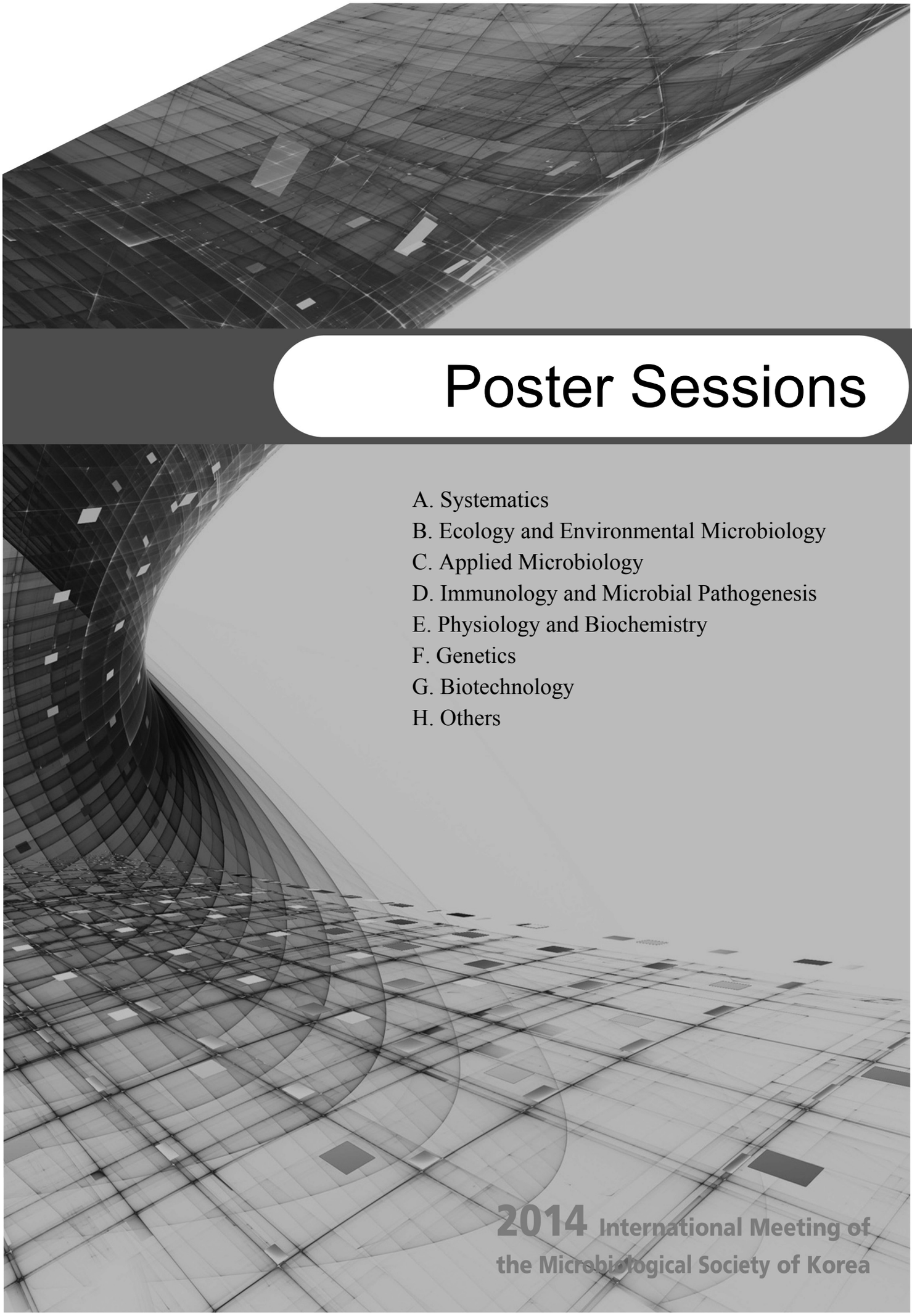
Namil Kim and Jongsik Chun

ChunLab, Inc.

RNA sequencing measures all gene expressions in specific species with a single experiment. RNA-Seq makes it possible to obtain more accurate and diverse information whether the genes are expressed, the level of expression or how the expression varies under certain conditions.

DNA microarray measures the hybridization of the cDNA on the microarray slide, however, RNA-Seq sequences the cDNA to map it against a reference gene in order to calculate the number of sequence reads. Sequencing results are mapped based on sequences from the reference genome. The number of sequence reads mapped for each gene are measured and normalized into RPKM values. The type of data that can be obtained from ChunLab's RNA-Seq service are Raw data as FASTQ file formats and analysis files as clt (*clt) file formats, which can be viewed and analyzed with ChunLab's CLRNASeq™ bioinformatics software. CLRNASeq™ provides various functions such as 1) mapping status browser 2) annotated RPKM table 3) scatter plot analysis and 4) clustering analysis (hierarchical clustering, K-Means clustering, SOM clustering).

RNA-Seq technology is more reproducible, convenient, and accurate than any other methods. To date, several protocols were designed for various applications. We will review current NGS technology in terms of RNA-Seq technology and out incredible service.



Poster Sessions

A. Systematics
B. Ecology and Environmental Microbiology
C. Applied Microbiology
D. Immunology and Microbial Pathogenesis
E. Physiology and Biochemistry
F. Genetics
G. Biotechnology
H. Others

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A001

A Polyphasic Investigation on a Novel Anaerobic Actinobacterium Isolated from Human Faeces

Jong-Sik Jin[1], Keun Chul Lee[2], In-Soon Park[2], Kwang Kyu Kim[2], Jong Seog Ahn[3], Yoshimi Benno[4], Masao Hattori[5], and Jung-Sook Lee[2*]
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A novel actinobacterial strain, designated CAT-2^T, was isolated from human faeces as a bacterium capable of dehydroxylating (+)-catechin derivative. Strain CAT-2^T was found to be strictly anaerobic, Gram-positive, non-motile and non-spore-forming coccobacilli. The major fatty acids were $C_{16:0}$ DMA (dimethy acetal), $C_{16:0}$, $C_{14:0}$, anteiso-$C_{15:0}$ and iso-$C_{14:0}$. The three predominant menaquinones were identified as MK-6 (menaquinene-6), MMK-6 (monomethylmenaquinone-6) and DMMK-6 (dimethylmenaquinone-6). The polar lipids were found to be diphosphatidylglycerol, phosphatidylglycerol and four unidentified glycolipid. The DNA G+C content of strain CAT-2^T was 68.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequence similarities showed that strain CAT-2^T belongs to the genus *Gordonibacter*, sharing the highest level of sequence homology with *Gordonibacter pamelaeae* DSM 19378^T (97.3%). Combined phenotypic, chemotaxonomic and phylogenetic characteristics supported the conclusion that the strain CAT-2^T represents a novel species, for which the name *Gordonibacter faecihominis* sp. nov. is proposed. The type strain is CAT-2^T (= KCTC 15204^T = JCM 16058^T).

A002

Neptunomonas daebuensis* sp. nov., Isolated from a Sediment of the Daebu-Island, Korea and Emended Description of Members of the Genus *Neptunomonas

Sung-Hyun Yang, Hyun-Seok Seo, Jung-Hyun Lee, Sang-Jin Kim, and Kae Kyoung Kwon*
Marine Biotechnology Research Center, Korea Institute of Ocean Science & Technology

A Gram-negative, aerobic, rod-shaped and non-motile marine bacterium, designated as MEBiC 06243^T was isolated from a sediment at the Daebu-Island of the Yellow Sea, Korea. The 16S rRNA gene sequence analysis revealed that strain MEBiC06243^T showed high similarity with the *Neptunomonas naphthovorans* NAG-2N-126^T (96.3%). Growth was observed at 10.2-38.7°C (optimum 30°C), at pH 6.0-9.0 (optimum 7) and with 0-7% (optimum 2.5) NaCl. The predominant cellular fatty acids were $C_{10:0}$ 3-OH (6.1%), $C_{12:0}$, (5.8%), $C_{16:0}$, (30.5%), $C_{18:1}\omega7c$ (21.6%) and summed feature 3 (30.7%). The DNA G+C contents is 41.4 mol%. The major respiratory quinone is Q-8. Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, two unidentified lipids, one unidentified amino-phospholipid and three unidentified aminolipids were detected as major polar lipids. On the basis of this polyphasic taxonomic data, strain MEBiC06243^T should be classified as a novel species in the genus *Neptunomonas* and it is proposed as *Neptunomonas daebuensis* sp. nov. The type strain is MEBiC 06243^T (=KCCM 42975^T =JCM 18291^T). Emended descriptions on the genus *Neptunomonas* were also given .
[Supported from KIOST & MBRB]

A003

***Pleiona flava* sp. nov., Isolated from the Sediment of Daebu-Island in the Yellow Sea, Korea**

Hani Jang, Sung-Hyun Yang, Hyun-Seok Seo, Jung-Hyun Lee, Sang-Jin Kim, and Kae Kyoung Kwon*
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A Gram-negative, non-motile, yellow pigmented and rod shaped strain, MEBiC 06907^T, was isolated from a sediment of Daebu-Island in the Yellow sea, Korea. Strain MEBiC 06907^T was oxidase positive and catalase negative. The strain is mesophilic, neutrophilic, and halophilic (require seawater component for growth). The 16S rRNA gene sequence analysis (98.1% similarity with *Pleionea mediterranea* DSM 25350^T) and DNA-DNA hybridization (26.12% with *P. mediterranea* DSM 25350^T) revealed that strain MEBiC 06907^T is a novel member of the genus *Pleionea*. The predominant cellular fatty acids were i-$C_{14:0}$, i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$, and summed feature 9 (comprised of $C_{16:0}$ 10-methly and/or $C_{17:1}\omega9c$). The DNA G+C contents is 46.7 mol%. The respiratory quinone is Q-8. The predominant polar lipids were phosphatidylethanolamine, phosphatidylglycerol, unidentified phospholipid, unidentified glycolipid, unidentified aminolipid and three unidentified aminophospholipids. On the basis of this polyphasic taxonomic data, strain MEBiC 06907^T should be classified as a novel species in the genus *Pleionea*, and proposed as *Pleiona flava* sp. nov. The type strain is MEBiC 06907^T.
[Supported from KIOST & MBRB]

A004

***Owenweeksia salterni* sp. nov., a Marine Bacterium Isolated from Seawater in the Saltern**

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A novel Gram-negative rod-shaped aerobic and motile strain, designated MEBiC 09403^T, was isolated from seawater in the solar saltern of the Jeolla province, South Korea. Strain MEBiC 09403^T produces orange-coloured colonies on Marine Agar and possess a single polar flagellum. On the basis of 16S rRNA gene sequence analysis, the closest relative was *Owenweeksia hongkongensis* DSM17368^T with 93.3% similarity and followed by *Phaeocystidibacter luteus* PG2S01T with 89.9% similarity. Growth was observed at 12.4–33.5°C (optimum, 21°C). When assayed with the API Zym systems acid phosphatase, alkaline phosphatases, leucine arylamidase and valine arylamidase activities were present. Based on phenotypic properties and phylogenetic data, strain MEBiC09403^T should be classified within the genus *Owenweeksia* and suggest *O. salterni* sp. nov. with strain MEBiC09403^T is proposed.
[Supported from KIOST & MBRB]

A005

***Flavihumibacter solisilvae* sp. nov., Isolated from Forest Soil**

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A Gram-staining-positive, strictly aerobic, yellow colony-forming bacterium, designated strain 3-3^T, was isolated from forest soil of Bac Kan Province in Vietnam. Cells were non-motile rods without gliding motility, showing oxidase- and catalase-positive reactions. Growth was observed at 20-37°C (optimum, 28°C) and pH 5.5-9.5 (optimum, pH 7.5). The major cellular fatty acids were iso-$C_{15:0}$, iso-$C_{15:1}$ G and summed feature 3 (comprising $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*). The G+C content of the genomic DNA was 49.5 mol% and the only isoprenoid quinone detected was menaquinone 7 (MK-7). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 3-3^T formed a tight phylogenetic lineage with *Flavihumibacter petaseus* T41^T with a bootstrap value of 100%. Strain 3-3^T was most closely related to *F. petaseus* NBRC 106054^T with 97.3% 16S rRNA gene sequence similarity and their DNA-DNA relatedness level was 9.4±1.2%. Based on phenotypic, chemotaxonomic and molecular features, strain 3-3^T represents a novel species of the genus *Flavihumibacter*, for which the name *Flavihumibacter solisilvae* sp. nov. is proposed. The type strain is 3-3^T (=KACC 17917^T = JCM 19891^T).

A006

***Chujafilamentum soli* gen. nov., sp. nov., Isolated from Soil**

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[1]Agricultural Microbiology Division, National Academy of Agricultural Science, [2]Department of Food Science and Biotechnology, Sungkyunkwan University

A Gram-staining-negative, aerobic, non-flagellated and filament-shaped bacterial strain, KIS 55-21^T, was isolated from a soil sample of Chuja Island, Republic of Korea. Phylogenetic trees based on 16S rRNA gene sequences showed that strain KIS 55-21^T was closely related with *Metallibacterium scheffleri* DKE^T. Strain KIS 55-21^T exhibited the highest 16S rRNA gene sequence similarity value of 92.6% to *Metallibacterium scheffleri* DKE^T. Strain KIS 55-21^T contained Q-8 as the predominant ubiquinone, iso-$C_{17:0}$, summed feature 9, anteiso-$C_{17:0}$ and $C_{16:0}$ as the major fatty acids, and PE, APL, PG, DPG and PME as the main polar lipids. The DNA G+C content of strain KIS 55-21^T was 65.9 mol%. On the basis of the data presented, strain KIS 55-21^T is considered to represent a novel genus and species, for which the name *Chujafilamentum soli* gen. nov., sp. nov. is proposed. The type strain is KIS 55-21^T (=KACC 16971^T).
[This study was carried out with the support National Academy of Agricultural Science, Rural Development Administration, Republic of Korea.]

A007

Pragia protaetiae* sp. nov., Isolated from the Gut of *Protaetia brevitarsis

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A bacterium (A-27^T) was isolated from the midgut of *Protaetia brevitarsis*. On the basis of a 16S rRNA gene sequence similarity, strain A-27^T was shown to belong to *Proteobacteria*, related to the genus *Pragia*, and shared 97.08% similarity with the only type species of this genus, *Pragia fontium* DSM 5563^T. DNA-DNA similarity between strain A-27^T and *Pragia fontium* DSM 5563^T was less than 25%. DNA G+C content was 43.87 mol%. Strain A-27^T grew at 0.5–5% (w/v) NaCl, at 10–42°C and at pH 5.0–10.0. The chemotaxonomic data (major ubiquinone, Q-8; major polar lipids, phosphatidylethanolamine and an unknown phospholipid; and major fatty acids, $C_{16:0}$ (30.3%), summed features 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*; 22.5%), $C_{14:0}$ (14.1%), and summed features 2 ($C_{12:0}$ aldehyde/unknown 10.928; 10.3%) supported the affiliation of strain A-27^T to the genus *Pragia*. The results of genotypic and phenotypic tests allowed differentiation of strain A-27^T from the validly published *Pragia* species. A-27^T therefore represents a new species, for which the name *Pragia protaetiae* sp. nov. is proposed, with the type strain A-27^T (= KCTC 42010^T = JCM 19876^T).
[Supported by grant from the KRIBB Research Initiative Program]

A008

Seasonal Comparison of Bacterial Communities Associated with the Marine Sponge, *Hymeniacidon flavia*

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The community structures of the sponge associated bacteria, *Hymeniacidon flavia* collected from Jeju Yongmeori coast on Agust (summer) and Decenber (winter) 2013 were compared by the PCR-DGGE based on cultivation independent method. The sequences derived from each observed DGGE bands belonged to the uncultured bacteria and had 87-100% similarities to the known bacterial sequences. The bacterial communities associated with *H. flavia*, collected in summer, showed 6 DGGE bands and they represents *Alphaproteobacteria* and *Gammaproteobacteria*. Another sponge collected in winter, showed 8 bands and they had *Alphaproteobacteria*, *Gammaproteobacteria* and *Firmicutes*. DGGE band patterns showed seasonal differences in the two sponges and phylum *Firmicutes* was found only in the sponges collected in winter.

A009

***Antarctobacter jejuensis* sp. nov., Isolated from Seawater in Jeju, Korea**

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A novel bacterium, designated strain 13-2-B6^T, was isolated from seawater in front of Songak Mountain on Jeju Island, South Korea. On the basis of 16S rRNA gene sequence similarity, strain 13-2-B6^T was shown to be phylogenetically closely related to the type strain of *Antarctobacter heliothermus*, the sole species of the genus *Antarctobacter*. The similarity of the 16S rRNA gene sequences of the strain 13-2-B6^T and *A. heliothermus* EL-219^T was 96.85%. Strain 13-2-B6^T grew optimally at 25-30°C, pH 7.5-8.0 and in presence of 3% (w/v) NaCl. Strain 13-2-B6^T contained ubiquinone Q-10 as the predominant isoprenoid quinones and $C_{18:1}$ $\omega7c$ / $C_{18:1}$ $\omega6c$ as the major fatty acids. The G+C content of strain 13-2-B6^T was 62 mol%. Based on the phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain 13-2-B6^T is considered to represent a novel species of the genus *Antarctobacter*, for which the name *Antarctobacter jejuensis* sp. nov. is proposed. The type strain is 13-2-B6^T (=KCTC 42009^T).
[Supported by grant from the National Fisheries Research and Development Institute]

A010

Seasonal Differences of Bacterial Communities Associated with the Marine Sponge, *Callyspongia elegans* Based on DGGE

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Seasonal differences of bacterial communities associated with the marine sponge *Callyspongia elegans*, were analyzed by the PCR-DGGE based on culture-independent method. The 16S rRNA gene sequences derived from the DGGE bands showed 81–100% similarities to the known bacterial species in the public database. The bacterial community structure of *C. elegans* sponge (131mur20) collected in winter was composed of 3 phylum, 6 classes: *Proteobacteria* (*Ahpha-*, *Beta-*, *Gamma-*, *Delta-*), *Cyanobacteria* and *Chloroflexi*. Another sponges (138mur2, 138mur3 and 138mur4) collected in summer, were composed of 2 phylum, 4 classes: *Proteobacteria* (*Ahpha-*, *Beta-*, *Gamma-*) and *Cyanobacteria. Deltaproteobacteria* and *Chloroflexi* were found only in the winter sponge and there are seasonal differences between the community structures of same sponge, *C. elegans*.

A011

Structure of ribosomal RNA Gene and Phylogeny of *Nosema* Isolates in Korea

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The ribosomal RNA (rRNA) gene region of the four *Nosema* sp. isolates (C01, C02, C03 and C04) from *Pieris rapae* in Korea has been examined. Complete DNA sequence data (3779 bp) of the rRNA gene of *Nosema* sp. C01 are presented for the small subunit gene (SSU rRNA: 1236 bp), the internal transcribed spacer (ITS: 37 bp), and the large subunit gene (LSU rRNA: 2506 bp). The secondary structures of *Nosema* sp. C01 SSU and LSU rRNA genes are constructed and described. The SSU rRNA showed a hypervariable V4 region identified four additional stems including a pseudoknot. Phylogenetic analysis based on the SSU rRNA suggests that the four isolates belong to the 'true' *Nosema* group. In contrast to the *Vairimorpha* /*Nosema* clade, the members of the group are highly divergent.

A012

***Paenibacillus cucumis* sp. nov. Isolated from Greenhouse Soil**

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Strain CO 4-7^T was isolated from greenhouse soil cultivated with cucumber in Korea. The 16S rRNA gene sequence of strain CO 4-7^T showed the highest sequence similarity with *Paenibacillus contaminans* CKOBP-6^T (94.2%) among the type strains. The strain CO 4-7^T was a strictly aerobic, Gram-staining-positive, endospore-forming, and motile rod-shaped bacterium. The strain grew at 10-45°C (optimum, 30°C), at pH 6.0-7.5 (optimum, pH 6.5) and in the presence of 0-5% NaCl (optimum, 0.5%). The DNA G+C content of strain CO 4-7^T was 48.5 mol%. It contained MK-7 as the major isoprenoid quinone and anteiso-$C_{15:0}$ (51.8%), $C_{16:0}$ (12.7%), and iso-$C_{16:0}$ (8.6%) as the major fatty acids. On the basis of evidence from our polyphasic taxonomic study, it was concluded that strain CO 4-7^T should be classified within a novel species of the genus *Paenibacillus*, for which, the name *Paenibacillus cucumis* sp. nov. is proposed. The type strain is CO 4-7^T (=KACC 17444^T =JCM 19515^T).
[Supported by grants from "Research Program for Agricultural Science & Technology (Project No. PJ90712702)" of the National Academy of Agricultural Science, Rural Development Administration, Republic of Korea.]

A013

***Oryzihumus terrae* sp. nov., Isolated from Soil**

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Gram-stain-positive, aerobic, non-flagellated bacterium, designated KIS22-12^T, was isolated from a soil sample, Republic of Korea. Cells were non-spore-forming, coccus showing catalase-positive and oxidase-negative reactions. Strain KIS22-12^T contained MK-8(H_4) as the predominant menaquinone, and $C_{17:1}$ ω8*c*, iso-$C_{15:0}$ and anteiso-$C_{15:0}$ as the major fatty acids. Strain KIS22-12^T contained DPG, PI, three unknown PLs, one unknown AL and one unknown lipid. Peptidoglycan type is A1γ. The G+C content of the genomic DNA was 69.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain KIS22-12^T formed a phyletic lineage with *Oryzihumus leptocrescens* KV-628^T. 16S rRNA gene sequence similarity between two strains 96.5%. On the basis of phenotypic, chemotaxonomic and molecular properties, strain KIS22-12^T represents a novel species within the genus *Oryzihumus*, for which the name *Oryzihumus terrae* sp. nov. is proposed. The type strain is KIS22-12^T (=KACC 16543^T).
[This study was supported by National Academy of Agricultural Science, RDA.]

A014

***Parafilimonas terrae* gen. nov., sp. nov., Isolated from Greenhouse Soil**

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Agricultural Microbiology Division, National Academy of Agricultural Science

Strain 5GHs7-2^T was isolated from a soil sample in South Korea. 16S rRNA gene sequence analysis of strain 5GHs7-2^T indicated that the isolate belonged to the family *Chitinophagaceae* and exhibited the highest sequence similarities with the genus *Terrimonas* members (89.2-92.6%), the genus *Sediminibacterium* members (90.8-91.4%), the genus *Chitinophaga* members (89.2-91.7%), *Filimonas lacunae* YT21^T (91.7%), the genus *Segetibacter* members (90.2-91.6%), *Parasegetibacter luojiensis* RHYL-37^T (90.9%) and *Flavihumibacter petaseus* T41^T (91.2%). The major cellular fatty acids of the novel strain were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{15:1}$ G. The polar lipid profile consisted of a large amount of PE, and small amounts of several unknown ALs, APL and lipids. The only respiratory quinone of strain 5GHs7-2^T was MK-7, and the DNA G+C content was 47.6 mol%. On the basis of the evidence presented, it is concluded that strain 5GHs7-2^T represents a novel species of a new genus, for which the name *Parafilimonas terrae* gen. nov., sp. nov. is proposed. The type strain of *Parafilimonas terrae* is 5GHs7-2^T (KACC 17343^T).
[This study was supported by National Academy of Agricultural Science, RDA.]

A015

Paenibacillus acervicinus* KUDC4121 sp. nov., Isolated from Rhizosphere Soil of a *Acer okamotoanum

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Strain KUDC4121 (=DSMZ 24950, KCTC 13870) was isolated from the rhizosphere of *Acer okamotoanum*, a native plant in Ulleungdo island in September 2006. The G + C content of genomic DNA was 48.28 mol%. The major fatty acids were anteiso-$C_{15:0}$ (63.78%) and iso-$C_{16:0}$ (10.73%). The closest type strain was *P. chondroitinus* DSM 5051^T with 97.8% similarity followed by *P. alginolyticus* DSM5050^T (97.6%), *P. pocheonesis* Gsoil 1138^T (97.5%), *P. frigoriresistens* YIM 016^T (97.5%) and *P. pectinilyticus* RCB-08^T (97.2%). The DNA-DNA relatedness between strain KUDC4121 and the reference strain was all relatedness ratio were lower than 70%. Strain KUDC 4121 comprised Gram positive, motile, rods and is capable of growth from 18–37 ºC and from pH 6.0–7.5 with optimal growth conditions of 30ºC and pH 7.0. It grows on tryptic soy agar media containing up to 0.5% NaCl (w/v). Based on combined phenotypic properties and phylogenetic and genetic data, strain KUDC4121 can be considered as a novel species of the genus *Paenibacillus*.

A016

A Bacterium Representing Novel Species in the Genus *Roseomonas*, Isolated from Freshwater of Woopo Wetland

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A Gram-stain-negative, non-motile, aerobic and pink pigmented bacterium, designated strain WW53^T, was isolated from wetland freshwater (Woopo wetland, Republic of Korea). Strain WW53^T grew at 15-40°C (optimally at 37-40°C), pH 6-8 (optimally at pH 7) and 0-0.5% of NaCl. Catalase and oxidase activities are present. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain WW53^T formed a distinct lineage within the genus *Roseomonas* and was the most closely related to *Roseomonas stagni* HS-69^T (95.3% 16S rRNA sequence similarity). The predominant fatty acids are Summed feature 8 ($C_{18:1}$ ω6*c*/$C_{18:1}$ ω7*c*) (32.5%), $C_{16:0}$ (18.5%) and Summed Feature 3 ($C_{16:1}$ ω6*c*/$C_{16:1}$ ω7*c*) (9.6%). The major quinone is ubiquinone 10 (UQ-10). On the basis of phenotypic-, chemotaxonomic data and phylogenetic inference, strain WW53^T should be classified into the genus *Roseomonas*, as a member of a novel species, for which the name *Roseomonas woopoensis* sp. nov. is proposed. The type strain is WW53^T (=KCTC 32534^T).
[This research was supported by the project on survey and excavation of Korean indigenous species of the NIBR under the Ministry of Environment, Republic of Korea.]

A017

Burkholderia jiriense sp. nov. Isolated from Forest Soil

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A Gram-negative, obligate aerobic bacterium designated as JRM2-1T was isolated from forest soil of Jirisan Mountain and its taxonomic position was investigated based on the polyphasic taxonomy. On the basis of 16S rRNA gene sequence analysis, the closest neighbor of the strain JRM2-1T was *Burkholderia terrae* KMY02^T (97.2%). The major cellular fatty acids were $C_{16:0}$, cyclo-$C_{17:0}$ and cyclo-$C_{19:0}$ $\omega 8c$. Polar lipid analysis showed that the polar lipids profile of the strain JRM2-1^T was consisted with diphosphatidylglycerol, phosphatidylglycerol, phosphatidylenthanolamine, several unidentified amino lipids and unidentified amino-phospholipid. Major isoprenoid quinone was Q-8. The G+C content of the strain JRM2-1T was 63.7 mol%.
On the basis of polyphasic taxonomical investigation, the strain JRM2-1^T was proposed to be classified as a novel species in the genus *Burkholderia* for which the name *Burkholderia jiriense* sp. nov is proposed.

A018

Lewinella jeungdonensis sp. nov., Isolated from the Seawater in Korea

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A Gram-staining-negative, rod-shaped, orange colored strain HME9321^T, was isolated from the seawater in Yellow sea, Korea. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain HME9321^T grouped with members of the genus *Lewinella.* The 16S rRNA gene sequence similarity of strain HME9321^T was related to *Lewinella marina* MKG-38^T (94.4 %) and *Lewinella lutea* FYK2402M69^T (91.5 %). The major fatty acids were summed feature 3 (comprising $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; 35.0 %), iso-$C_{15:0}$ (21.0 %) and summed feature 9 (comprising iso-$C_{16:0}$ 10-methyl and/or $C_{17:1}$ $\omega 9c$; 15.8 %). The DNA G+C content was 58.4 mol%. On the basis of the evidence presented in this study, strain HME9321^T represents a novel species of the genus *Lewinella*, for which the name *Lewinella jeungdonensis* sp. nov., is proposed the type strain HME9321^T (= KACC 17619^T = CECT 8419^T).

A019

Erythrobacter persica sp. nov., Isolated from Sea Water in Korea

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A rod-shaped, Gram-staining-negative and orange pigmented bacterium, designated strain HME9302^T, was isolated from sea water of the Yellow sea in Korea. The major fatty acids were summed feature 3 (comprising $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; 28.3%), summed feature 8 (comprising $C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$; 26.7%), $C_{14:0}$ 2-OH (18.7%) and $C_{16:0}$ (10.7%). Phylogenetic tree based on 16S rRNA gene sequence showed that strain HME9302^T formed a lineage within the genus *Erythrobacter*. The strain HME9302^T was closely related to *Erythrobacter jejuensis* CNU001^T (95.8% sequence similarity), *Erythrobacter seohaensis* SW-135^T (95.3%) and *Erythrobacter citreus* RE35F/1^T (95.3%). The DNA G+C content was 62.0 mol%. On the basis of the evidence presented in this study, strain HME9302^T represent a novel species of the genus *Erythrobacter*, for which the name *Erythrobacter persica* sp. nov., is proposed the type strain HME9302^T (=KACC 17617^T = KCTC 32463^T = CECT 8417^T).

A020

Paenibacillus chungnamensis sp. nov., Isolated from the Root of *Oenothera biennis*

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A novel Gram-staining negative, aerobic and rod-shaped bacterium designated strain DT7-4^T was isolated from the root of evening primrose (*Oenothera biennis*). Colonies were pale grey, round and flat. The strain grew at 15-37°C (optimum = 30°C), at pH 6-7, and also in the presence of 0-2 % (w/v) NaCl (optimum = 0%). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain DT7-4^T is most closely related to *Paenibacillus phyllospaerae* KACC15578^T and *Paenibacillus taihuensis* THMBG22^T sharing the 16S rRNA gene sequence similarity of 96.3%. The major cellular fatty acid of strain DT7-4^T was anteiso-$C_{15:0}$ (45.4%). The cellular polar lipids were composed of DPG, PE, PG and unidentified polar lipids. The diamino acid found in the cell wall peptidoglycan was meso-diaminopimelic acid. The DNA G+C content of strain DT7-4^T was 50.8 mol%. The biochemical analyses distinguished the strain from related species. Based on the results of the polyphasic taxonomic analysis, strain DT7-4^T should be classified into genus *Paenibacillus* as a member of a novel species, for which the name is *Paenibacillus chungnamensis* sp. nov. proposed. The type strain is DT7-4^T (=JCM 19573).

A021

Garumicola koreensis gen. nov., sp. nov., Isolated from Saeu-jeot: Traditional Korean Fermented Shrimp

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A Gram-staining-positive, halophilic, non-motile cocci, aerobic bacterium, designated strain SJ5-4^T, was isolated from *seau-jeot*. Growth of strain SJ5-4^T was observed at 15-40°C, at pH 6.0-9.5 and in the presence of 1-17% (w/v) NaCl. Anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$ and $C_{16:0}$ as major fatty acids. The G+C content of the genomic DNA was 61.8 mol%. MK-7, MK-8 and MK-9 were detected as the isoprenoid quinones. The 16S rRNA gene sequence similarity was closely related to the genus *Nesterenkonia* with a 93.1-94.8%. However, phylogenetic analyses showed strain SJ5-4^T formed a distant phyletic lineage from members of the genus *Nesterenkonia*. Phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol were detected as the polar lipids. The peptidoglycan type contained lysine, 2, 4-diaminobutyric acid, alanine, glutamic acid and aspartic acid, which also supported that strain SJ5-4^T could be differentiated from the genus *Nesterenkonia*. Based on phenotypic and genotype features, strain SJ5-4^T represents a novel genus for which the names *Garumicola koreensis* gen. nov., sp. nov., is proposed. The type strain is SJ5-4^T (=KACC 16909^T=JCM 18572^T=DSM 28238^T).

A022

Description of _Hymenobacter wooponensis_ sp. nov., Isolated from Freshwater of Woopo Wetland in Korea

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A non-motile, Gram-stain-negative, red-pink colored bacterium, designated WM78^T, 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 *Hymenobacter*, with the closest relatives being *Hymenobacter gelipurpurascens* DSM 11116^T (97.71% 16S rRNA gene sequence similarity), *Hymenobacter perfusus* LMG 26000^T (96.12%) and *Hymenobacter rigui* KCTC 12533^T (95.89%). Cells grow on NA, PCA, GYEA and R2A, but not on MA and TSA. Growth occurred at 10-40°C (optimum, 30°C) and pH 6.0-8.0 (optimum, 7.0). Oxidase-negative and catalase-positive. Strain WM78^T contained iso-$C_{15:0}$, anteiso-$C_{15:0}$, Summed feature 3($C_{16:1}$ w6c and/or $C_{16:1}$ w7c) and Summed feature 4(iso-$C_{17:1}$ B and/or iso I) as the major fatty acids. The major respiratory quinone is menaquinone 7 (MK-7). Strain WW78^T represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter wooponensis* sp. nov. is proposed.
[This research was supported by the project on survey and excavation of Korean indigenous species of the NIBR under the Ministry of Environment, Republic of Korea.]

A023

Sequence Validation for the Identification of the White-Rot Fungi _Bjerkandera_ in GenBank

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White-rot fungus, *Bjerkandera* is cosmopolitan species with wide potential for industrial application and bioremediation. As industrially important strains of *Bjerkandera adusta* are preserved as cultures or dried fragments, knowing authentic and precise sequences of *Bjerkandera* is crucial for accurate application and understanding of the species. After rigorous morphological examination of *Bjerkandera* specimens of Korea, we used internal transcribed spacer (ITS) region and 28S nuclear ribosomal large subunit (LSU) as DNA markers to build a framework for molecular identification of *Bjerkandera*. The phylogenetic analysis of Korean *Bjerkandera* specimens showed clear distinction between two species, *B. adusta* and *B. fumosa*. Based on this framework, we examined the accuracy of sequences available at GenBank. The BLAST search results revealed that many possible *Bjerkandera* sequences in the database are misidentified and unidentified. This study provides a robust reference sequences for sequence-based identification of *Bjerkandera* and further demonstrates credibility of sequences uploaded on GenBank.

A024

Caulobacter rigui sp. nov., Isolated from Freshwater of Woopo Wetland in Korea

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A non-motile, rod-shaped, non-spore-forming, white-colored and aerobic bacterium, designated strain WW137^T, was isolated from freshwater of Woopo wetland (Republic of Korea). Cells were Gram-stain-negative, catalase-positive and oxidase-negative. The temperature and NaCl ranges for the growth of strain WW137^T were 4-32°C and 0-1%, respectively. A phylogenetic tree based on 16S rRNA gene sequences showed that strain WW34^T forms a lineage within the genus *Caulobacter* (97.4–96.8%, sequence similarity) and formed a distinct branch with the clade comprising *C. henricii* KACC 10986^T (97.4%), *C. segnis* KCTC 12505^T (97 %) and *C. vibrioides* KCTC 3405^T (96.8 %). The major cellular fatty acids of strain WW137^T were summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*; 28.1%), $C_{16:0}$ (15.4%) and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; 14%). On the basis of the data presented in this study, strain WW137^T represents a novel species, for which the name *Caulobacter rigui* sp. nov. is proposed. The type strain is WW137^T.
[This research was supported by the project on survey and excavation of Korean indigenous species of the NIBR under the Ministry of Environment, Republic of Korea.]

A025

***Psychroserpens marinus* sp. nov., Isolation from Antarctic**

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A Gram-stain-negative, yellow-pigmented, aerobic, rod-shaped, non-motile bacterium, designated strain PAMC 27130^T, was isolated from marine sediments of the Ross Sea, Antarctica. Optimal growth of strain PAMC 27130^T was observed at 15°C, pH 7.0 and the presence of 2% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain PAMC27130^T belonged to the genus *Psychrosepens* and was closely related to *Psychrosepens mesophilum* (97.2% sequence similarity), *Psychrosepens damuponensis* (94.7% sequence similarity) and *Psychrosepens burtonensis* (94.2% sequence similarity). The major respiratory isoprenoid quinone was menaquinone-6 (MK-6) and major polar lipids were phosphatidylethanolamine. On the basis of genotypic and phenotypic data collected in this study, it is proposed that strain PAMC 27130^T represents a novel species of the genus *Psychrosepens*, for which the name *Psychrosepens* marinus sp. nov. is proposed.

A026

Physiology and Genomic Characteristics of Strain IMCC13023, a Marine Actinobacterium Isolated from Arctic Seawater

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It has been known that a part of marine actinobacterial populations are closely related to several freshwater lineages of actinobacteria. However, studies on those marine actinobacteria have been hampered by the lack of relevant isolates. Here we report on the polyphasic taxonomy and genome characteristics of IMCC13023 that was isolated from Arctic seawater but was closely related to 'Candidatus Aquiluna rubra', a freshwater actinobacterial clade, with 97.46% of 16S rRNA gene sequence similarity. Optimal growth of strain IMCC13023 was observed at 15℃, pH 7-8, and in the presence of 1% NaCl. Some biochemical characteristics, major fatty acids, and polar lipid profiles differentiated the strain from other genera in the Actinobacteria. Illumina sequencing followed by combinatorial PCR resulted in the complete genome sequence of 1.36 Mb, the smallest genome size ever reported among free-living actinobacteria, with G+C content of 51.7 mol%. An actinorhodopsin gene was predicted from the genome, suggesting a photoheterotrophic metabolism.

A027

Purification of Tiny Colony throughout the Long Term Incubation and Identification of Novel Bacteria

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The number of colonies usually increased throughout the 50 days in a stepwise manner that can be simulated by a colony forming curve (CFU). The typically tiny colonies (<0.5 mm diameter) that appeared in less than 900 h of incubation. Cells were picked from 22 tiny colonies and subcultured in fresh medium. Three isolations designated PKR-39, PKR42 and 5KR71 were affiliated with an uncultivated lineage within the *α-proteobacteria* and the nearest cultivated neigh-bours were bacteria in the genera in the family *Methylocystaceae* (92.5~93.8% 16S rRNA gene sequence similarity) and *Beijerinckiaceae* (92.7~93.5%). The strain PKR-39^T was a chemoorganotrophic bacterium, which was incapable of growth on C1 substrates. Atmospheric nitrogen fixation and nitrate reduction were negative. The strain contained the ubiquinone Q-10 and major cellular fatty acids were $C_{18:1}$ ω7*c* and $C_{19:1\ cyclo}$ ω8*c*. The major polar lipid was phosphatidylethanolamine. The DNA G+C content was 62.3 mol%. On the basis of the information described above, the new genus and species, *Panacibacter rhizosphaerae* gen. nov., sp. nov. is proposed in the order *Rhizobiales*. The type strain is PKR-39^T (=KACC 17632^T =NBRC 109815^T).

A028

***Nocardioides soli* sp. nov. Isolated from Soil**

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One strain, designated KIS31-44^T, was isolated from a soil sample collected from Dokdo Island, South Korea. The strain is Gram-stain-positive, aerobic, non-spore-forming and non-motile. It grows optimally at 28-30°C, at pH 7.0 and 0% NaCl. 16S rRNA gene sequence analysis showed that strain KIS31-44^T belonged to the genus *Nocardioides* and shared the highest sequence similarities with *Nocardioides aestuarii* JC2056^T (95.5%) and *Nocardioides terrae* VA15^T (95.0%). The major fatty acids of strain KIS31-44^T were $C_{17:1}$ ω6*c*, $C_{18:1}$ ω9*c*, summed feature 3 ($C_{16:1}$ ω6*c* and/or $C_{16:1}$ ω7*c*), iso-$C_{16:0}$, $C_{18:0}$ 10-methyl (TBSA), $C_{16:0}$ 2-OH, $C_{17:0}$ 10-methyl and iso-$C_{16:1}$ H. The major isoprenoid quinone was MK-8 (H_4). The strain contained diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as the major polar lipids. The peptidoglycan structure was A3γ-type with ll-diaminopimelic acid. Based on these data, the isolate represents one novel species in the genus *Nocardioides*, for which the name *Nocardioides soli* sp. nov. (type strain KIS31-44^T = DSM 27142^T = KACC 17309^T) is proposed.

A029

Characterization of *Pelagicola* sp. TJ5 a Novel Alphaproteobacterium Isolated from Marine Sample

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A gram-negative, motile, aerobic and short-rod to ovoid bacterium, designated as strain TJ5, was found to grow optimally at temperature 30°C, at pH 7.0–8.0 in the presence of NaCl 2.0–3.0% (w/v). It contained Q-10 as the predominant ubiquinone and C18:1°C *ω7c* as predominant fatty acid. The determined major polar lipids were phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content of strain TJ5 was 58.6% and its DNA-DNA relatedness between strain TJ5 and closest type strain *Pelagicola litorisediminis* D1-W8^T was 13%. Phylogenetic data and 16S rRNA gene sequence exhibit that strain TJ5 is belonging to genus *Pelagicola* and showed the similarity values of 97.12% with *Pelagicola litorisediminis* D1-W8^T and 97.06% with *Roseovarius aestuarii* SMK-122^T. Based on phenotypic, chemotaxonomic and phylogenetic data, strain TJ5 is considered to represent a novel species of genus *Pelagicola.*

A030

Isolation and Characterization of *Paracoccus* sp., KF89^T Isolated from Tongyeong Sea-Water

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A new bacterial strain, designated as KF89^T, was isolated from Tongyeong seawater, South Korea. Strain KF89^T is Gram-negative, non-spore-forming, motile, rod- to cocci shaped bacterium. Growth occurred between 10 and 35°C (optimum 32°C) and at pH 6.0-9.0 (optimum pH 7.0) and at NaCl 0-7% (optimum NaCl 2%). The 16S rRNA gene sequence analysis identified this strain as a member of the genus *Paracoccus* that belongs to the phylum *Proteobacteria*. The highest degree of gene sequence similarities were determined to be with *Paracoccus homiensis* DD-R11^T (97.19%), The major quinone is ubiquinone Q-10; the DNA G+C content is 57%; the major fatty acid is $C_{18:1}$ *ω7c* (85.33%); polar lipid profile contained phosphatidylglycerol (PG), phosphatidycholine (PC), diphosphatidylglycerol (DPG), unidentified phospholipid (PL), unidentified amino lipid (AL), and unidentified lipids (L1-3). Furthermore analysis, I will research DNA-DNA hybridization and more enzyme activities. Based on the results, obtained during this study suggest, that the strain KF89^T could represents a novel candidate in the genus *Paracoccus*.

A031

Characterization of *Neptunomonas* sp. nov., Isolated from Tongyeong Sea Water of Korea

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Three bacterial strains were isolated from Tongyeong sea water of Korea. The isolated strains, 2R1^T, 2R7 and 2R12 are Gram-negative, rod-shaped, motile, and aerobic bacteria. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel strain 2R1^T was most closely related to *Neptunomonas naphthovorans* NAG-2N-126^T (97.97% sequence similarity), strain 2R7 was most closely related to *N. naphthovorans* NAG-2N-126^T (98.08% sequence similarity), strain 2R12 was most closely related to *N. naphthovorans* NAG-2N-126^T (98.06% sequence similarity). Three strains are positive for catalase. The major cellular fatty acid of 2R1^T is 18:1 *ω7c* (41.543%), 16:0 (25.24%), 16:1 *ω7c*/16:1 *ω6c* (17.81%), and minor fatty acids are 10:0 3OH (5.45%), 2R7 is 18:1 *ω7c* (36.16%), 16:0 (27.73%), 16:1 *ω7c*/16:1 *ω6c* (18.79), and minor fatty acids are 12:0 (5.75%), 2R12 is 18:1 *ω7c* (36.23%), 16:1 *ω7c*/16:1 *ω6c* (28.53%), 16:0 (22.11%), and minor fatty acids are 10:0 3OH (4.14%). Three strains contained Q-8 as the predominant ubiquinone. Based on the phenotypic, genotypic and phylogenetic results, the strains 2R1^T, 2R7, and 2R12 represent novel candidate belonging to the genus *Neptunomonas*.

A032

Characterization of *Donghicola* sp. nov Isolated from Sea Water of Tong-Yeong in Korea

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A novel strain, FJ12^T is Gram-negative, aerobic, motile, rod-shaped bacteria which was isolated from sea water, Tong-Yeong, Korea. Phylogenetic analysis based on 16S rRNA gene sequences reveal that the novel strain include *Donghicola* genus, closest species is *Donghicola eburneus* (95.37% similarity), *Aestuariihabitans beolgyonensis* (95.31%), *Sulfitobacter ponitiacus* (94.91%), *Roseisalinus antaricticus* (94.76%), *Charonomicrobium ambiphototrophicum* (94.74%), and *Marivita byusanesis* (94.66 %). The major cellular fatty acids are C18:1 *ω7c* (61.64%), C16:0 (16.44%), 16:1 *ω7c*/16:1 w6c (9.71%), C10:0 3OH (5.5%), C18:0 (2.11%), and C19:1 *ω6c* (2.11%). The DNA G+C content of novel strain FJ12^T is 69 mol%. Strain FJ12^T contained Q-10 as the predominant ubiquinone. Based on phenotypic characteristics, phylogenetic data, physiological and chemotaxonomic data, FJ12^T should be classified as a novel *Donghicola.*

A033

Isolation and Characterization of Novel *Tumebacillus* sp., Isolated from Ukraine Soil

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Gram positive, aerobic, non-motile, spore forming, and rod-shaped bacteria designated as strains U13, U14 and U27, was isolated from soil from Ukraine. Three strains were isolated based on serial dilution method on R2A agar plate and based on the 16S rRNA gene sequencing, they are belong to the genus *Tumebacillus* and closely related to *Tumebacillus ginsengisoli* Gsoil 1105^T (95.2~95.5%), *T. flagellatus* GST4^T (94.7~95%), *T. permanentrifrigoris* Eur1 9.5^T (93.2%) and *Alicyclobacillus pohilae* MP4^T (90.8%). Growth observed between 25~42℃ (optimum 30~37℃), pH 5~9 (optimum 6~9), and able to tolerate 1% of NaCl (optimum 0%). Chemotaxonomic data revealed that the strains have menaquione 7(MK-7) as predominant quinone, and cell wall amino acids were type A1γ. Fatty acids profile of the strains were anteiso-C15:0 (9.16~10.1%), iso-C15:0 (71.3~75.8%), and iso-C16:0 (5.4%). The chemotaxonomic, morphological, and phenotypic characteristics of the strains clearly suggest that the strains are belonged to the *Tumebacillus* and represent a novel candidate of the genus. Based on the polyphasic classification data, the strains should be classified to be a novel species of the genus *Tumebacillus.*

A034

Novel Aerobic and Thermophilic Bacteria Enriched with Macromolecular Carbohydrates

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Diverse microorganisms are reported from marine environment, displaying various physico-chemical ecosystems which are three dimensional, however, many of which still not identified. Thermophilic bacteria were enriched with 0.1% of lignin or cellulose at 50ºC. The 74 isolates affiliated with *Bacillales* (65%), *Proteobacteria* (16%), *Actinobacteria* (8%), *Bacteroidetes* (7%), and *Archaea* (4%) and approx. 20% of isolates constitutes novel taxon. Some of the isolates closely related with previously reported thermophiles but some has been not known as thermophilic or thermotolerants. Amongst, four isolates produces deep branch in each phylogenetic group shows highest similarity with *Caldalkalibacillus uzonensis* (MEBiC 09487, 89%), *Mechercharimyces asporophorigenens* (MEBiC 07976, 91%), *Pararhodobacter aggregans* (MEBiC 09520, 90.7%), or *Rhodothermus profundi* (MEBiC 09517, 88%). The four isolates were neutrophilic and halophilic and showed 50℃ as optimum growth temperature. The results implied that novel microorganisms could be enriched by combination of high temperature and recalcitrant substrates.
[Supported by MBRB of MOF, Korea, KIOST in-house program (PE99212) and CAP of KRCF]

A035

***Catenulispora purpureus* sp. nov., Isolated from Forest Soil**

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An actinomycete strain, designated strain SA-246^T, was isolated from forest soil sample collected from Chungnam, South Korea. Applying a polyphasic approach, the isolate was identified as a member of the genus *Catenulispora* using morphological and chemotaxonomic characteristics, including the presence of $_{LL}$-diaminopimelic acid, glutamic acid, alanine and glycine. Whole-cell hydrolysates contained predominantly rhamnose, mannose, ribose, arabinose, galactose and glucose. The major menaquinones were MK-9 (H_4), MK-9 (H_6) and MK-9 (H_8). 16S rRNA gene sequence analysis revealed similarity to *C. yoronensis* TT N02-20^T (98.7%), *C. subtropica* TT 99-48^T (98.2%), *C graminis* BR-34^T (97.4%), *C. rubra* Aac-30^T (97.4%) and *C. acidiphila* ID139908^T (97.3%), respectively. Analysis of its 16S rRNA gene sequence, DNA–DNA hybridization studies and the results of physiological tests showed that this strain represents a novel species of *Catenulispora*, for which the name *Catenulispora purpureus* sp. nov. is proposed. The type strain is SA-246^T (=KACC 17878^T =NBRC 110074^T).

A036

***Novosphingobium daejeonensis* sp. nov., Isolated from Ground Water**

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A Gram-negative, strictly aerobic, non-motile, non-spore-forming, yellow colored and rod-shaped bacterium, designated E-II-3^T, was isolated from ground water at Daejeon in Korea. Strain E-II-3^T grew between 4 and 45°C (Opt. at 28°C), between pH 6.0 and 9.0 (Opt. at pH 7.5) and at salinities of 0–2.0% (w/v) NaCl (Opt. at 0.5% NaCl). On the basis of 16S rRNA gene sequence analysis, strain E-II-3^T was shown to belong to the genus *Novosphingobium* and showed closest phylogenetic similarity to '*Novosphingobium ginsenosidimutans*' FW-6 (97.7%) and *Novosphingobium aromaticivorans* F199^T (96.9%). The major polar lipids were phosphatidylglycerol, diphosphatidyl-glycerol, phosphatidylethanolamine, phosphatidylcoline and sphingoglycolipid. The predominant ubiquinone was Q-10. The major fatty acids were $C_{18:1}$ ω7*c* (34.0%), $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2OH (23.8%) and $C_{17:1}$ ω6*c* (19.3%). The DNA G+C content of this novel isolate was 62.7 mol%. On the basis of polyphasic evidence, strain E-II-3^T represents a novel species of the genus *Novosphingobium* for which the name *Novosphingobium daejeonensis* sp. nov. is proposed.
[Supported by a grant from the Regional Subgenebank Support Program of RDA]

A037

Chitinophaga ginsengihumi sp. nov., Isolated from a Soil of Ginseng Rhizosphere

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A novel strain designated SR18^T was isolated from the rhizosphere soil of a ginseng in Korea. Cells were Gram-staining-positive, motile by gliding, catalase-positive and oxidase-negative, non-spore-forming rods. The isolates grew aerobically at 15-45°C (opt. at 28°C), pH 5.5-7.5 (opt. pH 6.0) and 0-3.0% (w/v) NaCl (opt. 1.5% NaCl). Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain SR18^T belongs to the genus *Chitinophaga* with the sequence similarity of 97.2% and 97.0% to *Chitinophaga japonensis* 785^T and *Chitinophaga rupis* TCS5-B1^T, respectively. The predominant menaquinone was MK-7. Major fatty acids were iso-$C_{15:0}$ and $C_{16:1}$ *ω5c*. The polar lipids contained phosphatidylethanolamine, unidentified phospholipids, unknown aminolipids and unknown lipids. The genomic DNA G+C content was 45.3 mol%. DNA-DNA relatedness between strain SR18^T and *C. japonensis* NBRC 16041^T was 28.5-32.4%. On the basis of polyphasic analysis from this study, strain SR18^T represents a novel species of the genus *Chitinophaga* for which the name *Chitinophaga ginsengihumi* sp. nov. is proposed.
[Supported by a grant from the Regional Subgenebank Support Program of RDA]

A038

Lentibacillus namhaensis sp. nov., Isolated from Myeolchi-jeot

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A Gram-positive, moderately halophilic aerobic bacterium, designated strain SL-MJ2^T, was isolated from myeolchi-jeot, Korean traditional fermented seafood, that was fermented using *Engraulis japonicus* caught from south sea (Nam Hae) in Korea. Colonies were beige-colored on marine agar plate and cells were non-motile rods without flagellum. Growth was observed between 15 and 45°C (optimum, 37°C) and between pH 6.5 and 8.5 (optimum, pH 7.0). The G+C content of the genomic DNA was 43.6mol%. Phylogenetic analyses based on 16S rRNA gene sequence indicated that strains SL-MJ2 ^{T} formed a phyletic lineage within the genus *Lentibacillus* and was most closely related to *Lentibacllus juripiscarius* JCM12147 with 95.1% of 16S rRNA gene sequence similarity. On the basis of phenotypic, chemotaxonomic and molecular properties, strain SL-MJ2^T represents a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus namhaensis* SL-MJ2^T is proposed. The type strain is SL-MJ2^T.

A039

Molecular Diversity of Rumen Microbial from the Rumen of Korean Native Cattle (HanWoo)

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Methane (CH_4) is produced in the rumen by methanogens (*Archaea*) mainly through reduction of carbon dioxide by hydrogen, released by protozoa, fungi, and bacteria during degradation of the feed. The microbial diversity of rumen was investigated in Korean native cattle (HanWoo; *Bos taurus coreanae*) emitted methane considered to be an important contribution factor to global warming and climate change. The rumen microbial population was evaluated using 16S rRNA gene libraries, qRT-PCR and NGS to compare methane emission group and non-methane emission group for enteric production of methane from nine Korean native cattles. All selected strains that were isolated from the rumen fluid were classified into 5 genera and 10 species for anaerobic strains, 9 genera and 27 species for aerobic strains and 2 genera and 5 species for methanogen strains based on the study of pure cultures and analysis of small subunit rRNA gene sequence data. Strain GH1 (anaerobic strain) and JH1 (methanogen strain) were then selected to be characterised for 16S rRNA gene sequence similarity, phenotypic, chemotaxonomic and phylogenetic analysis.
[Supported by the Agriculture Science & Technology Development]

A040

Introduction of the Value Evaluation Study of Actinobacterial Resources Based on the Microbial Products

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Amongst prokaryotes, actinomycetes remain a rich source of new natural products, including useful antibiotics, antitumour agents, and so on. Even we have amount of actinobacterial resources, but the utilization and application are insufficient. It is necessary to evaluate microbial resources for the researchers that can utilize them well.
The purpose of this study is to support and help researchers, by constructing of the infra-structure and mass-analysis of the microbial characteristics. We focus on analyzing sequences for molecular taxonomy and cultural characteristics. In order to improve their values, we are assembling each microbial strain information data such as 16S rRNA sequences, enzymes and antimicrobial activities, growth conditions depend on temperature, pH, NaCl and LC/MS profiles. Microbial products are being prepared and the microbial culture packages of taxonomic or functional groups are being manufactured for mass-distribution. More than 13,000 actinobacterial strains are collected until now. And these will be helpful to the researchers who need high-throughput screening assay.
[This research was supported by Ministry of Science, ICT and Future Planning (MSIP).]

A041

New Fungal Species of *Penicillium* from *Viscum album* var. *coloratum*

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Mistletoe (*Viscum album* var. *coloratum*) plant has been used as an important traditional Chinese medicine. Two isolates of *Penicillium* were purely isolated from a sample of stored mistletoe kept in the refrigerator at 5°C. Based on the morphological characteristics and rDNA ITS, beta-tubulin and calmodulin genes sequence data, the fungus was provisionally identified as a new *Penicillium* species showing a separate phylogenetic status.The colony of the two isolates, EML-WPF1 and 2 on YES agar was radially sulcate, velutinous, grey green, clear droplets on surface and orange to blackish green on reverse side. The penicillate form of the isolates was two-stage branched (terverticillate) on the medium. Sequence analysis by BLAST indicated that the fungus was closest to *Penicillium* spp. (accession number, *Penicillium* sp. HQ225717 and *Penicillium* sp. HQ637354, *Penicillium* sp. KF021538 and *Penicillium* sp. JX996973) with 99, 94-95, 93% sequence similarities of ITS, beta-tubulin and calmodulin genes, respectively. Our results showed that the fungus isolated from mistletoe plant is new to science.
[Supported by grants from NIBR.]

A042

New Record of *Penicillium viridicatum* from *Viscum album* var. *coloratum* in Korea

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One isolate of *Penicillium* was purely isolated from a sample of stored mistletoe plant kept in the refrigerator at 5°C. Based on the morphological characteristics and rDNA ITS, beta-tubulin and calmodulin genes sequence data, the fungus was provisionally identified as a known *Penicillium* species, *P. viridicatum* belonging to Fasciculata clade. The colony of the fungus on YES agar was radially sulcate, velutinous, bluish green and beige light brown on reverse side. The penicillate form of the fungus was two or three-stage branched (terverticillate) and the conidia was subglobulose or globulose on the medium. Sequence analysis by BLAST indicated that the isolate was closest to *P. viridicatum* (accession numbers, AF033478, AY674295 and GQ979712) with 99% ITS, beta-tubulin and calmodulin sequence similarity. Our study showed that this is the first record of *Penicillium viridicatum* on stored mistletoe plant in Korea.
[Supported by grants from NIBR.]

B001

Polar and Alpine Microbial Collection (PAMC): A Culture Collection Dedicated to Polar and Alpine Microorganisms

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The number of microbial strains isolated from polar and alpine areas is increasing and they are recognized as valuable resources in fundamental studies, such as ecology, physiology, and -omics. Thus, the necessity of culture collection dedicated to the polar and alpine microorganisms has increased. Korea Polar Research Institute (KOPRI) established the Polar and Alpine Microbial Collection (PAMC) to share biodiversity information and bio-resources collected from polar and alpine areas in science and public communities. Approximately 2,000 out of 6,500 strains maintained in PAMC have been identified and belonged primarily to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Many of the microbial strains of PAMC can grow at low temperature and produce proteases, lipases, and/or exopolysaccharides. PAMC provides search tools based on keywords such as taxonomy, geographical origin, habitat and physiological characteristics. Biological materials and information provided by PAMC will be important resources for those who have had no opportunity to visit polar and alpine areas and are expected to contribute to the development in the extreme life sciences (PE14080).

B002

Stable Isotope Probing and Metagenomic Reconstruction Reveals Pathways of Aromatic Hydrocarbon Metabolism by an Uncultivated Denitrifying Bacterium in the Genus *Herminiimonas*

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Stable isotope probing is a cultivation-free methodology that provides information about the identity of microorganisms participating in assimilatory processes in complex communities. In this study, a *Herminiimonas*-related bacterium was identified as the dominant member in a denitrifying enrichment culture fed ^{13}C-toluene. The draft genome of the uncultivated toluene-degrading bacterium was obtained after applying pyrosequencing to the heavy DNA fraction, and assembly of 131 contigs. Metabolic reconstruction of aromatic hydrocarbons (toluene, benzoate, *p*-cresol, 4-hydroxybenzoate, phenylacetate and cyclohexane carboxylate) degradation indicated that the bacterium might be specialized in anaerobic hydrocarbon degradation, which is novel for the order of *Burkholderiales* within the class *Betaproteobacteria*. Under oxic conditions, the benzoate oxidation gene cluster (BOX) system is likely involved in the degradation of benzoate via benzoyl-CoA. Putative mobile genetic elements associated with these catabolic genes were highly abundant—suggesting gene acquisition by *Herminiimonas* via horizontal gene transfer.
[Supported by Mid-career Researcher Program through a NRF and BK21+ program.]

B003

Microbial Flora in Photobioreactors Culturing Microalga, *Chlorella* sp. KR-1

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To understand the effects of microorganisms in photobioreactors culturing microalgae, which is emerging as a feedstock of biofuels, microbial community structure was investigated by pyrosequencing technique in the reactors culturing *Chlorella* sp. KR-1. In addition to this, several dominant microorganisms were isolated from the reactors after dilution of the culture solution by a plate method. In the reactors, *Chlorella* sp. KR-1 was cultured in an artificial inorganic medium supplemented with 10% carbon dioxide in air as a carbon source. The meta-genomic DNAs for the pyrosequencing were obtained from biomass samples derived from the seven photobioreactors on four different periods. According to the pyrosequencing results, gamma proteobacteria were dominant at the initial stage of the cultivation while alpha proteobacteria were dominant on cultivation time. Major isolates by the plating method were identified by blast search of NCBI with the sequencing results of 16S rRNA gene. They are *Sphingomonas* sp., *Leifsonia* sp., *Flectobacillus* sp., and *Herbasprillium huttiense*.
[Supported by grants from NRF2013011775.]

B004

pH Caused the Constitutional Shift of Bacterial Community in Soil Ecosystem

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In order to compare bacterial community structure of mountain soil with pH of 5.2 (Jiri Mountain) with those of pH of 7.7 (Jang Mountain located close to big city), their DNAs were extracted, amplified using PCR with 27F/800R primers and pyrosequenced using Roche 454. The results were expressed in ratio (%) of numbers of same reads to those of total reads on phylum level. As results, in the Jiri Mountain soil with acidic pH (5.2), almost half was Proteobacteria (49%), and followed by other 11 different phyla. By contrast, in Jang Mountain soil three dominant phyla, Actinobacteria (28%), Proteobacteria (26%), Bacteroidetes (23%) made up 78% and followed by only 4 other phyla. So it suggests that the ecologically stable Jiri Mountain showed more diverse bacterial community than ecologically less stable Jang Mountain even with fairly optimal pH (7.7). As supported by the above mentioned result, the diversity of species composition evaluated by Shannon- and Simpson-index was higher in Jiri Mountain than Jang Mountain. The finding shows that the large gap of pH caused a large shift in their bacterial community structure.
[Supported by Daegu University Research Grant 2011]

B005

Evaluation of Microbial Community Depending on Setting Anodic Potential in Microbial Fuel Cell

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Microbial fuel cells (MFCs) are innovative processes that use microorganisms as a catalyst to produce an electrical current from a variety of organic matters. The anodic potential in MFCs controls the theoretical energy gain for these microorganisms. To investigate microbial community variation depending on setting potential, we set four different anode potentials (-0.3, -0.2, -0.1, +0.1 V vs. Ag/AgCl) using a potentiostat also used acetate and wastewater as substrate. The microbial community analysis base on DGGE profile showed significantly different between acetate-fed MFCs (A-MFCs) and wastewater-fed MFCs (W-MFCs). *Geobacter* sp. (99% identity), which was most closely related to the exoelectrogen, and *Zoogloea* sp. (100% identity) were detected in all case of A-MFCs. The anode community at the most positive potential (+0.1 V) revealed high band intensity of *Geobacter* sp.. In contrast, W-MFCs showed that *Variovorax paradoxus* (99% identity), which was found in activated sludge, was most dominant species, but representative exoelectrogens such as *Geobacter* sp. were not detected.
[This work was supported by the MFC R&BD center co-founded with K-water, Hanhwa E&C and Taeyoung E&C]

B006

Divergence of Bacterial Community Catalyzing Autotrophic Denitrification at Biocathodes of Bioelectrochemical Systems with Either Abiotic or Biotic Anodes

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Bioelectrochemical systems (BESs) were operated to investigate the structure of bacterial community catalyzing autotrophic denitrification at the biocathodes when using abiotic and biotic anodes. Acetate was used as electron donor in BESs with biotic anode, whereas a direct current power supply was used as energy source in BESs with abiotic anode. The laboratory results showed that the highest efficiency (78.0%) of autotrophic denitrification was achieved when electron transfer from the biotic anode chamber of BESs was used. Data from denaturing gel gradient electrophoresis and principal component analysis indicated that the bacterial community catalyzing autotrophic denitrification in BESs using abiotic anodes was entirely different with those in BESs using biotic anodes. The results of phylohenetic analysis suggested that denitrification in BESs with abiotic anode could be attributed to *Nitratireductor* sp., *Shinella* sp., and *Dyella* sp., whereas the dominant bacterial denitrifiers in BESs with biotic anode were found to be *Pseudomonas* sp., *Curtobacterium* sp., and *Aeromonas* sp.
[This work was supported by National Research Foundation of Korea grant funded by the Korea government]

B007

Comprehensive Analysis of Soil Bacterial Community Structure in King George Island, Maritime Antarctica

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In recent years, applications of molecular methods to study microbial ecology have allowed the extension of our knowledge that Antarctica contains unexpected high diversity of bacteria and their complex of community. Total 263 samples from 51 sites were collected from December of 2010 to February of 2012. Among these samples, we here presented the preliminary results with 85 samples. On the basis of the 16S rRNA genes amplicon using NGS, total 172,198 sequence reads were obtained and 9,673 OTUs were defined using 97% similarity cutoff. *Actinobacteria* (21.4%), *Proteobacteria* (20.0%) and *Acidobacteria* (11.3%) were dominant across all habitats. Interestingly, candidate phylum AD3 (4.0%) was abundant in several soil samples, which has not been recognized in previous studies. The bacterial community structures confirmed habitat specific with significant patterns that the predominant phylum is *Bacteroidetes* in coastal soil, *Proteobacteria* in upper layer soil and *Actinobacteria* in lower layer soil. These findings may be inferred that the bacterial specific adaptation to these terrestrial environments was affected by the underlying soil parameters.
[Supported by grants from KOPRI]

B008

Comparative Analysis of Bacterial Community Composition in Lakes of the Dry Valleys, East Antarctica

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McMurdo Dry Valleys (MDVs) are located in the coldest and the driest on earth as the ice-free region. To date, despite biogeochemical constraints, many studies have shown that the molecular diversity was examined of cold active microorganisms and reported their important roles in these lakes. In the current study, the community composition of bacteria were investigated the perennial ice-covered lakes in the fine scale using 454-pyrosequencing; Fryxell (FRX), Hoare (HOR) and Miers (MIE). Based on 16S rRNA gene, total 124,803 sequences were quality filtered using 97% similarity cutoff, and 55 phyla were recovered containing the major phyla *Actinobacteria* (29%), *Bacteroidetes* (24.4%) and *Proteobacteria* (14%). In the phylum level, the bacterial taxonomy were highly shown the heterogeneous communities. Microbial community composition varies not only between lakes, but also along the depth gradients within lake. Taken together, the diversity of microbial communities in lakes of MDVs provided a crucial evidence to understand the impact of microbial ecological roles in these ecosystems.

B009

Comparative Proteomic Analysis of Extracellular Vesicles (EVs) from the Hyperthermophilic Archaeon, *Thermococcus onnurineus* NA1

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Thermococcus onnurineus NA1 is able to produce extracellular vesicles (ToEVs) under anaerobic cultivation at 80°C. EVs were purified through ultra membrane filtration and CsCl-density gradient ultracentrifugation. Two major bands (B & C) were obtained and theirs proteomes were further analyzed by LC MS/MS spectrometry. It was revealed that the ToEVs of band B and C contained 294 and 426 proteins, respectively. Ratio of membrane protein to total protein in bands B and C ToEVs was slightly higher than that of parental cells. The analysis of predicted metabolic function of proteins from two major bands based on the COG database indicated that there was no big discrepancy between them. Proteomic comparison among parental cell, band B and C ToEVs using Venn diagram showed overlapping zone where we could find 238 proteins. The total numbers of protein which was found from EVs but not from parent cells were 15. The role of specific proteins mainly packaged from parental cell to ToEVs will be discussed.

B010

Genome-wide Transcriptional Profiling of *Acinetobacter oleivorans* DR1 in Response to Different Class Antibiotics

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To understand common and specific gene expression patterns in *A. oleivorans* DR1, we investigated RNA-seq-based transcriptomic profile under different class of antibiotics (ampicillin; Amp, kanamycin; Km, tetracycline; Tc, and norfloxacin; Nor). Genes related to transporters, integrase, membrane proteins and stress response were commonly upregulated under 4 antibiotics conditions. Metabolic pathway related to arginine and proline biosynthesis was specifically upregulated in Km and Tc conditions, SOS response and DNA repair genes were induced by Nor, and lipid transporters by Amp. All antibiotics changed membrane permeability, filamentation, and ROS production. Gene expression patterns were consistent with the phenotypic changes such as ROS generation, motility, cell morphology, growth defect and DNA repair. Interestingly, loss of base-excision repair ability was observed with Tc and Km. The most dramatic phenotypic changes were observed under Km: enhanced motilities, increased fimbrial expression, elongated cell shape and loss of DNA repair capability. This study provides novel insight into antibiotics resistance of environment-originated *Acinetobacter* species.

B011

Biosorption of Ionic Dye and Precious Metal from Aqueous Solution by *Bacillus* sp. JB-007 Biomass

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Jeonju Biomaterials Institute

The main aim of this work was to evaluate the biosorption capacity of *Bacillus* sp. JB-007 (JB-007) biomass for the removal of anionic dye Reactive Red 4 (RR 4) and recovery of precious metal gold (Au (I)), as the industrial materials. In the pH edge experiments, the adsorption of RR 4 and Au (I) increased in acidic pH. The equilibrium isotherm experiments indicated that JB-007 biomass exhibited the maximum uptake for RR 4, i.e. 192.03 mg/g and 91.90 mg/g for Au (I) at pH 2.0. Of the two isotherm models considered, the Langmuir model provided a better description of the experimental isotherms. Kinetic experiments revealed the RR 4 and Au (I) sorption processes were found to be very rapid, and the equilibrium of the sorption processes could be reached within approximately 10 min. In order to identify the binding sites for the dye and precious metal molecules, the biosorbent was analyzed by FTIR analysis. These results indicate that *Bacillus* sp. JB-007 biomass has good properties as an industrial biosorbent for the removal of anionic dye and recovery of precious metal from wastewater.

[This subject is supported by Korea Ministry of Environment as "The Eco-Innovation project"]

B012

PCR-DGGE-based Analysis of Bacterial Community Change in Agricultural Soil Stimulated with Red Clay

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Red clay could be used to promote bacterial growth and was proven to have biostimulation effect on diesel-polluted soils. However, high concentration of red clay in soil could have inhibitory effect on some bacterial population. To explain the relationship between concentration of red clay and bacterial population change, agricultural soils were collected and incubation was conducted at 20°C as a slurry in the microcosm for 7 weeks after red clay amendment [0, 0.1, 0.5, 1, and 5% (w/v)]. Ribosomal gene fragments were amplified with PCR using soil DNA and were loaded on denaturing gradient gel electrophoresis (DGGE) to analyze bacterial community change. Our data showed that different group of bacterial community became dominated by different concentration of red clay. The highest diversity was detected in the sample treated with 0.1% red clay, as the number of bands was greater than other samples. Interestingly, some strains flourished only under a particular concentration of red clay. High concentration of red clay (5%) appeared to be toxic to some bacterial species because less DGGE bands were detected. Bacterial community is being currently investigated by sequencing DGGE bands.

B013

A Novel Function of Extracellular Vesicles Produced from Rhodopsins-containing Flavobacterium *Sediminicola* sp. YIK13

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We isolated and purified the extracellular vesicles (EVs) produced from rhodopsins-containing a parent bacterium, *Sediminicola* sp. YIK13 (S13), using by ultra membrane filtration followed by CsCl density equilibrium ultracentrifugation. The production rate of S13EVs was estimated by 3.97 $\times 10^{13}$ ml^{-1} from the total number of parental bacteria, 6.67×10^{9} ml^{-1}. The buoyant density of S13EVs ranged from 1.3730 to 1.4065 g cm^{-3}. S13EVs showed a spherical shape. The range of diameter was between 88 to 227 nm and the median size was 117 nm. Interestingly, S13EVs contained orange color pigment as same as parental cells. The absorbance of S13EVs indicated the presence of carotenoid and PR pigments which were certainly packaged from parental cells. S13EVs showed the light driven proton pump activity as similar as the parental cells although the EVs represented stronger proton inward pump activity rather than that of parental cells. This is the first report of extracellular vesicles of which membrane bound pigments like cartenoid and PR were packaged from the parental bacteria, and those photo proteins of EVs generated the proton motive force which could be utilized for the ATP synthesis.

B014

Comparative Analysis of Fecal Microbiome in the Different Swine Group by Pyrosequencing

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Complex microbial communities in the swine gastrointestinal tract play a critical role in health and disease. To detect a core of microorganisms in the gastrointestinal tract in two different groups separated by meat quality and weight grades, we used high-throughput 16S rRNA gene-based pyrosequencing. At high levels of taxa, two bacterial populations (Bacteroidetes and Firmicutes) were dominated and shared between difference groups. However, significant differences between groups were found at the genus level. The genera Lactobacillus and Oscillibacter in level 2 (0.4-1.4% of the classified reads) were higher than respective genera in level 1. The proportion of the genus Roseburia in level 1 (4.4%) was higher than the proportion in level 2 (1.5%). These results suggest that the genera Clostridium, Oscillibacter and Roseburia work as core microorganisms. Moreover, the genera Roseburia and Clostridum in level 1 produce both linoleic acid and short chain fatty acid that contribute to swine health and development. In conclusion, the presence of core bacteria in the swine gut positively affects to increase amount and meat quality with the reduced body fat in swine.

B015

Changes of Stress Shock Proteins DnaK and GroEL by Explosive RDX in *xenA/xenB* knock-out *Pseudomonas* sp. HK-6 Mutants

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Our previous research has demonstrated that stress shock proteins, 70-kDa DnaK and 60-kDa GroEL, are induced in wild-type *Pseudomonas* sp. HK-6 in response to sublethal concentrations of explosive hexahydro-1, 3,5-trinitro-1,3,5-triazine (RDX) [Chang *et al*. (2004) *Appl. Microbiol. Biotechnol.* 65:323-329]. In the present work, the changes of stress shock proteins in *xenA/xenB* knock-out *Pseudomonas* sp. HK-6 mutants in response to the explosive RDX were investigated. Bacterial growth and RDX degradation by wild-type and *xenA/xenB* knock-out mutants of *Pseudomonas* sp. HK-6 with time in the presence of different concentrations of RDX was monitored. The stress shock proteins, which contribute to the resistance of the cytotoxic effect of RDX, were diminished at different RDX concentrations in growing cultures of *xenA/xenB* knock-out *Pseudomonas* sp. HK-6 mutants, whereas the stress shock proteins were gradually induced in wild-type *Pseudomonas* sp. HK-6. The proteins were identified as 70-kDa DnaK and 60-kDa GroEL by SDS-PAGE and Western blot using the anti-DnaK and anti-GroEL monoclonal antibodies.
[This research was supported by NRF (#2010-0022225)].

B016

Enhanced Functionality of Mixed Lactic Acid Bacteria Isolated from *Dongchimi*, Korean Fermented Watery Kimchi

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The aim of this work was to investigate the several functionalities of lactic acid bacteria (LAB) and to compare the enhanced functionalities in individual LAB (*e.g.*, DK-3, DK-6, DK-13) and these three mixed LAB as probiotics isolated from Korean fermented watery Kimchi, *Dongchimi*. We monitored growth rate, production of organic acids (*e.g.*, lactic acid and acetic acid) as metabolites, and pH change during the growth as well as functional properties including antioxidant activity, nitrite scavenging, β-galactosidase, and antimicrobial activity. At the end of the incubation period, all cultures produced organic acids and showed acidic pH. Functionality values obtained from the mixed LAB cultures have been shown to be higher than those of individual LAB cultures. These studies demonstrate that the functionalities in mixed cultures compared to individual cultures can be enhanced. Both BIOLOG system and 16S rRNA sequencing were conducted to identify the LAB strains, which were assigned to *Leuconostoc mesenteroides* DK-3, *Leuconostoc dextranicum* DK-6, and *Lactobacillus curvatus* DK-13, respectively.

B017

Bacterial Community Successions during Fermentation of Anchovy (Myeolchi-jeot) with Different Anchovy Sizes

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To investigate microbial communities of *myeolchi-jeot*, made by the fermentation of highly salted [25% (w/v)] anchovy in Korea, four sets of samples were prepared using anchovy (*Engraulis japonicas*) with different sizes and fishery zones and their bacterial and archaeal abundances, pH, and bacterial communities were analyzed. Bacterial community analysis using pyrosequencing revealed that *Photobacterium*, *Vibrio*, and *Psychrobacter* belonging to the phylum *Proteobacteria* were dominant at the beginning of the fermentation (day 0) and the bacterial communities were significantly different depending on *myeolchi-jeot* samples during the early fermentation period, but eventually members of *Tetragenococcus*, halophilic LAB, belonging to the phylum *Firmicutes* became predominant. The bacterial community changes in myeolchi-jeot samples prepared by smaller size anchovy occurred more rapidly. In conclusion, bacterial successions in *myeolchi-jeot* were different during the early fermentation period depending on anchovy size and fishery zones, but eventually *Tetragenococcus* became predominant in all samples regardless of anchovy sizes and fishery zones during the late fermentation period.

B018

Endolichenic Fungal Communities Associated with Dominant Lichens in the Maritime Antarctica

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King George Island is the largest island in the South Shetland Islands, the maritime Antarctic zone. Bryophyte and lichen are one of the microhabitats richest in microfungi in the Antarctic environment. We isolated 60 endolichenic fungi from 44 lichens belonging to 10 family and 22 lichen species collected from King George Island, using the surface sterilization method. Total 60 endolichenic fungi isolates were grouped into 37 fungal strains by ITS nuclear ribosomal gene sequencing. Phylogeny analysis represents 37 endolichenic strains belonging to the phyta Ascomycota and five classes and ten orders. We compared 37 endolichenic fungi with 16 endophytic fungi isolated from bryophytes and evaluated whether these fungi represent distinct ecological guild of flexible symbiotrophs capable of colonizing lichens or bryophytes indiscriminately. We found that identical fungal isolates were detected from the lichen and bryophyte. This result suggested that evolutionary adaptation of these microorganisms was not simply governed by host specificity. We firstly reported ecologically flexible endophytic fungus that occurs both in lichen and bryophyte in the maritime Antarctica.

B019

Bacterial Populations in the Leaves of the *Prunus* Species

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The surface of leaves called as phyllosphere is the main microbial habitats on the earth. In this study, we performed a metagenomic analysis to identify bacterial populations in the leaves of the *Prunus* species. Four different 16S rDNA libraries were prepared and subjected for sequencing using the FLX 454 Plus system. We obtained a total of 163,686 rRNA reads. The 16S rRNA sequences from the plant organelles such as mitochondria and chloroplasts were dominant. According to the bacterial species, 473, 527, 425, and 614 operational taxonomic units (OTUs) were identified for apricot, cherry, peach, and plum, respectively. We identified a total of seven phyla belonging to nine classes including 31 orders which were further divided into 76 families and 159 bacteria species. We compared the identified bacteria in each sample at the genus level. Only 23 genera out of 159 genera were commonly identified in four phyllospheres. In particular, several alphaproteobacteria such as *Sphingomonas* and *Methylobacterium* were dominant in the phyllosphere of four fruit trees. Taken together, our metagenomic analysis revealed strong bacterial diversity in the leaves of the *Prunus* species.

B020

Growth Promotion of Tomato under Drought Stress and Drought-Related Gene Expression by Some Rhizobacteria

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To select rhizobacteria that can promote plant growth under drought stress, many bacteria were isolated from the rhizopheres of wild plants grown at barren land. Among them one strain which secreted high concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase that can tolerate drought stress by degradation of stress hormone ethylene and indole-3-acetic acid (IAA) that can enhance plant growth, was selected and identified as *Enterobacter ludwigii* SJR3. Strain SJR3 could produce 45.79 mg/L of IAA and show ACC deaminase activity of 13.76 μmol α-ketobutyrate/h/mg protein. Strain SJR3 could also produce gibberellin and zeatin and solubilize insoluble phosphate. When tomato seedlings were treated with SJR3 and grown for 21 days under drought stress, root length of tomato plants increased by 26.44% than that of the uninoculated control. Shoot/root ratio was also lower than the control. Expressions of drought-related genes, *DREB2, DREB3, ACS4* and *ACS6* of tomato treated with SJR3 strain were lower than those of the uninoculated control plants under drought stress conditions, which indicated that the tomato inoculated with strains acquired dehydration resistance.

B021

Characterization of Heterotrophic Nitrification and Aerobic Denitirification by *Alcaligenes faecalis* NS13

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Ammonium removal from wastewater is important to prevent eutrophication of aquatic environments. To solve the problem of high cost of chemical and physical methods of N removal, environment-friendly measure using microbes can be an alternative one. We isolated a heterotrophic bacterial strain NS13 from wastewater, which showed the high removal rate of ammonium and was identified as *Alcaligenes faecalis* by 16S rRNA gene sequence analysis. This bacterium could remove over 99% in a heterotrophic medium containing 140 mg/L of ammonium at pH 6~9, 25~37°C and 0~4% of salt concentrations within 2 days. Strain NS 13 showed the higher ammonium removal at the higher initial ammonium concentration in the medium with the removal of 55.2, 99.9 and 210.4 mg/d during 4 days at 300, 600 and 1500 mg/L of initial ammonium concentration, respectively. *A. faecalis* NS13 could reduce nitrate by nitrate reductase which was confirmed by detection of nitrate reductase gene *napA* by PCR. One of denitrification metabolite N_2O was also detected from the headspace of bacterial culture. This bacterium was speculated to perform the heterotrophic nitrification and aerobic denitrification at the same time.

B022

Growth Promotion of Tomato by Application of Immobilized *Arthrobacter woluwensis* ED in Alginate Beads

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In order to increase the persistence of plant growth promoting rhizobacteria (PGPR) in rhizpsphere soil, the growth of tomato was examined after the application of *Arthrobacter woluwensis* ED immobilized in alginate bead. When tomato seedlings were treated with *A. woluwensis* ED of 1 x 10^6 cells g $soil^{-1}$ and incubated for 30 days, shoot and length, fresh and dry weight of tomato plants treated with the suspended inoculants significantly increased by 36.2, 59, 51.1 and 37.5%, respectively compared to those of the uninoculated control. The treatment of the immobilized bacteria increased those by 42, 67.4, 62.5 and 60.4%, respectively compared to those of the control. The effects of the inoculation on soil bacterial community and the fate of the inoculated bacteria were monitored by DGGE analysis. The DNA band intensity of *A. woluwensis* ED in the tomato rhizosphere treated with the immobilized inoculants showed the maximum at 1 week after inoculation and the decreasing rate was less than that of the suspended inoculants. Encapsulation of PGPR in alginate beads may be more effective than liquid inoculant for the plant growth promotion and survival of PGPR at plant rhizosphere.

B023

Screening of Plant Growth-promoting Rhizobacteria as Biological Control Agents against *C. acutatum* in Pepper

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Plant growth-promoting rhizobacteria (PGPR) are colonizing plant root and rhizosphere with stimulating the plant growth, suppressing the pathogen and reducing the damages caused by stressful environmental conditions. In this study, we screened rhizospheric soil in pepper to examine their microbiome and characteristics related to plant health. From the results, total 150 bacteria were isolated from rhizosperic soil of pepper field in Mir-Yang, Korea. All bacterial isolates were identified by 16S rDNA sequences and phylogenetic analysis was performed for comparative purpose. To determine the plant growth promotion characteristics, all isolates were tested for phosphate solubilization, production of siderophore and indole-3-acetic acid (IAA). Among total 150 bacteria, 28 isolates had the ability to produce indole-3-acetic acid (IAA), 92 isolates were able to the produce siderophore, and 65 isolates solubilized phosphate. In addition, we conducted *in vitro* antifungal assay to find the good biological agents against *Colletotrichum acutatum.* Forty four strains among total 150 isolates showed antifungal activity.
[Supported by NRF: No. 2011-0011565]

B024

Evaluation of the Effect of Exogenous Phenylacetic Acid Application on Growth and Induced Systemic Resistance of Tobacco Seedlings against *Pectobacterium carotovorum* subsp. *carotovorum*

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The non-indole compound phenylacetic acid (PAA) can influence plant-pathogen interactions. PAA was identified as one of the metabolites of the plant growth-promoting rhizobacteria (PGPR) *Ochrobactrum lupini* KUDC1013 that induced systemic resistance (ISR) of tobacco against the leaf soft rot pathogen *Pectobacterium carotovorum* subsp. *carotovorum* (PCC). In the present study, the effect of exogenous PAA application on the growth and ISR of tobacco against PCC were examined. The lowest disease incidence was observed in plants treated with 0.5 mM PAA. Activities of PDF1.2 genes in transgenic tobacco plants carrying plant defensin gene fused with the beta-glucuronidase (GUS) reporter gene (PDF1.2::GUS) and of the defense related enzyme, phenylalanine ammonia-lyase (PAL) were enhanced. PAA also modulated the expression of defense related genes. High concentrations of PAA decreased the primary root length however, increased PAA concentrations led to an observable increase on lateral root formation. Altogether, this study supports the role of PAA in plant growth development and in eliciting plant defense signaling.

B025

Plant Growth-Promoting Effect and Elicitation of Systemic Resistance in Pepper by Volatile Organic Compounds

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Volatile organic compounds (VOC) which are defined from their properties of high vapor pressure at room temperature and low boiling point are also found as elicitors of systemic induced resistance (ISR) in plants. The strain *Ochrobactrum lupini* KUDC1013 was previously reported to elicit induced systemic resistance (ISR) in tobacco and pepper. The crude supernatant obtained from this strain contained linoleic acid (LA), phenylacetic acid (PAA) and 1-hexadecene which elicited ISR against leaf soft rot in tobacco. We studied the effects of different concentrations of the three chemicals for the elicitation of systematic resistance against fungus *Stemphylium lycopersici*, which induces gray leaf spot disease in pepper. And we performed *in-vitro* antifungal assay using different concentration gradients of three chemicals against *S. lycopersici*. To investigate the influence of above three chemicals on growth-promoting, the seed germination was assayed in pepper.
[Supported by NRF: No.2011-0011565]

B026

Analysis of Pathogenic Microorganism's Contamination on Production Environment of Tomato in Korea

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The purpose of this study was to analyze microbiological hazards for cultivation environments and personal hygiene of tomato farms at the growth and harvesting stage. A total of 1944 samples including air born, soil or medium, mulching film, harvest basket, groves and irrigation water etc. were collected from farms. As a result, total *S. aureus* and *B. cereus* in all samples were detected 0~6.7 and 0~5.9 log CFU, respectively. Among the total bacteria of tomato farms, *S. aureus* (glove: 0~4.0 log CFU/100 cm^2, harvest basket: 0~5.0 log CFU/100 cm^2, soil or culture media: 0~6.1 log CFU/g, mulching film: 0~4.0 log CFU/100 cm^2), *B. cereus* (glove: 0~4.0 log CFU/100 cm^2, harvest basket: 0~4.3 log CFU/100 cm^2, soil or culture media: 0~5.9 log CFU/g, mulching film: 0~4.7 log CFU/100 cm^2) were detected in all samples. In particular, the amount of *S. aureus* and *B. cereus* detected in tomato farms was less than the minimum amount required to produce a toxin that induces food poisoning. In this way, the degree of contamination of food poisoning bacteria was lower in the production environment of the Korea tomato, but problems can be caused by post-harvest management method.

B027

Isolation and Characterization of Bacteria from Lake Alginskoe in Russia

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We isolated bacteria from Lake Alginskoe in Russia. The isolated bacteria were analyzed 16S rRNA sequence, Enzyme activities and growth rate were tested. As the results, total 43 species bacteria belonging to 3 taxonomy groups (Proteobacteria, Actinobacteria, Firmicutes) were isolated from Lake Alginskoe. And most isolates were halophilic bacteria. Moreover 2 isolates were new species. Enzyme activities, amylase, cellulase, lipase and protease of isolates were analyzed. Twenty two isolates had one or more enzyme activities at least. And four isolates had three enzyme activities (*Cupriavidus pauculus*, *Bacillus safensis*, *Brachybacterium paraconglomeratum*, *Bacillus aryabhattai*). And the growth rate of isolates in different salinity were analyzed. *Cupriavidus pauculus* showed high growth rate at 0% salinity. But *Cupriavidus pauculus* showed the growth rate of decreased significantly with the increase of salinity. *Bacillus safensis* showed faster growth rate than other species in highly saline condition. *Brachybacterium paraconglomeratum* and *Bacillus aryabhattai* increased the growth rate in 0~7% of salinity, the growth rate was faster than the other species.

B028

Metagenomic Analysis of Bacterial and Viral Diversity in the Intestines of Abalone (*Haliotis discus hannai*) and Spoon Worm (*Urechis unicinctus*)

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Abalone is one of typical invertebrate aquacultured in South Korea and the abalone production has been sharply increasing as well as mortality rate caused by microorganisms. In case of spoon worms, they inhabit almost all around the world, but they still have difficulty to be cultivated artificially. In this study, bacterial diversity in abalones and spoon worms were revealed using the recently revised metagenomic tools and the results from this study are expected to be applied to the probiotic applications for abalone and spoon worm aquaculture as fundamental informations. Results showed that *Mycoplasma* species took over 70% in the whole bacterial population in abalone intestine, whereas *Vibrio* species occupied about 10%. In spoon worm samples, Lactic acid bacteria such as *Lactococcus* and *Leuconostoc* species were dominant occupying 63.6% in the whole bacterial population. Viral diversity analysis revealed that viral compositions of both abalone and spoon worm samples were mainly occupied by ds DNA viruses such as *Myoviridae*, *Sipoviridae* and *Podoviridae* belonging to *Caudovirales* and ss DNA viruses such as *Chlamydia* phages related to *Microviridae*.

B029

Comparison of Plant Growth-Promoting Rhizobacteria (PGPR) Isolated from Rhizospheric Soils in Pepper under High Salinity and Normal Farm Field Conditions

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This study established the plant growth-promoting rhizobacteria (PGPR) in the rhizosphere of pepper plants grown in highly saline and normal conditions from Mir-Yang, Korea. The value of electrical conductivity in rhizospheric soils showed 5.41 ds/m for high salinity and 2.11 ds/m for normal field, respectively. Total of 479 strains were isolated and partial identified using the 16S rRNA gene sequencing. All of these isolates were investigated for PGP characteristics such as production of Indole-3-acetic acid (IAA), siderophore, and ACC deaminase and phosphate solubilization. Among the isolates from high salinity conditions, 15.6% were able to produce IAA, 41.7% of isolates produced siderophore and 33.7% were able to solubilize phosphate. Forty nine point eight percent of isolates were able to utilize ACC. On the other hand, isolates from normal conditions, 25.9% produced IAA, 60.0% produced siderophore, and 52.4% were able to solubilize phosphate. Fifty six percent of isolates were able to produce ACC deaminase. Our results showed that the number of PGP isolates from normal conditions was higher than that with high salinity conditions.
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B030

Production of Polyhydroxyalkanoates (PHA) from 4-Methoxybenzoate Using *Sphingobium chungbukense* DJ77

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In the present study, we report the conversion of aromatic compound, 4-methoxybenzoate (4-MBA), into the aliphatic biopolymer polyhydroxyalkanoates (PHA) using *Sphingobium chungbukense* strain DJ77. PHA production was investigated using an aromatic compound 4-MBA at different concentrations (1, 3, 5, 8 and 11 mM), and production efficiency was compared with non-aromatic compound i.e., acetate at 8 mM. The strain DJ77 showed the growth on 4-MBA and the accumulation of PHA with simultaneous 4-MBA degradation. Using 4-MBA at 8 mM as a sole carbon source in Minimal-Salts-Basal (MSB), maximum amount of chloroform-soluble PHA (68%) was extracted from dry cell mass, followed by 5 mM (56%), 3 mM (51%), 1 mM (44%), and 11 mM (38%). The cell grown on acetate at 8 mM showed higher PHA production (79%) compared with 4-MBA. The functional groups present in the produced bioplastics were confirmed using FT-IR spectroscopy. These results make the strain DJ77 is excellent candidate for the generation of PHA from aromatic hydrocarbons as a feedstock providing a route for converting hydrocarbon wastes into usable biological polyesters.

B031

Optimization of Biodegradation Conditions with a Sea Tidal Flat BTEX-degrading Bacterium, *Janibacter* sp. SB2, Using Experimental Design Method

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The objective of this study was to isolate a BTEX (benzene, toluene, ethylbenzene and *o*-, *m*- and *p*-xylene)-degrading bacterium from contaminated sea-tidal flat and to optimize its degradation condition for efficient degradation of BTEX compounds. An enrichment culture was established using seawater containing BTEX compounds and the enriched microbial community was monitored by DGGE profiling, which indicated that *Janibacter* species was dominated during the enrichment. Strain SB2 corresponding to the major band, able to degrade all six BTEX compounds, was isolated and characterized. NH_4Cl, NaH_2PO_4, cell mass and BTEX concentrations were used as independent variables and a statically significant (R^2 =0.8933, P <0.0001) quadratic polynomial mathematical model was suggested. In a slurry system containing 3.0×10^7 cells/L, 45.5% BTEX degradation for the initial concentration of 240 mg/L BTEX was observed under the optimum condition of NH_4Cl and NaH_2PO_4 at 60 h; 32.2% BTEX degradation was observed for untreated samples.

B032

Studies on the Strategies to Cope with High Salinity Environments through the Genome Analysis of *Salimicrobium jeotgali*

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To cope with high salt environment, microorganisms have selected several strategies. *Salimicrobium jeotgali*, isolated from 'myeolchi-jeot', a traditional salted and fermented anchovy of South Korea, is one of halophilic bacteria which can survive up to 24% NaCl. In high salt environment, *Salimicrobium jeotgali* has various strategies for survival. We compared the ratio of acid to basic membrane protein in *Salimicrobium jeotgali* between other gram-negative bacteria. Ratio of Acid/ basic membrane protein in *Salimicrobium jeotgali* is higher than other. Transporter has in an important role as protect in osmotic pressure *Salimicrobium jeotgali* has three NhaC-type and four NhaP-type Na+/H+ antiporter to maintain Na+ homeostasis. A compatible solute is a substance compatible with the cellular metabolism that accumulates in the cytoplasm to balance external osmotic pressure. Ectione, Glycine-betaine and Glutamine which are uptaken from external to internal by ProU, OpuC and BCCT-type transporter and made by biosynthesis pathway in the cell are used *Salimicrobium jeotgali* as major compatible solutes.

B033

Diversity and Activity of Ammonia Oxidizing Archaea and Bacteria at Estuary Ecosystem

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Ammonia oxidation is a key part of nitrogen transformation in the estuary ecosystem. It is essential to understand the distributions of ammonia oxidizing archaea and bacteria in these sediments and how they respond to changes in chemical properties along the river (freshwater-marine). Here, we investigated the distribution and abundance of bacterial and archaeal *amoA* genes in estuarine sediments in winter and summer. The abundance of bacterial *amoA* genes by qPCR was greater than the of archaeal *amoA* genes in all sites. Pyrosequencing data of bacterial and archaeal *amoA* revealed that the bacterial amoA OTUs were clearly different at terrestrial and tidal sediments. The *Nitrosospira* lineages 1, 3a, 3b, and 4 were dominated at terrestrial sediments, while *Nitrosospira* 15-like lineages were dominated at tidal sediments without season. However, archaeal amoA OTUs related with *Nitrososphaera*, *Nitrosopumilis*, and *Nitrosotalea* clusters were co-existed at terrestrial and tidal sediments. Salinity and pH strongly affected the AOB community. The results implied that AOB might play a more important role in estuary sediments.

B034

Investigation of Effective Cell Disruption Methods for Lipid Extraction from Microalgae

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The main goal of this study is to find out an efficient microalgal cell disruption method to get high lipid yield. Four microalgae (*Chlorella vulgaris* CCAP211/11B, *Botryococcus braunii* LB572, *Dunaliella salina* and *Nannochloropsis oculata* CCAP 849/1) were tested. The highest total lipid extraction was obtained at optimized conditions under microwave cell disruption (at 150℃, 2,450 MHz and 15 min,) and autoclave cell disruption (at 121℃, for 1.5 MPa, and 30~60 min). The total lipid extraction was obtained using microwave disruption at optimum conditions were 22.3% (*C. vulgaris* CCAP211/11B), 48.9% (*B. braunii* LB572), 33.0% (*D. salina*) and 31.4% (*N. oculata* CCAP849/1). The fatty acid composition (presented in order of C16:0, C16:1, C17:0, C18:0, C18:1 and C18:2) obtained by microwave cell disruption of *C. vulgaris* CCAP 211/11B was 23.3±0.1, 2.5±0.2, 0.4±1.1, 1.8±0.3, 25.1±0.6 and 46.9±1.2; *B. braunii* LB572 was 29.3±0.4, 3.2±0.3, 0.1±0.1, 1.1±0.2, 41.8±0.5 and 24.5±0.5; *D. salina* was 25.2±0.2, 16.6±0.6, 10.9±0.3, 25.7±0.4, 3.2±0.1 and 18.4±0.3; *N. oculata* CCAP849/1 was 33.2±1.1, 30.9±0.1, 6.0±0.3, 22.1±0.6, 22.1±0.6, 7.3±0.7 and 0.5±0.2% (w/w), respectively.

B035

Enrichment and Genome Sequence of the Group I.1a Ammonia-Oxidizing Archaeon "*Ca.* Nitrosoterreus chungbukensis" Representing a Clade Globally Distributed in Deep Oligotrophic Soil Horizon

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The first step of nitrification, i.e., the oxidation of ammonia, was long considered to be performed exclusively by ammonia-oxidizing bacteria (AOB). However, the theory of soil and marine nitrification changed significantly after the discovery of AOA. This study reports the discovery of a chemolithoautotrophic ammonia oxidizer that belongs to a distinct clade of non-marine thaumarchaeal group I.1a, which is widespread in terrestrial environments. Extensive growth assays showed that strain MY2 is chemolithoautotrophic, mesophilic (optimum temperature 30°C), and neutrophilic (optimum pH 7 to 7.5). The genome size of strain MY2 was 1.76 Mb, similar to that of *N. maritimus* and "*Ca.* N. koreensis", and the repertoire of genes required for ammonia oxidation and carbon fixation in thaumarchaeal group I.1a was conserved. High representation of conserved orthologuous genes of signal transduction and motility in the non-core genome might be implicated in niche adaptation by strain MY2. On the basis of phenotypic, phylogenetic, and genomics characteristics, we propose the name "*Candidatus* Nitrosoterreus chungbukensis" for the ammonia-oxidizing archaeal strain MY2.

B036

Detection of Saxitoxin Gene in the Korean Freshwaters

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Saxitoxin causes the paralytic shellfish poisoning syndrome, which produced by dinoflagellates in the sea. Recently it was revealed that the freshwater cyanobacteria such as *Cylindrospermopsis raciborskii* also produced this toxin. In this study the PCR primers were designed from the gene sequences of stxG involved in the saxitoxin production. PCRs were conducted with the water samples taken from the Daechung Reservoir at the Hyenam and Chuso sites. The amplicons of PCR reaction were found in the samples taken on Sep. 2010. The saxitoxin, however, was not detected in these samples. These results indicated that the potential producer of saxitoxin exists in the analyzed reservoirs and the routine monitoring of the concentrations of saxitoxin and the existence of its producer is required to maintain the source of water supply safe. [Supported by grants from National Institute of Environmental Research]

B037

Comparison of the Relative Sensitivity of Protein Phosphatase Inhibition Assay to Congeners of Microcystins at Different Cominations of Enzymes and Substrates

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The protein phosphatase inhibition assay is one of the most popular method to measure the concentration of microcystins(cyanobacterial toxin) in the environmental samples because of its simplicity and consistency. In this study the extent of enzyme inhibition of combination of enzymes and subrstrates to microcystin congeners was examined. The protein phosphatase 1 and 2A as enzymes, methyl umbelliferone phosphate (MUP), p-nitrophenol phosphate (pNPP), phosvitin, and phopshorylase as substrates, and microcystin-LR, LA, YR, and RR were used in this study. The extent of enzyme inhibition was in the order of microcystin-LR, -LA, -YR, and -RR of the same concentration of congeners in all combinations. Moreover, the inhibitory proportion of each congener with the different combination of enzymes and substrates was not statistically different, indicating any combination can be used to analyze the concentrations of microcystins. The most recommendable combination for microcystin analysis is protein phosphorylase 1 and MUP because of its sensitivity and cost.
[Supported by grants from NRF and National Institute of Environmental Research]

B038

Confocal Laser Scanning Microscopic Observation of *Pseudomonas alkylphenolia* Pellicles

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Bacteria have a capacity to develop a niche-specific biofilm, a pellicle, to optimally occupy the air-liquid interface. *Pseudomonas alkylphenolia* forms unique circular pellicles in a diameter of 0.3-0.5 mm. The number of pellicles on the liquid surface is increased under a condition of decreased level of oxygen or with a supplement of glucose. Experiments with GFP-tagged cells showed that pellicle formation is due to clonal growth rather than assembly of existing cells. Mutagenesis study showed that a gene cluster, named *epm*, that shows similarity to the *alg* gene cluster, encoding alginate biosynthesis, is required. The GFP reporter gene coupled to the *epm* promoter was highly expressed at the upper layer and at the rim of the pellicles. The dead cells stained by EtBr were present at the lower layer of the pellicles and inside the zone expressing the active *epm* genes. This pellicle developmental process will provide an insight into bacterial sensing of an air-liquid interface and subsequent progress to floating macro-cellularity.
[Supported by grants from KRF]

B039

Bacterial Community Changes under the Temperature and Precipitation Manipulation Experiment in Cambridge Bay, Canada High Arctic

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According to the rapid rate of warming permafrost, increasing carbon dioxide (CO_2) will result in global temperature increases and hydrological cycle changes. Therefore, Arctic is sensitive ecosystems to climate change. We are studying on understanding how microbial community structure and soil organic carbon (SOC) will respond to climate change in the future. To examine the effects of climate factors on microbes and SOC, we established open top chambers (OTC) with total five replicated blocks containing four treatments (NWNP ; no-warming and no-precipitation as a control, NWP as no-warming and precipitation, WNP as warming and no-precipitation, and WP as warming and precipitation) in Cambridge Bay. There are two types of plot with monitoring plots and destructive plots. At the end of June 2012, this OTC set was installed after the ground-penetrating radar survey for the measurement of the active layer depth, and soils were sampled in each plot for baseline data in the both of plots. From 2013, soil will be sampled in the destructive sampling plots in order to monitor the changes of microbes and SOC.
[Supported by grants from Korea Polar Research Institute (PN13082) and NRF]

B040

Microclimate Monitoring Around Lichen Habitat in Barton Peninsular, King George Island, Antarctica

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Lichens ability to withstand extreme environmental conditions like polar area, they exist more than 70% of plant biodiversity in Antarctic terrestrial ecosystem. Barton Peninsula, King George Island has complicated geographic features and diverse flora specially lichen. Geographic difference remarkably affects microclimate characteristics. To study about how lichens responded and adapted to environmental change, we selected five long term monitoring sites. We measured air temperature, relative humidity, temperature of the lichen habitat, moisture contents and photosynthetically active radiation (PAR) from February 2013 to January 2014. Three sites were foamed patterned gradient of different kind of lichens. We observed 28 lichens included *Cladonia gracilis, Stereocaulon alpinum, Sphaerophorus globosus, Psoroma* sp. and *Ochrolechia* sp.. PAR reached 2338.7 uE in January, minimum was 1.2 uE and never zero. Air temperature was measured 20 celsius degree in the summer and -17 degree in the winter. Relative humidity ranged from 34 % to 100 %. Temperature of the lichen habitat was at least -15 to 13 celsius degree. Water content was logged at least 0.19 to -0.13 m^3/m^3.

B041

Cultivation and Draft Genome Sequence of Marine Verrucomicrobial Strain IMCC8625, Isolated from the East Sea

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The phylum Verrucomicrobia is widely distributed in marine environments, but only a limited number of verrucomicrobial isolates have been cultured from marine ecosystems. In this study, draft genome sequence and polyphasic taxonomy are reported for a verrucomicrobial strain IMCC8625 that was isolated from surface seawater of the East Sea by dilution-to-extinction cultivation. The 16S rRNA gene phylogeny revealed that strain IMCC8625 belong to the Verrucomicrobia subdivision 4 and is closely related to the genus *Coraliomargarita akajimensis* DSM45221^T (95.4%, similarity) of the family *Puniceicoccaceae*. Predominant fatty acids were revealed to be $C_{18:1}$ ω9*c* (23.0%), iso-$C_{14:0}$ (19.1%) and $C_{14:0}$ (10.2%). The genome sequence of IMCC8625 was obtained using llumina sequencing. The 2.84 Mb genome was assembled into 69 contigs, with N50 of 93 kb. The DNA G+C content was 54.3 mol%. The genome of strain IMCC8625 has been found to possess 2,621 protein coding genes, including a gene for a protein FtsZ, a cytoskeletal protein involved in cell division. Additionally we found the presence of many ABC transporters for proline/glycine betaine and amino acids.

B042

Metagenomic Analysis of Nitrogen Metabolism During a Full Scale Tannery Wastewater Treatment Process Bioaugmented with BM-S-1

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Biological tannery treatment technologies often require pretreatment for effective and efficient treatment, and are known to generate enormous amount of sludge. Here, we treated tannery wastewater using the novel consortium BM-S-1 without any pretreatment process. Our goal is to elucidate the functional gene structures and roles of microbial communities involved in the treatment process. We analyzed whole-metagenome sequencing data using Illumina MiSeq Sequencer to determine microbial community structures and functional genes associated with treatment of tannery wastewater at the different stages of buffering (B), primary aeration (PA), secondary aeration (SA) and sludge digestion (SD). The metagenome sequencing data demonstrated that the dominant phyla were Acidobacteria, Bacteriodetes, Actinobacteria and Firmicutes at the different stages. The genes involve in metabolisms of carbohydrates, protein and amino acids were more abundant compared to the genes associated with metabolisms of lipids, fatty acid, nitrogen, sulfur and phosphorus in B and PA. The genes associated with central carbohydrate and one-carbon metabolisms were prevalent in all the stages. Genes responsible for protein biosynthesis and degradation were more abundant in B than the other stages. Regarding nitrogen metabolism, genes associated with ammonia, nitrate and nitrite assimilation were more abundant than the genes responsible for denitrification and dissimilatory nitrite reductase. The dominant genii involved in nitrogen metabolism were *Burkholderia*, *Polaromonas*, *Albidiferax*, *Acidovorax*, *Geobacter*, *Dechloromonas*, *Pseudomonas*, and *Rhodopseudomonas*. Glutamate synthase, glutamate dehydrogenase, nitrous oxide reductase and nitrate reductase were distinctively observed depending on the kinds of these genii. Most of the metabolic processes were relatively more active in PA and B which corroborated with the chemical data (COD, T-N and T-P, etc.) obtained from all the stages during the treatment process. These results indicate that metagenome analysis of the microbial biomass of each treatment process could provide a comprehensive metabolic insight into mechanistic basis of the eco-friendly tannery wastewater treatment.

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B043

Cultivation and Genome Characteristics of Strain IMCC19250 Belonging to the LD28 Clade, Isolated from Soyang Lake

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The LD28 clade of the family *Methylophilaceae* is a betaproteobacterial lineage widely found in freshwater environments. Although many environmental sequences have been found to be affiliated with the LD28 clade, no cultured isolate has been reported so far. Here we report cultivation and genome sequencing of strain IMCC19250 of the LD28 clade, isolated from Soyang Lake. Strain IMCC19250 showed ≈95% 16S rRNA gene sequence similarity to HTCC2181, an isolate of marine methylotrophic OM43 clade. Addition of methanol was revealed to markedly promote the growth of IMCC19250, suggesting a methylotrophy as reported for HTCC2181. The draft genome sequence of IMCC19250 was determined by Illumina sequencing (250-bp paired-end), resulting in 5 contigs. Through combinatorial PCR-based contig ordering and gap filling, complete genome sequence was obtained. The IMCC19250 genome was 1.31-Mbp long with a G+C content of 36.6%. Genes for methylotrophy were predicted from the genome, including a gene for methanol dehydrogenase, as expected from the utilization of methanol. A putative xanthorhodopsin gene was also predicted, suggesting also a photoheterotrophic metabolism of the new strain.

B044

Coexistence of Multiple Endosymbionts in the Tubeworm *Lamellibrachia satsuma* Revealed by Comparative Metagenomic Analysis

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Lamellibrachia satsuma is a small vestimentiferan tubeworm, one of the common members of faunal communities in the Kagosima Bay. Two samples from different cluster of tubeworms of the seep were studied. Metagenomic library were constructed using Illumina HiSeq 2000 from the trophosome. Comparative metagenomics analysis carried out with the data annotated from MG-RAST. Analysis suggest that the tubeworm harbor mostly γ-proteobacterial endosymbiont in all tubeworms irrespective of location, but in one sample, presence of ε-proteobacterial endosymbiont provide a new direction regarding acquisition of endosymbiont by the tubeworm. The endosymbionts contain genes for oxidizing H_2S through the cytoplasmic enzyme AprA/AprB. Two carbon fixation pathways, the Calvin cycle and rTCA cycle also found, which has been reported in the endosymbiont of the various tubeworms. This study suggests that, endosymbiont uptake inorganic material from tubeworm and transform in to organic compounds despite the presence of different class of endosymbionts. Studying the role of coexisting endosymbionts from the metagenome is still a challenge, which is yet to be studied.

B045

Characterization of Bacterial Community Structure in Rhizosphere and Bulk Soil of a Ginseng Field Using Pyrosequencing

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We performed the bacterial community analysis to reveal bacteria composition in rhizosphere soil (RS) and bulk soil (BS) of ginseng using pyrosequencing based on the 16S rRNA genes. It was shown that the species richness and diversity indices were slightly higher in BS than in RS. At the phylum level, *Proteobacteria* was a predominant phylum (34.3~58.5%) in all samples and *Acidobacteria* (7.3~19.2%), *Actinobacteria* (9.3~14.6%), *Firmicutes* (1.6~7.3%) and *Bacteroidetes* (1.5~7.3%) were followed. The phyla, *Proteobacteria, Actinobacteria* and *Bacteroidetes* were relatively more abundant in RS. At the genus level, the genera *Sphingobium* (2.9%), *Pseudomonas* (2.5%), *Novosphingobium* (1.2%), *Microbacterium* (1.2%) and *Streptomyces* (0.8%) were detected much more in RS. It was revealed that sphingomonads, *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* were predominant genera in RS, indicating that ginseng is one of the best sources to isolate sphingomonads. This study suggests that the genera *Streptomyces* and *Pseudomonas* can be good sources for screening microbial agent to control some diseases of ginseng.

B046

Phylogenetic Composition of Bacterioplankton Cultivated from Soyang Lake Using Dilution-to-extinction Approach

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High throughput cultivation based on dilution-to-extinction showed a great potential to isolate uncultured oligotrophic marine bacteria. In this study we report the application of dilution-to-extinction culturing to freshwater bacterioplankton. Similar to marine systems, many important freshwater bacterial clades have been reported to be uncultured yet, including LD12 of the *Alphaproteobacteria*, LD28 of the *Betaproteobacteria*, and acI of the *Actinobacteria*. A water sample of Soyang Lake was examined using dilution-to-extinction culturing with very low nutrient-amended lake water as culture media. Most of the 313 strains cultivated from 720 inoculated wells belonged to the *Betaproteobacteria* and *Alphaproteobacteria*, with a very small number of *Actinobacteria* and *Gammaproteobacteria*. Only 73 strains formed colonies on 1/3R2A and/or 1/10R2A agar plates. Interestingly, we successfully isolated several major freshwater bacterial groups, particularly acI of the *Actinobacteria*; *Limnohabitans* and *Polynucleobacter* of the *Betaproteobacteria*, which suggests the potential of dilution-to-extinction culturing in cultivating representatives of previously uncultured freshwater bacterial groups.

B047

Etiological Agent of Fish Eead in the Rivers of Inje: Pathogenic Bacteria, Organic Substrate and Water Temperate

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Inje County, located in the Taebaek Mountains of Gangwon Province in South Korea, is famous for its pristine landscapes and untouched nature. Three major rivers flow through the area: Inbuk River, Buk River and Naerin River. Recently, there was an episode that fish died an masse in Inbuk River. We study the area to try to determine the cause of the deaths. We isolate and identify the bacterial community found on the dead fish in Inbuk River in June 2013 by isolating and sequencing the 16S ribosomal DNA marker. Dominant bacteria identified on the dead fish were *Aeromonas veronii* (32 isolates, 37%) and *A. hydrophlia* (30 isolates, 35%). To investigate and understand the seasonality and geography of the etiological agents of fish disease, we take water samples from several points of the three rivers in Inje between June 2013 and March 2014. Through targeted PCR method using specific primers, we discover the existence of these two pathogenic bacteria in many points in the river. We measure temperature and levels of organic compounds and find that fish death may be correlated with water temperature and total amount of organic substrate such as Phosphate (P) and Nitrate (N).

B048

Comparative Metagenomic Investigations of Microbial Communities and Functions in Deep Marine Sediment of Ulleung Basin, East Sea

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Marine sediments are the largest global reservoir of methane, and it was known that most of methane is biologically consumed at sulfate methane transition zone (SMTZ) by anaerobic oxidation of methane (AOM). We have compared the taxonomic composition and functional gene diversity between surface and SMTZ sediment obtained at Ulleung Basin, East Sea, by using pyrosequencing data. Community analysis based on homology search of the protein-coding genes showed the majority of genes derived from *Deltaproteobacteria*, which may reflect the greater access to sulfate reduction metabolism in the both sediments. However, there was a significant overrepresentation of the methane related class *Methanomicrobia* and *Archaeoglobi* was found in the SMTZ. Compared to surface sediment, the functional gene analysis showed that a significant difference in microbial methane metabolism was found in SMTZ. The annotated genes related with methane metabolism are especially enriched in the SMTZ, which were originated from ANME-1 by using reference mapping analysis. This study may serve as a relationship between AOM and functional capability of ANME-1 members.

B049

Isolation and Characterization of *Limnohabitans* from Freshwater Environment

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The genus *Limnohabitans* was previously identified as one of the major genera inhabiting 5 major freshwater bodies of Korea based on pyrosequencing analysis. In this study, strains of *Limnohabitans* were isolated and their taxonomic properties were examined. Members of *Limnohabitans* are known as free-living and abundant in neutral or alkaline aquatic habitats. The symbiotic relationship between *Limnohabitans* and algae has also been reported. Based on these reports, strategies for the isolation of *Limnohabitans* were established. Samples were taken from locations of Keum River where algal growth was observed. Terminal restriction fragment length polymorphism (T-RFLP) analysis was carried out to confirm the presence of *Limnohabitans* in the samples. The filtration-acclimatization method (FAM) and dilution-acclimatization method (DAM) were used for the isolation. Three strains of *Limnohabitans* were isolated, and the 16S rRNA gene sequence similarity indicated that the strains were mostly related to *Limnohabitans curvus* MWH-C5^T at the sequence similarity of 99.1-99.9%. The ongoing study includes phenotypic characterization of the isolates to elucidate their possible roles in environment.

B050

Genotypical Diversity of *Escherichia coli* Strains Isolated from Surface Waters and Sediments in the Yeongsan River Basin, South Korea

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In this study, genotypical diversity of 2,880 *Escherichia coli* strains obtained from surface waters and sediments of the Yeongsan River basin in South Korea was examined by using the horizontal fluorophore-enhanced rep-PCR (HFERP) DNA fingerprinting technique. Environmental samples were collected bimonthly from February to December 2013, and water quality parameters and soil properties were measured for each water and sediment samples. Multidimentional scaling (MDS) analyses based on HFERP DNA fingerprint data revealed the genotypical trends of *E. coli* strains, and self-organized maps (SOMs) analyses and redundancy analysis (RDA) were performed to determine environmental factors affecting *E. coli* genotypes. The aim of this study was to: 1) examine the genotypical relationship between *E. coli* populations in surface water and sediment of the river seasonally and regionally, and 2) determine correlations between *E. coli* genotypes and environmental factors in the environments. Our results indicate that that an ecological approach needs to be considered in order to obtain a better understanding of *E. coli* community dynamics in the environments.

B051

Degrading of Aromatic Compounds by Photosynthetic Bacteria under Dark Condition

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Most widely caused from petroleum or gasoline storage tank leaks underground, LNAPLs are highly toxic volatile organic compounds (VOCs) such as BTEX, which can and have been negatively affecting humans as well as the environment. This study observed the removal of benzene, toluene and ethylbenzene using a more stable and environment friendly method by non-pathogenic photosynthetic bacteria. Ultimately, purple non-sulfur bacteria, *Rhodopseudomonas capsulatus*, showed efficient degradation of 20 mg/L of BTE. 1 g/L (w/v) of cell mass was inoculated to MSM (pH 6.8±0.2) with 0.1% di-sodium succinate under aerobic condition. In 8 days, 85.51% of BTE was removed. Analysis of degradation efficiency was performed using headspace method via GC-FID. Molecular level experiments, such as Real-Time PCR were also used to identify and confirm the target gene responsible for the degradation of BTE in aerobic condition. Oxygenase was identified as a common intermediate in the aerobic degradation pathway of BTE. Molecular level experimental results allow us to know what is happening during bioremediation of BTE under aerobic conditions.

B052

Anaerobic Biodegradation of TCE by *Desulfovibrio* sp. T1 Isolated from Vietnamese Wastewater River-sediment

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An anaerobic bacterium which has slightly curved rod to spiralled shape, monotrichous polar flagella was identified as *Desulfovibrio* sp. T2, based on the 16S rRNA gene sequencing. The strain T2 was originally isolated from Vietnam wastewater river sediment and showed high efficiency to degrade TCE anaerobically. The strain degraded TCE completely while utilizing pyruvate, lactate, yeast, fumarate and propionate but not glucose or acetate. In the optimum condition: pH 7.2- pH 7.4, 30℃, pyruvate was used as the sole carbon source at the concentration 10 mM, strain T2 (*Desulfovibrio desulfurican*) showed complete degradation of 100 ppm (in 65 ml clear bottle containing 30 ml media) after 65 h. In average, the strain removed 3.3 ppm TCE every hour. The chloride release was 78.25 mg/L. On the other side, without vitamin B12, the degradation of TCE was completely inhibited, suggesting that vitamin B12 is the essential co-factor of TCE-degrading enzymes. Based on its high TCE removal efficiency, it can be applied *in-situ* biodegradation at TCE polluted field for complete remediation.

B053

Isolation, Identification and Characterisation of Antifungal Activity by *Pseudomonas* 43-4 against *Cylindrocarpon destructans*

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Panax Ginseng (C.A.Meyer) is a medicinal crop with high demand all over the world. The major producers of ginseng are China, Russia, Japan, Korea, and America. The crop is used to cure ailments in respiratory tract, anti-aging, and in treatment of cancer. The bacteria were isolated by serial dilution method. All the isolated strains were screened against phytopathogen *Cylindrocarpon destructans*. Identification of the strains was performed by 16S rRNA partial sequencing. *Pseudomonas* sp. are ubiquitous bacteria commonly present in the ginseng Rhizosphere. The strain 43-4 showed the maximum antifungal activity and was characterized by morphological, physical and biochemical tests. The bacteria was cultured on various media such as nutrient broth, brain heart infusion broth, potato dextrose broth, Luria-Bertani broth to test maximum antagonist effect. The best media was chosen for the culture filtrate assay. Further studies focuses on the isolation of the antifungal compound and identification.

B054

***Bacillus* sp. NWO Isolated from Oysters (*Crassostrea gigas*) with a Broad Range of Enzyme Activities for the Removal of Organic Compounds from Environment**

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The marine bacterium, *Bacillus* sp. NWO isolated from oysters (*Crassostrea gigas*) aquacultured in Wando seawater was analyzed owing to broad range of enzyme activities which can be used for industrial application. It was creamy in yellow colour, Gram-negative, facultative aerobic, non-motile, fermentative, and rod-shaped. Strain NWO grew at the temperature range of 15-50℃, at the salinity range of 0-11% (w/v) NaCl, and at pH range of pH4-8. Strain NWO was revealed the closest to both *Bacillus methylotrophicus* CBMB205^T and *B. amyloliquefaciens* subsp. *ptantarum* FZB42^T with 99.9% similarity based on 16S rRNA. Biochemical test revealed that it exhibited positive enzyme activities of acid- and alkaline-phosphatase, DNase, cellulase, amylase, protease, esterase (C4) and esterase lipase (C8), which can be applicable to the removal of organic compounds from environment.

B055

Conversion of 4-Methoxybenzoate into Bioplastics by *Comamonas testosteroni* P19

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Bioplastics production from an aromatic compound, 4-methoxybenzoic acid (4-MBA), was investigated at different concentrations (1, 3, 5, 8 and 11 mM) using *Comamonas testosteroni* strain P19. The strain showed highest (64%) bioplastics production when 4-MBA at 5 mM was supplied and the production efficiency was followed by 8 mM (58%), 3 mM (48%), 1 mM (46%), and 11 mM (38%) of 4-MBA. After optimizing substrate concentration (4-MBA), further experiments were carried out for optimization of nitrogen concentration. Nitrogen limitation was found to have a significant effect for bioplastics production, determining 50 mg/L of ammonium sulphate as optimum. The functional groups present in the produced bioplastics were confirmed using FT-IR spectroscopy. Results from this study indicated that strain P19 was capable of utilizing 4-MBA as sole carbon and energy sources, and converting 4-MBA into useful bioplastics under nitrogen limitation condition.

B056

Identification of Genes for Metabolism of 4-Methoxybenzoate in *Comamonas testosteroni* P19

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The microorganism *Comamonas testosteroni* strain P19 was capable of utilizing 4-methoxybenzoic acid (4-MBA) as sole carbon and energy sources. Using plasposon mutagenesis, a mutant strain (N28) which lost the ability to degrade 4-MBA was identified. A gene encoding demethylase was knocked out in the mutant strain. Two ORF were organized consecutively encoding monooxygenase and fusion protein containing domains for oxidoreductase and ferredoxin. In real-time PCR analysis, the demethylase gene expression was specifically induced by 48 folds responding to 4-MBA. The demethylase genes (two ORF) were cloned into pQE31 vector and the recombinant *E. coli* successfully transformed 4-MBA into 4-HBA in resting cell assay. Results from this study showed that 4-MBA was metabolized through demethylation converting to 4-hydroxybenzoate which was catalyzed by demethylase in strain P19.

B057

Cyclobacterium holothuriaincola sp. nov., Isolated from a Sea Cucumber (_Holothuria_ sp.) Aquaculture Farmland

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The taxonomic position of a novel bacterium, designated SD70, isolated from a sea cucumber (*Holothuria* sp.) aquaculture farmland was determined. It was a Gram-negative, aerobic, non-motile, and horseshoe-shaped. The optimal growth conditions for strain SD70 were pH 7.0-8.0, 25-37ºC and 3.0-5.0% (w/v) NaCl. 16S rRNA gene sequence analysis revealed that strain SD70 was placed into the genus *Cyclobacterium*. Strain SD70 exhibited 16S rRNA gene sequence similarities of 97.3–8.5% to the type strains of species of the genus *Cyclobacterium*. The phylogenetic and genetic distinctiveness and several differentiating phenotypic properties revealed that strain SD70 was separated from other species of the genus *Cyclobacterium*. Strain SD70 exhibited no enzyme activities for degradation of casein, starch, cellulose and Tween 40, 60, 80 except for Tween 20. On the basis of the data presented, strain SD70 represents a novel species of the genus *Cyclobacterium*, for which the name *Cyclobacterium holothuriaincola* sp. nov. is proposed. Notably, it exhibited enzyme activities such as esterase (C4), esterase lipase (C8), and lipase (C14), all of which are responsible for the degradation of lipid.

B058

Characterization of Phosphate-solubilizing Bacteria _Enterobacter_ sp. 04-P-1 with Antimicrobial Activity against Fungal Phytopathogen

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Phosphorus is an essential nutrient in plants, but it is easily converted to insoluble form in soil. Thus, P fertilizer is used in amounts excessive to those actually required by plants. It causes not only negative effects on the environment but also economic problems. In this study, a phosphate-solubilizing bacterium strain 04-P-1 isolated from forest soil was able to convert insoluble P in soil. The 16S rRNA gene sequence analysis showed that the isolate was most similar to the type strain of *Enterobacter amnigenus* (ATCC 33072^T). The P-solubilizing activity of the isolate was confirmed, as the tri-calcium phosphate medium became more acidic (pH of 4.44) and available phosphate was increased to 596.6 ppm. In sterilized soil, available P increased 3.5-fold when the isolate was inoculated compared to the non-inoculated control. In addition, the isolate showed antimicrobial activity against *Botrytis cinerea*, which causes gray mold. Thus, we can expect the bacteria to reduce the use of phosphate fertilizers in soil with the insoluble phosphate and the isolate will be useful to biotic pesticide.
[Supported by grants from Agenda Program (PJ00858002), Rural Development Administration]

B059

Isolation of a New Species and Three New Recorded Fungi from Dokdo Island, Korea

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Dokdo island is located in the northeastern part of Ulleungdo, extremity of Korea and known as volcanic island. In total, 53 fungal isolates were collected from the soil sample, using dilution plate technique. The isolates were identified on the basis of morphological characteristics and rDNA ITS sequence analysis. From the results, a new species and three new recorded fungi were established. When the sequences of EML-DDSF4 isolate were compared with related species retrieved from NCBI BLASTN, it was closest to *Mortierella oligospora* (accession number, JX976032) with 100% sequence similarity and tentatively identified as *M.* cf. *oligospora*. The EML-MF30-1 isolate was closest to *Clonostachys* cf. *rosea* (accession number, KC313107) with 97% sequence similarity and the EML-IF9 isolate was closest to *Metarhizium guizhouense* (accession number, HM055445) with 98% sequence smilarity. The EML-IFS45 isolate was closest to *Absidia* sp. (accession number, JQ683214) with 92% sequence smilarity, showing that the isolate is new to science. In addition, three species of *M. oligospora, C. rosea* and *M. guizhouense* represented new records of fungi from Dokdo island, Korea.
[Supported by grants from NIBR.]

B060

Two New Records of Seed-borne Fungi from Korea

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During the course of investigation and detection of seed-borne fungi, a total of 332 isolates were collected from the grains including rice, barley, corn and wheat. Out of them, two species of *Bipolaris spicifera* and *Sordaria lappae* represented new records from Korea. A mitosporic fungus, *B. spicifera* was isolated from wheat seed. The shape of the spores were straight, oblong, cylindrical and dark brown with three distosepta on PDA medium. The dimension was 0.7-0.9 (av. 0.8) µm wide × 1.5-2.3 (av. 2.1) µm long. An ascomycetous fungus, *S. lappae* was isolated from barley seed. The fungus produced round-shaped perithecium 1 week after culture on PDA at 27℃. The perithecium released lots of asci through ostiole. A maturing cylindrical ascus in which all 8 ascospores were linearly arranged. For investigation, the seeds were surface treated with 2% NaOCl for 30 seconds, washed in D.W and plated on moist blotter directly or after deep-freezing for 1hr. The detection rates of major genera represented *Alternaria* spp. (29.5%), mycelia sterilia (18.7%), *Penicillium* spp. (16.7%), *Aspergillus* spp. (15.7%) and others (19.4%).
[Supported by grants from NIBR and KOFAC.]

B061

Gnotobiotic Culture of Brine Shrimp with Bacteria Enhances Growth and Development of the Shrimps

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Brine shrimp is an aquatic crustaceans belonging to a genus of *Artemia*. This organism is widely used for testing the toxicity of chemicals. In this study, a brine shrimp, *Artemia salina* was infected with various pathogenic bacteria for the bacterial virulence analysis. Interestingly, despite of the virulence, some brine shrimps surviving the infection were found to grow bigger and faster. Among bacterial strains tested in this study, *Pseudomonas aeruginosa* and *Echerichia coli* showed the growth-promoting effect on the brine shrimp. To explain this phenomenon, the symbiosis of these bacteria in shrimps was addressed by counting and observing live bacterial cells in the brine shrimp gut. Both *E. coli* and *P. aeruginosa* could survive in the brine shrimp gut, but *E. coli* was able to survive only for limited period whereas *P. aeruginosa* survived more and longer in the gut than *E. coli*. These results strongly suggest that *P. aeruginosa* survives in the brine shrimp gut, probably as a symbiont and this brine shrimp-*P. aeruginosa* may be an artificial model system to study the symbiosis.

B062

Sterilization Efficacy of a Pipe Type Water Treatment System Using Low Temperature Plasma

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Plasma is loosely described as an electrically neutral medium of positive and negative particles. Plasma contains free charges (electrons, ions), free radicals, excited molecules, photons (UV) and generate a transient electric filed. Several studies have demonstrated the efficacy of sterilization by atmospheric nonthermal plasma. Recently a Korean company has made a pipe type water treatment system using low temperature plasma to effectively sterilize waterborne pathogens. In order to evaluate the sterilization efficacy of the system in swimming pool water and tap water, a variety of experimental model bacteria, fungus, and viruses for human pathogens, including *E. coli*, *B. subtilis*, *S. aureus*, *C. albicans*, hepatitis A virus, bovine herpes virus, and porcine parvovirus were all selected for this study. From the inactivation study, it was found that the pipe type water treatment system using low temperature plasma can be an alternative measure to sterilize waterborne microorganisms in swimming pool and ballast water.
[Supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Small and Medium Business Administration in 2013.]

B063

Characteristics of Anaerobic Methane Oxidation Process at the Ulleung Basin, East Sea of Korea

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Despite the fact that other electron acceptors are more energetically favorable (nitrate, nitrite and so on) the most thoroughly investigated hypothesis for anaerobic oxidation of methane (AOM) is reverse methanogenesis using sulfate as the terminal electron acceptor. In this study, we investigated the characteristics of the AOM process that can occur in a variety of ways in the sediment of the Ulleung basin which include methane hydrate. Quantitative real time PCR analysis of methyl coenzyme M reductase (*mcr*A) gene revealed the highest copy number at sulfate methane transition zone (SMTZ), where it comprised 25% of the number of archaeal 16S rRNA gene. Nitrate reductase (*nar*G) gene was dominant compared to the nitrite reductase (*nir*S and *nir*K) and the dissimilatory sulfite reductase (*dsr*AB) gene. Phylogenetic analysis of *mcr*A, *nar*G and 16S rRNA gene clone libraries showed that ANME-1 related archaea and *Halomonas* were predominant. This results suggest that AOM process from the Ulleung basin is carried out by the syntrophic consortium of ANME-1 and *Halomonas* which is known to be a member of denitrifier.
[Supported by grants from KIOST]

B064

Isolation and Identification of Insect Pathogen *Serratia marcescens* in *Protaetia brevitarsis seulensis* (Kolbe) from Korea

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According to efficiency of the larvae of *P. b. seulensis* about anti-oxidant, anti-hepatic disorder, and anti-diabetic effect, *P. b. seulensis* were reared increasingly in local, Korea, and *Protaetia brevitarsis seulensis* (Kolbe) has being focused on a protein alternate and functional food with pharmacological effect in Korea. The purpose of this study is the evaluation of the the reared larvae of *P. b. seulensis* from Gyeong-gi in Korea were infected with Spo-1 and identification of the bacteria is *Serratia marcescence* using 16S rRNA PCR, electro-microscope and verifying through Bioassay.

B065

Cyanobacterial Diversity and Seasonal Changes in Paldang Reservoir (Korea) Explored by Microscopy and 454 Pyrosequencing

Boopathi Thangavelu, Ponmani Thangaraj, Ruoyu Guo, Man-Duck Lee, Won Bae Yu, Min-Ah Lee, and Jang-Seu Ki*
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Cyanobacteria are the major component of the bloom forming community that has to be monitored frequently. Hence, we have analyzed molecular diversity and seasonal changes of cyanobacteria in Paldang reservoir using morphological and 16S rRNA pyrosequencing. The samples were collected from four stations during Mar.-Dec. 2012. Totally, 40 phylotypes of cyanobacteria were identified after comparing 49,131 pyrosequence reads. The cyanobacterial genera such as *Anabaena*, *Aphanizomenon*, *Microcystis*, *Synechocystis* were predominantly present in the samples. However, majority of cyanobacterial sequences (65.9%) identified here were of uncultured origin. In contrary, morphological identity of cyanobacteria revealed different pattern which resolved eight cyanobacterial genera. Seasonal pattern of cyanobacterial community was also observed, with no occurrence in Mar. and Dec. The relative abundance of cyanobacterial sequences was observed as high in Aug. These suggested that pyrosequencing approach can reveal cyanobacterial diversity that undetected morphologically, and can be used as a reference for studying and monitoring of cyanobacterial communities in Paldang Reservoir.

B066

Biodegradation of Polycyclic Aromatic Hydrocarbons in the Marine Bacterium, *Novosphingobium pentaromativorans* US6-1

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Novosphingobium pentaromativorans US6-1 is a halophilic marine bacterium able to degrade PAHs. Genome sequence analysis revealed that the large plasmid pLA1 present in *N. pentaromativorans* US6-1 consists of 199 ORFs and possess putative biodegradation genes that may be involved in PAH degradation. Up-regulated biodegradation enzymes were quantitatively compared. Among the PAHs, phenanthrene induced the strongest up-regulation of extradiol cleavage pathway enzymes such as ring-hydroxylating dioxygenase, putative biphenyl-2,3-diol 1,2-dioxygenase, and catechol 2,3-dioxygenase in pLA1. These enzymes lead the initial step of the lower catabolic pathway of aromatic hydrocarbons through the extradiol cleavage pathway and participate in the attack of PAH ring cleavage, respectively. However, *N. pentaromativorans* US6-1 cultured with p-hydroxybenzoate induced activation of another extradiol cleavage pathway, the protocatechuate 4,5-dioxygenase pathway, that originated from chromosomal genes. These results suggest that *N. pentaromativorans* US6-1 utilizes two different extradiol pathways and plasmid pLA1might play a key role in the biodegradation of PAH in *N. pentaromativorans* US6-1.

B067

Effect of Surface Water on Microbial Community Structure of Alluvial Aquifer Groundwater in Long Term-Operated River Bank Filtration Site

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Municipal and agricultural demands of groundwater derived construction of river bank filtration systems to overcome draw down of water level, which use extraction wells in a distance from a water body, such as river, to purify the drawn-off water by passing it through the geological media for use as drinking water. For more than 7 years, river bank filtration has been operating in the alluvial aquifer in proximity of Nakdong River as a water supply for Daesan Filtration Plant, Changwon, South Korea. This study aims to evaluate changes in microbial community structure in the subsurface environment affected by surface water infiltration into groundwater at the river bank filtration site. Along with the geochemical and hydrological data indicating obvious intrusion of river water into the aquifer, pyrosequencing analysis of groundwater DNAs suggested significant changes in the microbial community structures. The results imply that changes of environmental condition, here surface water into groundwater, can change the microbial community structure in a prolonged time period, and further biogeochemical reactions in the geologic media.
[Supported by KIGAM project (14-3211)]

B068

Bacterial Community Diversity in the Guts of Three Xylophagous Insect Species

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Insect guts present distinctive environments for microbial colonization, and bacteria in the gut potentially provide many beneficial services to their hosts. Especially, the gut bacterial community associated with xylophagous insect larvae were of interest for potential biotechnological applications in lignocellulose degradation. In this study, we used pyrosequencing to characterize bacterial diversity and community structure in the guts of three different insects. The dominant bacterial phyla/classes were *Firmicutes* (18.5%) and α-*Proteobacteria* (17.1%) in giant rhinoceros beetle (*Allomyrina dichotoma*), *Cloroflexi* (31.0%) in giant stag beetle (*Dorcus titanus*), and *Spirochaetes* (32.7%) and *Bacteroidetes* (32.5%) in termite. The principal coordinates analysis (PCoA) showed that individual insect species harbored unique gut communities. Guided by this preliminary result, we are exploring the gut microbiome in more detail for their health improvement and cultivable bacterial diversity from their gut for application in the field of bioenergy.

B069

Study on the Improvement of Drinking Water Quality of Vulnerable Area as Small-Scale Water Supply Facilities

Jihye Kim, Bo-ram Lee, Siwon Lee, Sujeong Park, Hyen-Mi Chung, and Weon hwa Jheong*
Water Supply and Sewerage Research Division, National Institute of Environmental Research

This study developed a complex water treatment system that removes 99.99% of pathogenic microorganisms such as Norovirus through laboratory and on-site experiments. When the system was applied on-site, it was found that the water quality and maintenance were stable in general. Although Norovirus was detected in a groundwater source of one small-scale water supply facility (Gangwha), in the treated water the virus was not detected, which was the first case that Norovirus was removed through the treatment system on-site. We also conducted a complete survey on the areas supplied with water treated by the new system. According to the results, microorganisms were detected in some households because of secondary pollution caused by damage to water pipes, which requires appropriate measures. Renovation of worn-out water treatment facilities and prioritization of investments considering water quality properties could improve small-scale water supply facilities. In particular, it is expected that the new water treatment system can be a good way to provide safe drinking water and the remote alarm system will help effectively manage the water treatment system.

B070

Study of Microbial Diversity in the Bioaerosols in South Korea

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Water Supply and Sewerage Research Division, National Institute of Environmental Research

The dynamics of the Atmosphere's microbial inhabitants have potential implications for human health. To evaluate microbiology aerosol, we used an aerosol sampler (SPM) and a 1.2 μm pore size GF/C filter (110 mm diameter, Whatman) to collect samples in the NIER (National Institute Environment Research). They were cultured on an R2A agar and a TSA plates for identification. Using a 16S rRNA, we detected and monitored bacterial populations. As a result, the mostly found genus is *Bacillus*. Other genera of bacteria detected are *Methylobacterium, Bradhizobium* and *Streptomyces*. This concluded that monitoring requires bacteria to be present in a large variety of bioaerosols. As a future plan, we plan to further investigate the microorganism diversity in atmosphere.

B071

Comparison of Biodiversity of Bacterial Flora Inhabiting Rhizospheres of Indigenous Plants and Naturalized Plants in Oligotrophic Cover Soils over Ultramafic Rocks

Ju-Hun Kim, Minji Kim, and Young-Gun Zo*
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We hypothesized that the indigenous and naturalized plants have different bacterial community in their rhizosphere to make naturalized plants have relative advantages. To test the hypothesis, we identified rhizobacterial species and their metabolic functions. As the indigenous plants *Setaria viridis* and *Gypsophila oldhamiana* were used. As the naturalized plant *Ambrosia artemisiifolia* was used. Bacteria were isolated, and their 16S rRNA genes were sequenced. Number of viable bacteria in indigenous plants, *G. oldhamiana* and *S. viridis* were 4.1×10^4 CFU/g and 1.1×10^5 CFU/g at TSA media with neutral pH, respectively. The bacterial species commonly in rhizosphere of indigenous plants predominated by the oligotrophic bacterium *Ralstonia piketti,* and its dominance reached 44 ~ 60%. Number of viable bacteria in *A. artemisiifolia* rhizosphere was 3.9×10^5 CFU/g. *Enterobacter ludwigii* predominated by 54% in rhizosphere of *A. artemisiifolia*, *R. piketti* is known to promote growth of indigenous plants in oligotrophic soils. Functions such as nitrogen fixation with heavy metal tolerance appeared to be contribute to propagation of indigenous.
[Supported by grants from KRF and RDA.]

B072

Metabolic Succession Based on Structural Dynamics of Bacterial Community in Mesocosms of Carcass Landfills Treated with Quicklime

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When livestock carcasses are buried, quicklime is treated for preventing spread of pathogens in the landfill soil. Quicklime may kill soil bacteria, so soil ecosystem can be destroyed. It can lead to inhibition of livestock carcasses decomposition. Understanding of bacterial community structure and their metabolic processes is necessary for promotion of livestock carcasses decomposition in landfills. Mesocosms of carcass landfills were made using mixture of soil, meat (40% w/w) and quicklime (0-10%). We investigated succession process of soil bacterial community using 454 pyrosequencing of 16S rRNA gene. The succession of bacterial community in the mesocosms was observed genus *Clostridium* (47.8%), genus *Leuconostoc* (55.6%) and genus *Tissierella* (19.9%). Based on the succession of bacterial community and metabolism of the dominant species, metabolism of soil bacteria in the mesocomes followed amino acids fermentation, lactic acid fermentation and anaerobic respiration.
[Supported by KRF.]

B073

Fungal Diversities Associated with Thrips (Thysanoptera: Thripidae) Feeding on the Evening Primrose, *Oenothera bienniss* L.

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We investigated fungal diversity associated with the thrips by using 454 pyrosequencing of the internal transcribed spacer (ITS) region. We collected thrips species from evening primrose in Busan. The thrips were identified *Frankliniella intonsa* and *Thrips plami*. The fungal communities of gut and salivary gland in thrips were compared between *F. intonsa* and *T. plami*. By sequencing a total of 155,356 reads, we identified 113 different OTUs in the four samples. The OTUs related to the phylum Ascomycota were observed predominantly (21.2-93.5%) in 4 tissues. The Ascomycota had similar fungal patterns at the phylum and lower taxonomic levels. Individuals of the two thrips species had similar overall fungal communities in their guts. In case of salivary glands, *F. intonsa* had high count of *Myrothecium* while *T. plami* had high count of *Cladosporium*. Our work shows that the Ascomycota associated with thrips. Ascomycota are responsible for most of phytopathogenic fungal diseases that causes leaf spot and disorders, rots, and cankers in various plants. This association appears to have a potential application in insect pest control.
[Supported by grants from RDA.]

B074

Potential of Smartphones as a Reservoir of a Wide Variety of Bacterial Species

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Smartphones may act as a reservoir of a wide variety of bacterial species, many of which may have the potential to be pathogenic. Smartphones used by parents are of particular interest, as they may be implicated in the spread of pathogens to children. Smartphone accessories may also function as a pathogen reservoir. Although many of these items are subjected to very high hygienic standards, standard cleaning procedures or even guidelines for the use of smartphones are yet to be established. In this study, we cultured staphylococcal bacteria from smartphone surfaces. Results showed a correlation between isolated bacterium found on parents' mobile devices and respective questionnaires, demonstrating the cross-contamination potential of smartphones in the nurture environment. Out of 52 samples, Total coliform was 0.008 CFU/cm², the average abundance of the *Staphylococcus aureus* was 0.425 CFU/cm², the maximum value was 195 CFU/smartphone. Many studies have reported that the majority of people, including infant's parents, do not clean their smartphones. Therefore, good personal hygiene ought to be sought in development of smartphones and accessories.
[Supported by grants from KRF.]

B075

Impact of Ocean Acidification on Microbial Community and Its Function – Metagenomic Analysis in a Marine Mesocosm Experiment

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According to the reports from the Intergovernmental Panel on Climate Change, the concentration of atmospheric CO_2 will rise up to 1,000 ppm and the ocean pH will decrease 0.3 units due to dissolved CO_2. To anticipate the possible influence of the ocean acidification to the marine ecosystem, we performed a time-series comparative whole metagenomic analysis of the marine microbial communities between two CO_2 conditions (380 ppm and 1200 ppm) in a mesocosm experiment. In both conditions, *Gammaproteobacteria*, *Alphaproteobacteria*, and *Flavobacteria* were dominant bacterial phyla, and *Bacillariophata* and *Chrysophyceae* were dominant eukaryotes in the beginning. At the late stages, the bacterial community shifted into an *Alphaproteobacteria*-dominant structure, but the proportion of *Flavobacteria* was still high in elevated CO_2. At 380 ppm CO_2, *Dinophyceae* were dominant during the late stages, but their dominance did not appear at 1200 ppm CO_2. Moreover, there were notable changes in gene complements according to the CO_2 concentrations and time course. Details on microbe and gene dynamics will be presented.
[Financial support from the National Research Foundation (grant no. NRF-2011-0017670)]

C001

Antiviral Properties of Probiotic Mixtures against Rotavirus in the Rat

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We evaluated the anti-rotaviral activities of probiotic mixtures in a Sprague-Dawley rat. Litters of 12 pups per dam were randomly assigned to four groups; placebo, phosphate buffered saline (PBS), and two probiotic mixtures (PRO-1 and 2). All groups were inoculated with rotavirus at dose of 8 log plaque forming units per animal at 5 days old. After inoculation, PRO-1 and 2 groups were orally administered probiotic mixtures 1 or 2, respectively, at a dose of 8 log colony forming units once daily for 4 days from the day of inoculation, respectively. It was found that the weights of small intestines were greater in the PRO-1 and 2 groups than in either of the control groups. Villi were shortened and villous epithelial necrosis was exhibited by rotavirus infected rats, but these morphological changes were not observed in PRO-1 and 2 treated rats. Real time-quantitative PCR assay showed that critical threshold values were higher for PRO-1 and 2 fecal samples than for those of the control groups. Also, the mRNA transcript levels of rotavirus in small intestinal epithelial cells were lower in the PRO-1 and 2 groups.
[Supported by grants from Sahmyook University Research Fund (2012).]

C002

Probiotics for the Treatment of Viral Gastroenteritis in Children: A Randomized, Double-blind, Placebo-controlled Trial

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We evaluated the efficacy of probiotics for the treatment of acute viral gastroenteritis in children and against rotavirus *in vitro*. The antiviral activities of probiotic isolates on rotavirus infection were investigated in the Vero cell line using a plaque reduction assay. Of the tested probiotic strains, *Bifidobacterium longum* isolated from an infant showed the greatest inhibitory effect and *Lactobacillus acidophilus* showed the second-highest inhibitory effect. These probiotics were chosen for further clinical trials. Twenty-nine pediatric patients who presented with symptoms of viral gastroenteritis were enrolled in a double-blind trial and randomly assigned at admission to receive six probiotic strains (*B. longum*, *B. lactis*, *L. acidophilus*, *L. rhamnosus*, *L. plantarum*, and *Pediococcus pentosaceus*) at a dose of 9 log colony forming units or a comparable placebo twice daily orally for 1 week. These probiotics significantly shortened the duration of diarrhea as compared with a placebo (6.1 ± 0.5 vs 7.2 ± 1.9, $P = 0.030$). We suggest that these probiotics may be a useful for the treatment of rotaviral gastroenteritis or as an alternative therapy.
[Supported by grants from Sahmyook University Research Fund (2012).]

C003

Violacein from Novel *Pseudoduganella* Strain and Its Application to Kill *Staphylococcus aureus*

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C004

Isolation and Characterization of Lactic Acid Bacteria Inhibiting Harmful Pathogens

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Lactic acid bacteria (LAB) may inhibit growth of spoilage and pathogenic microorganisms. The antimicrobial LAB strains could be used for the alternative to antibiotics. Among several hundred strains isolated from various sources, ten isolates showed over 10 mm inhibition zone in the agar diffusion test against all the pathogens examined, such as *Escherichia coli*, *Clostridium perfringens, Listeria monocytogenes, Staphylococcus aureus*, *Salmonella typhimurium*. These LAB strains with highest antibacterial activity were identified as *Lactobacillus* sp., *Bifidobacterium* sp., *Pedioccus* sp. according to their 16S sequences. The characteristics of the lactic acid bacterial strains with particular functions such as antiviral activity, adherent ability to enterocyte, tolerances towards gastric juice, bile, heat, and cold-dry conditions were further investigated to select strains for probiotic use.
[Supported by MOTIE.]

C005

An Antagonistic Bacterium *Bacillus amyloliquefaciens* KB3 Isolated from Feces of *Allomyrina dichotoma* Tertiary Larvae Promotes Growth of Tomato and Pepper Seedlings

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A total of 1,000 bacterial strains were isolated from feces of *Allomyrina dichotoma* tertiary larvae. Among them, 190 strains were pre-screened for having potential antagonistic activity against plant pathogenic fungi, *Botrytis cinerea* and *Rhizoctonia solani*, and for having biosurfactant activity. The strain showing strong antifungal activity was named *Bacillus amyloliquefaciens* KB3 by morphological and biochemical properties as well as phylogenetic analysis with 16S rRNA sequences. The cultures of KB3 strain were effective in promoting shoot and root length, and biomass of tomato and pepper seedlings under gnotobiotic conditions compared to the control. Treatments with the cell-free culture supernatant (CFS), a suspension of KB3 cells in distilled water (KB3D), indole extracts, and lipopeptide extracts from KB3 cultures increased shoot and root growth, and biomass of tomato seedling. But no plant growth promotion was observed with treatment of a suspension of heat-killed KB3 in distilled water (KKB3D). By HPLC analysis, the presence of the auxin, indole-3-acetic acid, was detected from KB3 cultures. These results indicate that KB3 has an ability of promoting growth in plants.

C006

Characterization of *Staphylococcus haemolyticus* L62 Lipase Immobilized on Amine-Functionalized Magnetic Nanoparticles

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Staphylococcus haemolyticus L62 lipase has been immobilized onto amine-magnetic nanoparticle (AMP) by covalent cross-linking with glutaraldehyde. The physicochemical properties of the immobilized L62 AMP were evaluated by electron microscopy, Magnetic property measurement system, and Zeta potential system. It was verified that the magnetic nanoparticles aggregated to form about 1.6 μm size complex and displayed saturation magnetic value was 25.56 emu/g. The L62-AMP was investigated by studying the effect of temperature and pH on the activity and stability. In addition, its substrate specificities toward various synthetic *p*-nitrophenyl esters and natural oils were characterized. The L62-AMP showed an enhanced activity at high temperatures and at wide pH range, while it showed similar substrate specificity with free lipase. This L62-AMP could be recovered rapidly with external magnet and maintained above 90% of residual activity until 4-times of recovery.

C007

Transesterification of Plant Oils Using *Staphylococcus haemolyticus* L62 Lipase Displayed on *Escherichia coli* Cell Surface Using OmpA Signal Peptide and EstAβ8 Anchoring Motif

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Staphylococcus haemolyticus L62 (SHL62) lipase was displayed on outer membrane of *Escherichia coli* using OmpA signal peptide and auto-transporter EstAβ8 protein. The pOmpA-tu vector including OmpA signal sequence and EstAβ8 sequence was constructed. The localization of SHL62 lipase on outer membrane of *E. coli* was confirmed using immuno-fluorescence microscopy and flow cytometry analysis. SHL62 lipase activity of whole cells reached 2.0 U/ml culture (OD_{600nm} of 10) when it was measured by *p*-nitrophenyl caprylate assay after being induced by 1 mM IPTG for 24 h. Its optimum temperature and pH was 45°C and 10, respectively. It maintained more than 90% of maximum lipase activity up to 50°C and at the pH range of 5-9. The hydrolytic activity confirmed that *p*-nitrophenyl caprylate and corn oil were preferred substrates among various synthetic and natural substrates, respectively. Moreover, the displayed SHL62 lipase was used to produce fatty acid methyl esters from various plant oils through transesterification. These results suggest that the displayed SHL62 lipase can be used for biocatalytic applications.

C008

Biochemical Characterization of L-Asparaginase in NaCl-tolerant *Staphylococcus* sp. OJ82 Isolated from Korean Fermented Seafood

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L-asparaginase from Gram-positive bacterial species has been poorly explored. SoAsn was expressed in *Escherichia* coli BL21(DE3) with an estimated molecular mass of about 37.5 kDa by SDS-PAGE. Consistent with asparaginases in Gram-negative bacteria, our size exclusion chromatography demonstrated that SoAsn was a homodimer. We verified that SoAsn has optimal temperature near 37-50°C and thermal enzyme stability range is narrower than that of commercial *E. coli* Asn (EcAsn). Both SoAsn and EcAsn are active at pH9~pH10 although their overall pH-dependent enzyme activities are slightly different. Km value of SoAsn appeared to be 2.2 mM which has higher than that of EcAsn. Among eight metals tested for enzyme activity, cobalt greatly enhanced the SoAsn activity whereas Mg could be the most effective cofactor for EcAsn. Interestingly, SoAsn retained its activity more than 60% under 2 M NaCl, but the activity of EcAsn was reduced to be 48%. Taken together, SoAsn has different kinetics, cofactor requirement, and NaCl-tolerant from those of EcAsn.

[This work was supported by Next-Generation BioGreen21 Program (PJ0082082013), Rural Development Administration, Republic of Korea.]

C009

Linoleic Acid: A Potent Compound in *Withania somnifera* Inhibits Virulence Properties and Composition of *Streptococcus mutans* Biofilms

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This study investigated the role of polyunsaturated fatty acids present in *Withania somnifera* to show effect against virulence properties of *S. mutans* biofilms. Chemical characterization of hexane fraction (HF) was carried out by GC/MS which showed palmitic acid (PA), linoleic acid (LA) and oleic acid (OA) as major compounds. Effects of LA, OA, PA, sodium fluoride (NaF) and HF were tested against the acidogenic, aciduric and EPS formation ability of *S. mutans* biofilm cells. 100 μg/ml of each agent showed effect against the acidogenic ability of biofilm cells whereas HF, LA and OA showed strong inhibitory potential against the aciduric effect and EPS formation by biofilm cells. Mainly HF, LA, OA and NaF inhibited dry weight and water insoluble polysaccharide after twice daily 10 min treatment. Confocal images and COMSTAT analysis after twice daily treatment revealed that perfect inhibition of EPS of *S. mutans* biofilm by HF, OA, LA and NaF. Thus, these results suggest that LA might be the effective agents to reduce virulence properties of biofilm followed by the inhibition of dental caries.

C010

Strategy for Screening Metagenomic Resources for Novel Multifunctional Cellulolytic Enzymes Using a Robotic High-Throughput Screening System and Its Characterization

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There have been no screens for exocellulase owing to assay protocol limitations, the high cost of substrates, and low activity of exocellulase compared with endocellulase. This new HTS system enabled activity verification of more than 10^4 clones per day. We were able to obtain four exocellulase clones from about 30,000 metagenomic fosmid clones that had previously been prepared from sweet potato field soil microbes and rumen fluid. This powerful approach could be effectively applied to screen various metagenomic resources for new enzymes. The celEx-SF301, celEx-SF309, celEx-BR12, and celEx-BR15 revealed four ORFs predicted to encode proteins with amino acid sequence homologies to Glycoside hydrolase of *Candidatus Koribacter versatilis* Ellin345, β-galactosidase of *Granulicella tundricola* MP5ACTX9, Family 5 glycosyl hydrolase of *Prevotella ruminicola* 23, and cellulase of *Streptomyces hygroscopicus* ATCC 53653, respectively. Based on these findings, we believe that cellulases are efficient multifunctional enzymes that may prove useful for biotechnological applications.

C011

Characteristics of Mulberry Wine Using Traditional Fermentation Microorganism

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We investigated domestically made mulberry wine by using traditional fermentation microorganism and also observed its fermentative characteristics and antioxidant activity. *S. cerevisiae* B is traditional fermentation microorganism isolated from domestically grown *Rubus occidentalis* and not produce biogenic amines. Each *S. cerevisiae* B and Fermivin was inoculated into mulberry juice up to 1×10^9 CFU/ml/kg and it was incubated at 25℃ for 8 days. Final fermentation products of fermented mulberry juice from *S. cerevisiae* B presented 16.12% of alcohol, 10.1°Bx of sugar, and 4.38 g/L of acidity and final fermentation products from Fermivin presented 15.18% of alcohol, 11.1°Bx of sugar and 7.01 g/L of acidity. The content of total phenolic compounds of *S. cerevisiae* B (1969.96±10.25 mg/ml) observed higher than Fermivin (1901.69±17.38 mg/ml) and DPPH & ABTS radical scavenging activity showed similar figure on *S. cerevisiae* B and Fermivin. On the basis of all results, the possibility of industrial utilization of traditional fermentation microorganisms was confirmed by excellence on fermentation ability and antioxidant activity.
[Supported by the MOTIE, KIAT and HIRPE]

C012

Isolation and Characterization of Novel Genes Related with Calcium Carbonate Precipitation of *Paenibacillus polymyxa* E681 by Transposon-insertional Mutagenesis

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This study shows the isolation and characterization of mini-Tn10 insertional mutants derived from the calcium carbonate forming bacterium *Paenibacillus polymyxa* E681. We identified five transposon mutants showing an increased calcium carbonate precipitation on B4 solid medium. These five mutants were further characterized by quantitative test of calcium carbonate precipitation and pH increase. In the results, calcium precipitation of mutant strains was observed in 10 days after, but the precipitation of wild type E681 was not observed in same inoculation period. Among them, mutant 6-15 strain showed a higher increased pH than that of wild type E681, was finally selected. DNA fragments flanking the transposon insertion in five mutants were cloned and sequenced.

C013

A Novel CO-Dependent Transcriptional Regulator and Enhanced H_2 Production by an Engineered *Thermococcus onnurineus* NA1

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Genomic analysis suggested the existence of a CO-dependent transcriptional regulator composed of the CorQ with a V4R domain and the CorR with a DNA-binding domain of LTTR family in a carboxydotrophic hydrogenogenic *Thermococcus onnurineus* NA1. CorQR homologs were identified in three other *Thermococcus* strains and a *Candidatus Korarchaeum cryptofilum* OPF8. Using the mutant strains with deletion of *corQ* or *corR*, it was demonstrated that CorQR serve as a positive transcriptional regulator for expression of a gene cluster composed of a carbon monoxide dehydrogenase (CODH), a hydrogenase and a Na^+/H^+ antiporter. The mutant strain, MC02, with overexpression of *corQR* showed 2 to 7-fold higher transcripts and 2 to 4-fold higher proteins from the CODH gene cluster than the wild-type strain. The overexpression of the transcriptional regulator resulted in a 4-fold increase in H_2 production in a bioreactor culture compared to the wild-type strain. To the best of our knowledge, the engineered strain exhibited the highest H_2 production rate of 171 mmol/L/h and specific H_2 production rate of 237 mmol/g/h among CO-dependent H_2-producing microbes studied to date.

C014

***In Vitro* Evaluation of Antibacterial Activity of Plant Extracts against Clostridial Necrotic Enteritis Strains**

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Clostridium perfringens generate a variety of economically significant diseases as the causative agent of necrotic enteritis in poultry. This study was to evaluate the *in vitro* antibacterial activity of the plant extracts to prevent the clostridial necrotic enteritis from broiler chickens. Thirty wild plants were prepared by ethanol extraction method. Antimicrobial susceptibility testing was conducted by agar dilution methods. Two (*Fraxinus rhynchophylla Hance* and *Geranium koreanum Kom*.) of the 30 plant extracts showed excellent antibacterial activity against *C. perfringens* by modified spot-on-lawn method. The MIC values of *F. rhynchophylla Hance* and *G. koreanum Kom*. against strains were ranged from 128 to 256 μg/ml and 32 to 128 μg/ml, respectively. The MBC values of two extracts were ranged from 1,024 to 2,048 μg/ml and 256 to 1,024 μg/ml, respectively. The geometric mean of MBC against strains was 3-fold dilution higher than those of MIC. *F. rhynchophylla Hance* and *G. koreanum Kom.* showed outstanding antimicrobial activity against clostridial necrotic enteritis strains.
[Supported by grants from Ministry for Food, Agriculture, Forestry, and Fisheries, Korea (No. 311007-5)]

C015

Anaerobic Biodegradation of Sulfamethoxazole by Human Intestinal Bacteria *Eubacterium limosum* ATCC 8486

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Sulfamethoxazole (SMX) is one of the most commonly applied sulfonamide antibiotics. The widespread use and disposal of SMX have become a serious concern due to the potential antibacterial resistances. Hence, the effort to remediate SMX in environments has been increasing. In this study, we demonstrated SMX biodegradation by *Eubacterium limosum* which is human intestinal microbe. When 100 μM SMX and *E. limosum* were added into BHI media, about 80% of SMX was degraded within 7 days. Furthermore, three assumed metabolites were produced from SMX. Based on QTOF-LC/MS analysis, mass of the major unknown chemical compounds were 172, 210 and 244, respectively. This result suggest that SMX can be metabolized by *E. limosum* in human intestine and the staple concern with SMX biodegradation by this intestinal bacteria is the behavior of its metabolites in our body. This is the first report that individual bacteria could be related to SMX biodegradation under anaerobic conditions. For further work, identification of those metabolites would be accomplished to know their effect in our body.
[This work was supported by the NRF of Korea (NRF: 2010-0029224) grant.]

C016

Establishment of Improved Methods for Survivability of Freeze-dried *Vibrio* Pathogens in Long-term Preservation

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A wide range of bacteria is preserved with skim milk as a protectant in culture collection. However, *Vibrio* often fail to preservation in this way, resulting in loss of them. The aim of this study is therefore to search an effective protectant for *Vibrio* preservation and investigate its protective mechanism. The respective culture of *V. vulnificus, V. parahaemolyticus,* and *V. cholerae* at the exponential stage was suspended in skim milk and/or inositol solution as a protectant, and aliquoted in ampoules. After frozen and dried at -80°C under vacuum, the ampoules were stored for 2 weeks at 37°C for accelerated test. Freeze-dried bacteria was observed under the electronic microscope and their viability was determined by flow cytometry and colony count methods. The data from three species commonly showed the greatest viability when cultured in 3%NaCl-LB broth after stored with 5% inositol alone and this phenomenon was consistently observed to three non-pathogenic *Vibrio* species in the same condition. Therefore, our results suggest that 5%inositol could be a good candidate as a cryo- and lyo-protectant for *Vibrio* pathogens and 3%NaCl-LB positively helps them to reactivate and proliferate.

C017

Dual Effects of Non-thermal Dielectric Barrier Discharge Plasma on a Fungal Pathogen and Host Plant

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Non-thermal plasma generating various reactive species has demonstrated contradictory effects; inactivation and activation. Selective inactivation of plant microorganisms without damaging associated objects has been one of hot issues in sterilization by plasma technology. In this study, we analyzed the inactivation of plant fungal pathogen, *Fusarium oxysporum* f. sp. *lycopersici* as well as the induction of resistance in tomato host plant, *Solanum lycopercicum* by using plasma. Spore germination in saline was continuously reduced over incubation time after a 10 min argon (Ar) plasma treatment. Although majority of inactivated spores exhibited necrotic death, apoptotic spore death was also observed along with the increased expression of apoptosis related genes during incubation after Ar plasma treatment. The increased mRNA expression level of pathogenesis related (PR) genes was observed in roots of a susceptible tomato cultivar after treated with same dose of Ar plasma used in fungal inactivation.
[Supported by grants from NRF funded by the Korean government (MSIP) (2010-0027963, 2013R1A1A3011245) and also by RDA, Republic of Korea (PJ009891).]

C018

The Application of O_3 and Plasma to Bakanae Disease Control

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Selective sterilization by plasma is one of the current issues in plasma bioscience. Inactivation of microbes associated with foods and host organisms requires non-toxicity to foods and hosts. The objective of our study is to examine the potential of plasma and O_3 for selectively inactivating fungal pathogens infecting seed rice. For this, we first investigated the feasibility of using O_3 and plasma generated by arc discharge in water to inactivate *Fusarium fujikuroi*, a fungus causing rice bakanae disease which now becomes a problem in Korea. When fungal spores (1.2×10^{10}) in DI water were exposed to O_3 gas, germination was dramatically reduced within a minute. Reduction of 3 log scale in spore germination was also observed in water after a 10 min treatment with arc discharge plasma. Surface of spores was severely wrinkled in the treatment with ozone whereas many spores were crushed in the treatment with arc plasma. Less fungal growth was observed on seed rices after treatment with ozone or arc plasma.
[This work was supported by grants from NRF funded by the Korean government (MSIP) (2010-0027963, 2013R1A1A3011245) and also by RDA, Republic of Korea (PJ009891).]

C019

Production of Nitric Oxide Using a Microwave Plasma Torch and Its Role in the Development of *Neurospora crassa*

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Nitric oxide (NO) is known as a signalling molecule that regulate gene expression, mediate immune response and inflammation, and mediate tissue differentiation and organ development. The analytical theory indicates that the nitrogen monoxide density is nearly proportional to the oxygen molecular density and that the high-temperature flame is an effective means of generating nitrogen monoxide. In the study, we have measured the concentration of nitrogen monoxide produced by using different level of the oxygen input in units of cubic centimetre per minute. We applied nitric oxide produced from a nitrogen torch operated at a microwave power of 400W to fungal spores in saline and then examined the germination and sporulation. Our results demonstrated that the number of spores and the expression level of sporulation related genes such as acon-2, acon-3, acon-4 and acon-10 were elevated after the treatment with nitric oxide gernerated by using 400 sccm (standard cc per minute) O_2 flow.
[Supported by the National Foundation of Korea (NRF), (2010-0027963 and 2013R1A1A3011245).]

C020

Changes in the Yeast Flora during Alcohol Fermentation of Korean Persimmons

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This study were carried out to investigate the changes in the yeast flora during fermentation of Korean persimmon wine. Alcohol fermentation was carried out by inoculation of *S. cerevisiae* Fermivin and *S. cerevisiae* EC1118 cells. Persimmon juices prepared by filtration of crushed persimmon were adjusted to 24 °Brix with sucrose before inoculation of yeast cells. During the fermentation, physicochemical and microbiological changes were monitored and 20 yeasts each isolated at the various stages were analyzed by the PCR-RFLP for 16 days of fermentation period. Sugar content were 10~13.4°Brix and alcohol content were 8.8~12.8% after fermentation which were dependent upon the yeast strains. pH and total acid content were 3.9~4.1 and 0.3~0.4% during the fermentation of persimmon wine. Total 400 yeasts isolated during the fermentation were analyzed by PCR-RFLP. Internal transcribed spacer (ITS) region of the isolated yeasts was amplified by ITS1 and ITS4 primers and then, cut with restriction enzyme *Hae*III and *Hinf*I, which were resolve in the 1.5% agarose gel.
Keywords: PCR-RFLP, persimmon, Indigenous yeast

C021

Effects of a *Hanseniaspora uvarum* Isolated from Korean Grapes on the Quality of Wine Fermented Using Campbell Early Grape Must

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Grapes of Campbell Early cultivar, the major Korean domestic grape variety, contain low sugar and high acid contents. Wines fermented using Campbell Early grapes, in general, are weak in color and flavor. and contain undesirable flavor of fox aroma. In this study, it was found that *Hanseniaspora uvarum* is the major wild yeast in Korean grapes. Total 105 *H. uvarum* strains were isolated from Campbell Early (Sangju, Dansan, Yeongcheon, organic), MBA (Yeongcheon) and improved wild grapes (Dansan). When they were tested for alcohol and flavor production in a small scale grape juice fermentation. Several *H. uvarum* strains were selected based on the alcohol and flavor production in wine. And, their fermentation characteristics were analyzed using Campbell Early grape must.
Keywords: *Hanseniaspora uvarum*, Campbell Early, flavor

C022

Biodiversity of Wild Yeasts Isolated from Korean Honey Based on the rDNA PCR-RFLP and Sequences

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Wild yeasts were isolated from various Korean honeys and characterized by molecular techniques. Eighty yeast colonies each were isolated from four different honey samples such as multi-floral, black locust, chestnut and jujube tree honeys on the YPD plate containing sodium propionate. Diversity of the isolates was studied by the restriction fragment length polymorphism (RFLP) of PCR products amplified by using ITS1 and ITS4 primers. PCR-RFLP resulted that the isolates from each honey samples can be divided into 2-3 groups. When, typical strains of the group were selected and identified by the phylogenetic analysis, major yeasts were different from one honey sample another. Total three genera and five species were *Zygosaccharomyces rouxii, Zygosaccharomyces siamensis, Zygosaccharomyces mellis, Starmerella bombicola* and *Candida maqnolize*. Among the strains, *Z. siamemsis* were the most abundant yeast in the Korean honey especially in jujube tree (64/125 isolates) and chestnut honeys (45/96 isolates)
Keywords: Honey yeast identification, PCR-RFLP, 5.8S-ITS region

C023

Analysis of the Antimicrobial Effects of Non-thermal Plasma on Fungal Spores in Ionic Solutions

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Microenvironments surrounding microorganisms often modulate the effects of various anti-microbial agents. Increasing number of studies show that sterilization efficiency of plasma varies depending on the background environment surrounding microorganisms. In this study, we investigated the influence of NaCl in background media on anti-microbial effects of plasma using a model eukaryotic microbe, *Neurospora crassa* (filamentous fungus). Our data revealed that the presence of NaCl in the background solutions attenuated the deleterious effect of plasma on germination, internal structure, and genomic DNA of fungal spores. The protective effects of NaCl were not explained exclusively by pH, osmotic stability, or the level of reactive species in the solution. The presence of ions reduced plasma toxicity, which might be due to a reduced access of reactive species to fungal spores, and fungal spores were inactivated by plasma in a background fluid of non-ionic osmolytes in spite of the low level of reactive species.
[Supported by grants from NRF funded by the Korea government (MSIP) (2010-0027963, NRF-2013R1A1A3011245).]

C024

Building a Platform Technique for Rapid and Sensitive Detection of NADPH in Various Samples

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Intracellular redox and energetic status play a crucial role in cardiovascular diseases and metabolic disorders. The physiological status of reducing agents, such as NADPH and NADH, is required for the activity of antioxidant system. For these reasons, an accurate measurement of reducing and/or oxidizing cofactors enables clinical diagnosis of preanalytical phase. Although various methods have been used for this purpose, these are the high costs of experiment and large instrument requiring processes, also not suitable for rapid routine use. Thus, developing a miniaturized and economical device for the sensitive, specific and rapid determination of cofactors is highly desired. Here we present a fluorometric platform technique for the quantitative detection of NADPH. The system suggested here circumvents many issues of previous methods, because this system simply measured an enhanced fluorescence of NADPH by interaction only with mBFP (a metagenome-derived NADPH-dependent blue fluorescence protein). Using this platform technique, we can specifically measure the target analyte in complex samples derived from whole cells including blood, saliva, urine and environmental samples.

C025

Expression Analysis of Rice Pathogenesis-related Proteins Involved in Stress Response and Endophytic Colonization Properties of *gfp*-tagged *Bacillus subtilis* CB-R05

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Bacillus subtilis CB-R05, possessing antagonistic effects against several fungal pathogens is a diazotrophic plant-growth promoting bacteria marked with the *gfp* gene. To confirm the expression level of the PR proteins in rice inoculated with CB-R05, the expressions of four PR proteins (PR2, PR6, PR15 and PR16) were examined in this study, in the rice leaves treated with wounding stress over a time period. The results revealed that the PR proteins were generally more strongly expressed in the rice leaves inoculated with CB-R05 compared with the untreated control. The marked *gfp*-tagged CB-R05 strain was inoculated onto the rice seedlings under axenic conditions. Under the confocal laser scanning microscope (CLSM), the *gfp*-tagged CB-R05 bacterial cells were observed to penetrate the rhizoplane, especially in the elongation and differentiation zones of the rice roots and colonize the root intracellular. The CB-R05 population in the rice root rhizoplane was also monitored. These results show a very widespread colonization of the CB-R05 in the rice rhizosphere. Further attempts are under way to investigate the competition between the CB-R05 bacteria and the fungal pathogen *in vivo*.

C026

Antagonistic Activity of Potent Probiotic *Lactobacillus* Strain against Acne Pathogens

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Makgeolli is a Korean traditional alcoholic beverage fermented using steamed wheat and rice. In this study, we collected local makgeolli samples, and then isolated yeast and lactic acid bacteria (LAB). Makgeolli contained 10^6~10^8 CFU/ml of yeast and 10^4~10^6 CFU/ml of LAB on average. *Lactobacillus paracasei* subsp. *paracasei* HY7301 was also isolated from a makgeolli manufactured in Gyeonggi area. In this study, we investigated antagonistic activity of *L. paracasei* HY7301 against *Propionibacterium acnes* causing acne vulgaris. The culture supernatant of *L. paracasei* HY7301 inhibited growth of *P. acnes* virulent strains by about 80~90%. Also the growth of *P. acnes* was restrained by co-culturation with *L. paracasei* HY7301. These results suggest that *L. paracasei* HY7301 can be a useful probiotic microorganism for prevention acne vulgaris.
[Supported by grants from iPET]

C027

Cloning, Overexpression and Characterization of a Novel β-Galactosidase from *Leuconostoc mesenteroides* J18 Isolated from Kimchi

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The complete genome sequence analysis of *Leuconostoc mesenteroides* J18 revealed several β-galactosidase genes in this strain. A gene encoding protein (YP_005174294) of glycosyl hydrolase family with 478 amino acid residues was annotated to be β-glucosidase/6-phospho-β-glucosidase /β-galactosidase. To characterize this protein, the gene was PCR-amplified, cloned into *E.coli* DH5α, and overexpressed in the expression host *E. coli* BL21 (DE3) using pET28a(+) vector under the control of T7 promoter. The recombinant protein was overexpressed at 20°C and the crude enzyme was able to hydrolyze 4-nitrophenyl-β-D-galactopyranoside with a specific activity of 1.7 U/mg. No hydrolysis was observed with the substrate 4-nitrophenyl-β-D-glucopyranoside, suggesting that the recombinant enzyme possesses β-galactosidase activity. The recombinant protein was purified by Ni^{2+}-NTA affinity chromatography and produced a single band on SDS-PAGE with an approximate molecular weight of 55 kDa. Functional characterization of the enzyme is now in progress, since it did not show a significant amino acid sequence identity with other functionally characterized β-galactosidases.

C028

Isolation of Acetic acid Bacteria for the Production of Traditional Vinegar from Korean Wine and Their Properties

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Although industrial fermentation of vinegar has been well established, scientifically little has been known about traditional vinegar fermented from Korean wine. We tried to isolate acetic acid-producing strains from 26 brands of unrefined rice wine (makgeolli) produced by local microbreweries, which could reproduce flavors of traditional vinegar. To keep the high yield and preservation of vinegar, we selected 12 strains of acetic acid bacteria with excellent resistance against the toxicity of ethanol and sulfite. All of them grew well even in the presence of 350 mg/L of potassium metabissulfite used as a preservative. In spite of survival at the concentration of 20% (v/v) ethanol added as substrate, they produced vinegar optimally with production yields ranging from 9 to 47% at the concentration of 10% ethanol. As results of bacterial identification by16S rRNA sequencing, they belong to *Acetobacter cerevisiae*, *A. tropicalis*, *A. pasteurianus*, or *A. indonesiensis*, which are generally known as acetic acid-producers.
[This work was supported by the Ministry of Agriculture Food And Rural Affairs & Sunchang County.]

C029

Isolation of *Bacillus subtilis* Strains Suitable for the Fermentation of Soybean Product

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Some of *Bacillus* species play a key role in the fermentation of soybean products by producing a large amount of extracellular proteases. Through primary screening, we have isolated 65 *Bacillus* strains from 62 samples of traditionally fermented soybean products according to the selection criteria of *Bacillus* strains. The criteria for the selection include (1) antimicrobial activities against pathogens including *Bacillus cereus*, (2) no production of biogenic amines and degradation activities toward them, and (3) high production of extracellular proteases and amylases. Among them, we examined the strains that produce conjugate linoleic acid (CLA), which is marketed as a dietary supplement on the basis of health benefits, and then identified them using comparative analysis of 16S rRNA sequences. As a result, we could get 10 strains of *Bacillus subtilis* with production of CLA concentration above 250 ppm.
[This research was supported by High value-added Food Technology Development Program from Ministry for Food, Agriculture, Foresty and Fisherish, Republic of Korea (No. 313037-3).]

C030

Screening of Bacterial Strains Which Convert Major Ginsenoside Rb1 to Minors

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Codonopsis lanceolata is a folk medicinal plant in Asian countries, and it has bioactive compounds, ginsenosides, which are members of saponins. Many researchers have performed experiments towards converting major ginsenosides to the more active minor ginsenosides, like F2, Rg3, Rh2, and C-K. Ginseng contains a small percentage of these expensive minor ginsenosides. In the present work, for bio-conversion of ginsenoside Rb1 to minor ginsenosides, the bacterial strains having beta-glucosidase activation were screened from low price *C. lanceltata*. 139 strains were isolated from rhizosphere soil field. Among them, LSA71 was isolated and identified by esculin agar method for beta-glucosidase activity. It showed optimum growth rate with pH7 at 28℃. Strain LSA71 was closely related to *Ralstonia pickettii* ATCC 27511^T (98.96% similarity based on 16S rRNA sequence) and significantly transformed ginsenoside Rb1 to F2 and C-K.
[This research was supported by a grant NRF-2006-08790 funded by Ministry of Science, ICT and Future Planning of Korean Government.]

C031

Isolation of *Aspergillus oryzae* Strains Suitable for the Fermentation of Soybean Products and Their Properties

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Fungal species are major microbial sources to produce fermented soybean products, some of which excrete extracellular proteases and amylases that play a key role in fermenting and ageing process. Nevertheless, there have not been qualified mold strains universally applicable to soybean fermentation until now. We have isolated 158 mold strains from 94 brands of traditionally fermented soybean products and Korean traditional rice wine starters (Nuruk), and then identified them with morphological characteristics and with comparative analysis of base sequences of internal transcribed spacer (ITS) and 28S rRNA region. As a result, we could get 31 strains of *Aspergillus oryzae* with no production of aflatoxins and finally selected 3 strains among them after examining the excretion ability of extracellular amylases and proteases. We also compared the enzymatic properties of the strains between different amylases using kinetic analysis by high performance liquid chromatography (HPLC).
[This work was supported by the Sunchang Country (high value-added) Food Technology Development Program from Ministry for Food, Agriculture, Foresty and Fisherish, Republic of Korea (No. 311036-3)]

C032

Effect of *L. acidophilus* NS1 or *L. fermentum* NS2 on Plasma Cholesterol Level in Diet-induced Obese Mice

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Reductions of plasma total and LDL cholesterol are major factor to decrease the risk of cardiovascular diseases. The objective of this study was to investigate whether *Lactobacillus acidophilus* NS1 or *L. fermentum* NS2 effectively reduces plasma cholesterol level in mice fed high-fat diet. In animal study, seven-week-old male C57BL/6 mice were fed with a normal diet (ND), a high-fat diet (HFD) or a HFD with *L. acidophilus* NS1 or *L. fermentum* NS2 (ca. 1.0×10^8 cfu/ml) for 10 weeks. Total cholesterol and LDL cholesterol levels were significantly lower in mice fed with a HFD with *L. acidophilus* NS1 or *L. fermentum* NS2 than in those fed HFD. Expressions of SREBP2 and LDLR in the liver were dramatically reduced in mice fed HFD as compared to those of mice fed ND. These results suggest that the oral administration of *L. acidophilus* increased the expressions of SREBP2 and LDLR in the liver which were inhibited by high-fat intake, leading to a decrease in plasma cholesterol level. *L. acidophilus* NS1 could be useful probiotics for cholesterol-lowering dairy products and the improvement of hyperlipidemia and hepatic lipid metabolism.

C033

Change of Growth Ratio and Expression of Inductive Proteins in *Lactobacillus plantarum* L-67 under Cold Stress

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The physiological status of the cell and other environmental factors such as pH, salts and temperature may affect the mechanism of stress resistance. We report the survival of *Lactobacillus plantarum* L67 after freeze-thaw cycles, the expression levels of the *csp* genes (*csp*C, *csp*L and *csp*P) and ATPase activity in response to cold stress. As a result, only 56% of *L. plantarum* L67 cells without cold stress survived after four consecutive freeze-thaw cycles. However, 78% of *L. plantarum* L67 cells survived after the incubation temperature was downshifted to 5°C for 6 h under freeze-thaw conditions. In qRT-PCR and proteomic analysis, quantification of transcript levels of *cspP* gene was increased after reduction of the incubation temperature to 5°C. And 12 expressions of proteins were identified. This result indicates that exposing *L. plantarum* cells to low temperatures helps the cells to survive through subsequent freeze-thaw processes. Moreover, it may represent a further example of mechanisms for stress responses in *Lactobacillus plantarum*.

D001

Down-regulation of HIF-1α by Oncotropic H-1 Viral Infection Independently of VHL and RACK

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Hypoxia is a prevalent feature of solid tumors. Over-expression of HIF-1α, a transcription factor responsive to hypoxia, is frequently observed in aggressive tumors. Down-regulation of HIF-1α by virotherapy can thus be a good candidate strategy to treat these tumors. Herein we found that oncolytic H-1 parvovirus decreases protein levels of HIF-1α in pancreatic cancer cells under $CoCl_2$ or hypoxia. Down-regulation of HIF-1α by infection of H-1virus is regulated by proteasome-mediated pathway. Suppression of VHL or enforced expression of UCP failed to prohibit down-regulation of HIF-1α mediated by H-1 viral infection. Furthermore, suppression of RACK by siRNA did not inhibit H-1 viral infection-mediated decrease of HIF-1α. Although down-regulation of HIF-1α was observed under H-1 viral infection, higher levels of HIF-1α provided resistance of apoptosis to H-1 viral infection. We found that combined treatment with H-1 virus and YC-1, an inhibitor of HIF-1α enhances apoptosis of pancreatic cancer cells compared to treatment with H-1 virus or YC-1 alone. Accordingly, we propose that H-1 virus may be used together with YC-1 as a potential therapeutic agent against aggressive tumors.

D002

Activation of the Phosphatidylinositol 3-Kinase-AKT Pathway by Kaposi's Sarcoma-Associated Herpesvirus Viral Interferon Regulatory Factor 2

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Kaposi's Sarcoma-Associated Herpesvirus (KSHV) causes Kaposi's Sarcoma, a cancer primarily founded in AIDS patients. KSHV expresses four viral interferon regulatory factors (vIRF1-4). According to the previous study, vIRF2, encoded in K11.1 and K11, participates in caspase-3 mediated inactivation by interferon regulatory factor 3, thus vIRF2 acts as potent antagonist in the caspase-3 activity. Here we demonstrate that vIRF2 triggers phosphatidylinositol-3 kinase (PI3K) pathway, and AKT activation is followed. This activation comes from the induction of AKT phosphorylation on Threonine 308 but not on Serine 473. We also found that vIRF2 deregulates the transactivation activity of the forkhead box protein O3A (FoxO3A), FKHR transcription factor family member which play an important role in apoptosis. vIRF2 also inhibits FoxO3A mediated caspase-3 activity. Additionally, we found that vIRF2 inhibits extracellular signal regulate kinase (ERK) pathway. Our results suggest that KSHV vIRF2 activates PI3K-AKT pathway and inhibits ERK.

D003

Leucine Biosynthesis Is Required for Iron Homeostasis and Virulence in *Cryptococcus neoformans*

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Amino acid biosynthesis that is absent in mammals is considered an attractive target of antifungal treatment. Isopropylmalate dehydrogenase (Leu1) is an iron-sulfur cluster protein, required for leucine biosynthesis in *Saccharomyces cerevisiae*. Moreover, our previous transcriptome data showed that the expression of *LEU1* is regulated by iron availability in *Cryptococcus neoformans*. In this study, we aimed to characterize a role of leucine biosynthesis in iron homeostasis and virulence of the *C. neoformans*. We found that deletion of *LEU1* caused the cells to become leucine auxotroph and that intracellular iron levels were significantly distorted in the *leu1* mutant. The *leu1* mutants also displayed increased susceptibility to oxidative stress and cell wall/membrane disturbing agents, as well as attanuated virulence. The mutant lacking the beta-isopropylmalate dehydrogenase gene (*LEU2)*, which encodes an enzyme catalyzed in subsequent step of leucine biosynthesis, showed not only similar phenotypes to the *leu1* mutant but attenuated virulence. Overall, our results suggest that leucine biosynthesis is required for iron homeostasis and virulence in *C. neoformans*.
[Supported by awards from NRF.]

D004

Synergistic Effects of *Cinnamomum camphora* Leaves Extract against Clinical Isolated Methicillin-Resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has been emerging worldwide as one of the most important hospital and community pathogens. Therefore, new agents are needed to treat MRSA associated infections. *Cinnamomum camphora* (*C. camphora*) is a plant of family Lauraceae, and grown Jeju island in South Korea. In this study, antibacterial activites of *Cinnamomum camphora* leaves extract (CCE) were investigated in combination with antibiotics against clinical isolates of MRSA. The results showed that CCE was determined with MIC and MBC values ranging from 0.625 to 0.31 and 0.625 to 0.31 mg/ml, oxacillin from 0.5 to 1024 and 1 to 1024 μg/ml, ampicillin from 1.25 to 64 and 2.5 to 64 μg/ml. The combination of CCE with oxacillin or/and ampicillin were synergistic effect against all tested MRSA. This study suggests that CCE reduced the MICs and MBCs of antibiotics tested, that CCE in combination with antibiotics could lead to the development of new combination of antibiotics against MRSA infection.
[This research was supported by Basic Science Research Program through the NRF funded by the MKE-R0001028]
Keywords: *Cinnamomum camphora*, MRSA, Antimicrobial, Synergistic

D005

vIRF3 and vPK Encoded by Kaposi's Sarcoma-Associated Herpesvirus Inhibit T-Cell Factor-dependent Transcription via Different Pathway

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KSHV is associated with KS, PEL and MCD. Many studies revealed that host and viral factors interacts with each other. Wnt signaling have been shown that it has functional roles for oncogenesis in the dysregulated condition. Moreover, beta-catenin, a downstream of Wnt signal pathway, promotes TCF-dependent transcription producing cell cycle- and cancer-associated cellular factors, which contributes to cell proliferation and tumorigenesis. Here, we identified two viral factors, vIRF3 and vPK, which regulate TCF-dependent transcription. vIRF3 significantly inhibits TCF-dependent transcription in a dose-dependent manner. CBP-interaction motifs of vIRF3 are important for the inhibition of transcription activity. Our results show that vPK also inhibits TCF-dependent transcription in a dose-dependent manner. In the presence of vPK expression, interaction between beta-catenin and TCF4 was decreased. Although further study is required for finding detailed mechanisms, this study suggests that KSHV regulates host systems through several different pathways.
[Supported by grant from NRF and Bio-industry Technology Development Program, Ministry for Food, Agriculture, Forestry and Fisheries]

D006

Comparative Evaluation of the Liquid Culture System (BacT/Alert) and Löwenstein-Jensen (L-J) Medium for the Detection of *Mycobacterium tuberculosis* (*M. tb*) from Sputum Specimens

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This study was to compare the recovery of *M. tb* from sputum specimens of *M. tb* patient using the liquid culture system and L-J medium. 304 sputum specimens were processed for detection of AFB; 210 specimens were AFB smear positive, and 94 were AFB smear negative. In smear-positive specimens, detection rates were 90.0% and 94.3% on L-J medium and on liquid culture system, respectively. Two hundred eighteen isolates were recovered by at least 1 culture medium, and almost all were identified as *M. tb* and only one isolate was identified as *M. abscessus*. There was good concordance between culture results obtained on both culture media with an agreement of 94.8%. In the drug susceptibility test, of 217 isolates, 30 and 31 isolates showed resistance to INH by L-J medium and liquid culture system, respectively. There was good concordance between results of drug susceptibility using the two methods with an agreement of 97.7% for RIF, respectively. These results indicate that the liquid culture system (Culture/Drug Susceptibility Test) is more efficient and faster than L-J medium to diagnose *M. tb*.
[This research was supported from NRF (No. NRF-2013R1A1A2059687).]

D007

Synergistic Effect of Oleanolic Acid on Aminoglycoside Antibiotics against *Acinetobacter baumannii*

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Oleanolic acid (OA), a natural pentacyclic triterpenoid, has hepatoprotective, antitumor and weak anti-HIV, HCV activities. OA appeared to decrease motility and generate free radicals in *A. baumannii*. Fractional inhibitory concentration (FIC) measurement demonstrated that OA has synergistic effect only with aminoglycoside-antibiotics. Other antibiotics (ampicillin, rifampicin, norfloxacin, and tetracycline) have additive effect with OA. Our microarray and qRT-PCR confirmed that ATP synthesis, cell membrane permeability, glycosyltransferase, peptidoglycan-related and phage-related genes and DNA repair genes were up-regulated under OA. Deletion of highly induced genes: adk, encoding an adenylate kinase and des6, encoding a linoleoyl-CoA desaturase, increased FIC showing that adk and des6 genes contributed to synergistic effect of OA with aminoglycosides. Fluorescence-labeled gentamicin and 8-anilino-1-naphthalenesulfonic acid probe tests suggested that those genes (adk and des6) are involved in change of membrane permeability. Taken together, our data showed that the OA boosts up aminoglycoside uptake by changing membrane permeability in *A. baumannii*.

D008

Development of Culture-Enhancing Medium Containing Culture-Promoting Ingredients for *Mycobacterium tuberculosis* Culture

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In this study, we optimized the production process of culture-promoting ingredients from *M.* sp1 and produced culture-enhancing medium for *M. tb*. Then the medium were evaluated in Liquid culture system using *M. tb*. Optimization of the production process of culture-promoting ingredients from *M.* sp1 by adding lysozyme or glycine to the culture and disrupting the harvested cells with sonicator or French press. Quantification of protein in the whole cell extract prepared from *M.* sp1 to estimate the concentration of culture-promoting ingredients. Production of growth-enhancing media by adding 0, 0.2, 0.4, 1, 2 mg culture-promoting ingredients to 7H9 or Liquid culture btl. Growth analysis of *M. tb* on culture-enhancing medium (7H10) by CFU at various day (0~14 days). Evaluation of culture-enhancing medium using Liquid culture system. Determination of the TTPD of *M. tb* using Liquid culture system. Protocol standardization for production of culture-enhancing medium. Description of experimental procedures for optimized production of culture-promoting ingredients and culture-enhancing medium for *M. tb*. The TTPD was advanced by 21%.
[This research was supported from NRF (No. NRF-2013R1A1A2059687).]

D009

Antibiotic, Antibiofilm Activities and Cell Selectivity of the NRC-16 Peptide Derived from witch Flounder, *Glyptocephalus cynoglossus*

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In this work, we extended the search for the activity of peptide that showed antibacterial activity on clinically isolated bacterial cells and bacterial biofilm. We found that synthetic peptide NRC-16 displays antimicrobial activity and is not sensitive to salt during its bactericidal activity. Interestingly, this peptide also led to significant inhibition of biofilm formation at a concentration of 4-16 μM. NRC-16 peptide is able to block biofilm formation at concentrations just above its minimum inhibitory concentration while conventional antibiotics did not inhibit the biofilm formation except ciprofloxacin and piperacillin. It did not cause significant lysis of human RBC, and is not cytotoxic to HaCaT cells and RAW264.7 cells, thereby indicating its selective antimicrobial activity. In addition, the peptide's binding and permeation activities were assessed by tryptophan fluorescence, calcein leakage and circular dichroism using model mammalian membranes composed of phosphatidylcholine (PC), PC/cholesterol (CH) and PC/sphingomyelin (SM). These experiments confirmed that NRC-16 does not interact with any of the liposomes but the control peptide melittin did.

D010

Antibiotic, Synergistic Effects and Antibiofilm Properties of Chimeric Peptides against MR *Acinetobacter baumannii* Strains

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The increasing prevalence of drug-resistant pathogens highlights the need to identify novel antibiotics. The antibacterial activities, synergistic effects, and antibiofilm properties of the four chimeric AMPs were tested against *Acinetobacter baumannii*, an emerging Gram-negative, nosocomial, drug-resistant pathogen. Nineteen *A. baumannii* strains resistant to ampicillin, cefotaxime, ciprofloxacin, tobramycin, and erythromycin were isolated at a hospital from patients with cholelithiasis. All four peptides exhibited significant antibacterial effects (MIC = 3.12 to 12.5 μM) against all 19 strains, whereas five commercial antibiotics showed little or no activity against the same pathogens. The peptides also exhibited an ability to prevent biofilm formation, which was not seen with cefotaxime, ciprofloxacin, or erythromycin, though polymyxin also inhibited biofilm formation. Collectively, our findings indicate that the AMPs tested have no cytotoxicity but possess potent antibacterial and antibiofilm activities and may act synergistically with commercial antibiotics.

D011

Structural Analysis of *Pseudomonas aeruginosa* Flagellin, FliC

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Flagellin is a structural protein that polymerizes into bacterial flagellar filament. Flagellin from *Salmonella* species contain D0, D1, D2, and D3 domains, and have been extensively studied. The conserved D0 and D1 domains mediate filament assembly and targeted by innate immune receptors, including Toll-like receptor 5 (TLR5), whereas the variable D2 and D3 domains have been demonstrated not to be required for flagellar formation. To reveal the role of flagellin from other species in filament assembly and immune recognition, we have determined the crystal structure of the D1-D2 domains of *Pseudomonas aeruginosa* flagellin (*pa*FliC) at 2.1 A resolution. The *pa*FliC D1 domain is structurally similar to *Salmonella* flagellin and provided a major TLR5 binding site. In contrast, the D2 domain has a unique structure that has not been found in other flagellins. Our structure-based modeling study on the *pa*FliC filament suggests that, unexpectedly, the D2 domain contributes to filament formation potentially by interacting with the D1 domain from other subunits. In the model, the D2 domain was exposed to solution, suggesting that the D2 domain could play an important role in immunogenicity.

D012

A HPA3P2 Peptide with Antibacterial Activity without Cytotoxicity against MDRPA-infected Mice

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An earlier study indicated that HPA3, an analog of HP (2-20) derived from the N-terminus of *Helicobacter pylori* ribosomal protein L1, forms large pores and shows considerable cytotoxicity. However, HPA3P, in which a proline (Pro) is substituted for glutamic acid (Glu) at position 9 of HPA3, shows markedly less cytotoxicity. Unfortunately, HPA3P is not an effective antibacterial agent *in vivo*. We therefore designed a helix-PXXP-helix structure (HPA3P2), in which Pro was substituted for the Glu and phenylalanine (Phe) at positions 9 and 12 of HPA3, yielding a molecule with a flexible central hinge. As compared to HPA3P, HPA3P2 exhibited dramatically increased antibacterial activity *in vivo*. The changes in HPA3 behavior with the introduction of Pro likely reflects alterations of the mechanism of action: i) HPA3 forms pores in the bacterial cell membranes, ii) HPA3P permeates the cell membranes and binds to intracellular RNA and DNA, and iii) HPA3P2 acts on the outer cellular membrane component LPS. Collectively, these results suggest HPA3P2 has the potential to be an effective antibiotic for use against multidrug-resistant bacterial strains.

D013

Antimicrobial and Anti-inflammatory Effects of Cecropin A(1-8)-Magainin2(1-12) Hybrid Peptide Analog P5 against *Malassezia furfur* Infection in Human Keratinocytes

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The lipophilic fungus *Malassezia furfur* is a commensal microbe associated with several chronic diseases such as pityriasis versicolor, folliculitis, and seborrheic dermatitis. Because *M. furfur*-related diseases are difficult to treat and require prolonged use of medications, the treatment for *M. furfur*-related skin diseases is supposed to gain control over *M. furfur* growth and the inflammation associated with it, as well as to prevent secondary infections. In this study, we investigated the antifungal and anti-inflammatory effects of cecropin A(1-8)-magainin 2 (1-12) hybrid peptide analog P5 on *M. furfur*. The minimal inhibitory concentration of P5 against *M. furfur* was 0.39 μM, making it 3-4 times more potent than commonly used antifungal agents such as ketoconazole (1.5 μM) or itraconazole (1.14 μM). P5 efficiently inhibited the expression of IL-8 and Toll-like receptor 2 in *M. furfur*-infected human keratinocytes without eukaryotic cytotoxicity at its fungicidal concentration. P5 significantly downregulated NF-κB activation and intracellular calcium fluctuation, which are closely related with enhanced responses of keratinocyte inflammation induced by *M. furfur* infection.

D014

Characterization of Adenylate Kinase in *S. pneumoniae*

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Streptococcus pneumoniae (pneumococcus) infection claims 1.6 million deaths per year worldwide. Adenylate kinases (AdKs) constitute a major family of enzymes to regulate cellular ATP level; thus it plays a role in energy homeostasis of bacteria. However, it remains poorly understood about AdK characterization, localization and its function in pneumococcal diseases. Here we show that AdK from *S. pneumoniae* (SpAdK) is highly conserved among various strains and could generate bacterial intracellular ATP. The functional position of SpAdK was determined using point-mutations and adenylate kinase assay. Furthermore, essential role of adenylate kinase in pneumococcal normal growth was identified. On the other hand, SpAdK contains a conserved cell-wall anchored LPXTG motif and trans-locates to localize on pneumococcal cell-wall and extracellular environment. Taken together, our study revealed a functional activity of *S. pneumoniae* adenylate kinase in ATP generation and bacterial growth, and characterize its localization in the bacteria.

D015

Upregulation of ATF3 Stimulates Production of Cytokine during *Streptococcus pneumoniae* Infection

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Gram-negative and Gram-positive bacterial infections elicit significantly different host immune responses. However, the underlying mechanisms are not yet fully explored. ATF3 is a key repressor of cytokine expression observed in Gram-negative infections. This study reveals that ATF3 regulates innate immunity positively upon pneumococcus infection by enhancing TNF-α, IL-1β, and IFN-γ expression and modulating bacterial clearance. Therefore, ATF3 may represent a key differentiating factor between host immune responses to Gram-negative and Gram-positive infections.

D016

Ssd1 Functions Downstream of Cbk1 to Regulate Hyphal Morphogenesis in *Candida albicans*

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The NDR kinase Cbk1 regulates morphogenesis and polarized cell growth in yeast. However, it is poorly understood how Cbk1 regulates hyphal morphogenesis in *Candida albicans*. Recently, the mRNA binding protein Ssd1 was found to be a substrate of Cbk1 in *Saccharomyces cerevisiae*. In this study, we investigated the roles of Ssd1 in the hyphal growth of *C. albicans*. We found that *C. albicans* Ssd1 (*Ca*Ssd1) has a consensus motif for phosphorylation by Cbk1, and deletion of *CaSSD1* partially recovered the hyphal growth of *Cacbk1*Δ/*Cacbk1*Δ, which suggests that *Ca*Cbk1 negatively regulates the activity of *Ca*Ssd1 during hyphal growth in *C. albicans*. Furthermore, *in vitro* host cell invasion assay revealed that deletion of *CaSSD1* also recovered the ability of *Cacbk1*Δ/*Cacbk1*Δ to invade host cells. Taken together, this study reveals that the proper regulation of *Ca*Ssd1 by *Ca*Cbk1 is prerequisite for the normal cell morphogenesis and host cell invasion of *C. albicans*.
[2013R1A2A2A01014664]

D017

Evaluation of Transcriptional Responses of Apoptois Pathway Related Genes in Rock Bream (*Oplegnathus fasciatus*) Infected with *Megalocytivirus* (Family Iridoviridae)

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Rock bream iridovirus (RBIV), which is a member of the *Megalocytivirus* genus, causes severe mass mortalities in rock beam (*Oplegnathus fasciatus*) in Korea. In this study, we assessed apoptosis-related gene expression patterns in RBIV infected rock bream in high and low mortality conditions. In the both groups, significantly high levels of perforin, granzyme, Fas ligand and caspase 9 expression were observed in the kidney at several sampling points until 30 days post infection (dpi). Basal expression levels of Fas and caspases 8, 9 and 3 were observed accompanied by heavy viral loads. Inhibitor of apoptosis 1 (IAP1) significantly higher IAP1 expression was observed at 10 d (2.2-fold), 20 d (3.6-fold) and 22 dpi (2.0-fold) in low mortality group. In summary, perforin- and granzyme-related apoptosis initiation signals were activated; however, the Fas-induced apoptosis pathway did not efficiently respond. Up-regulated IAP1 in RBIV infected rock bream, which exhibited inhibited apoptotic responses in RBIV infected fish.

D018

Postantibiotic Effects and Postantibiotic Sub-MIC Effects of Chlorhexidine on *Streptococcus gordonii*

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Chlorhexidine is one of the most widely used biocides in antiseptic products. Postantibiotic effect (PAE) is difined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic. In this study, PAE, postantibiotic sub-MIC (PASME) and sub MIC effects (SME) of chlorhexidine on *Streptococcus gordonii* was investigated. The PAE was induced by 10x MIC of chlorhexidine for 5 min and chlorhexidine was eliminated by washing. The PASME were studied by addition of 0.1, 0.2 and 0.3x MICs during the postantibiotic phase of the bacteria, and the SME was studied by exposing bacteria to chlorhexidine at the sub MIC only. The mean PAE was 0.6 h, and the mean PASMEs were 0.7 h (0.1x MIC), 1.5 h (0.2x MIC), 2.7 h (0.3x MIC), and the mean SMEs were 0.1 h (0.1x MIC), 0.4 h (0.2x MIC), 0.9 h (0.3x MIC). The present study illustrates the existence of PAE, PA-SME and SME for chlorhexidine against *S. gordonii*, thereby extending the pharmacodynamics advatages of chlorhexidine.
[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 (2010-0025532).]

D019

ilvC* Mutant Is Potential Candidate Virulence Factor of *Streptococcus pneumoniae

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Streptococcus pneumoniae is responsible for significant morbidity and mortality worldwide. It causes a variety of life-threatening infections such as pneumonia. Preliminary microarray data had shown 7 fold increases in expression level of *ilvC* pneumococcal gene during wild type pneumococcal strain D39 infection to A549 cells. *ilvC* is keto-acid reductoisomerase that involved in biosynthesis of Branched Chain Amino Acid (BCAA) which is consisting of isoleucine, leucine, and valine. This work aimed to construct a deletion mutant and determine its growth rate, cytotoxicity, and *in vivo* survival rate after intranasal infection. The growth curve of the *ilvC* mutant showed comparable growth rate of D39, however, during stationary phase, it showed decreased level of growth especially in the presence of serum. Mice infected with the *ilvC* mutant showed higher survival rate than that of the wild type. The biosynthesis of BCAA was frequently identified in studies of pathogenesis. Taken together these results suggest that, *ilvC* associated with BCAA generation could play an important role in curbing pathogens of the respiratory tract and might be a potent candidate virulence factor.

D020

Macrophages Play a Key Role in the Protection by *Streptococcus pneumoniae pep27* Mutant Vaccine

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Streptococcus pneumoniae is responsible for high mortality worldwide. Because of several problems of current pneumococcal vaccines, like serotype shifts, a new type of vaccine is needed. In previous study, *i.n.* immunization of the *pep27* mutant showed protection from heterologous lethal challenge. To elucidate the underlying mechanism, humoral and cellular responses in immunized and control groups were compared. Although the level of IgG in the immunized group was increased, there was no passive-immunity. Moreover, when CD4+ and CD8+ T cells of immunized mice were depleted, followed by lethal challenge, the mice did not show mortality. However, BALF from the immunized mice showed higher level of IFN-γ when exposed to D39. Phagocytic activities of BM-derived macrophages from the immunized mice were increased when exposed to D39 *in vitro* as well. In addition, FACS showed spleen-derived monocytes from the immunized mice differentiated much rapidly into macrophages than those from the control. Overall, these results suggested that IFN-γ-activated macrophages, but not T and B cells, could be important for the protection from lethal infection after i.n. immunization with the *pep27* mutant.

D021

Extended Longevity and Robust Early-stage Development of *Caenorhabditis elegans* by a Soil Microbe, *Lysinibacillus sphaericus*

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Caenorhabditis elegans, originally isolated from soil, is a nematode used in host-microbe interaction research. While human pathogenic bacteria have been actively studied in *C. elegans*, no bacterial species that provides beneficial effects on *C. elegans* has been reported. Here, we tested several bacterial soil isolates and further characterized the effects of *Lysinibacillus sphaericus* on *C. elegans* growth-related phenotypes. Worms fed with *L. sphaericus* lived significantly longer than those growing with *E. coli* OP50. Juvenile-stage growth was also highly stimulated by *L. sphaericus*. In addition, significantly elevated fertilization was observed in worms fed with *L. sphaericus*. Furthermore, growth with *L. sphaericus* resulted in the production of larger numbers of progeny than the growth with OP50. Worms grown with *L. sphaericus* were highly resistant to oxidative and osmotic stress. Microarray analysis demonstrated that genes encoding cuticle collagen were highly upregulated in *L. sphaericus*-fed worms, supporting our findings with regard to enhanced resistance and rapid development. Together, our results reveal a novel mode of growth that involves healthy aging of nematodes.

D022

Identification of Non-Streptococcal Organisms from Human Dental Plaque Grown on the *Streptococcus*-Selective Medium Mitis Salivarius Agar

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Mitis-salivarius (MS) agar has been used in microbial epidemiological studies because oral streptococci can be selectively grown on this medium. In this study, we identified non-streptococcal organisms grown on MS agar plates by polymerase chain reaction (PCR) amplification and sequencing of the 16S ribosomal RNA (rRNA) gene. Eighty bacterial colonies on MS plates were isolated from plaque samples, and bacterial identification was achieved with the rapid API-20 Strep system and mini API reader. The bacterial colonies identified as non-streptococci by the API system were selected for further identification. The 16S rRNA gene was amplified by PCR and verified using DNA sequencing analysis for identification. Among the 11 isolated non-streptococcal strains, 7 strains were identified as *Actinomyces naeslundii* and 4 strains were identified as *Actinomyces oris* using Blastn. In this study, we showed that some oral *Actinomyces* species can grow on *Streptococcus*-selective MS agar plates.
[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 (2010-0025532).]

D023

Comparison of Identification Methods for α-Hemolytic Streptococci by 16S rRNA Gene Sequencing and Rapid ID 32 Strep

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Recently, it has been reported that agreement of identification of α-hemolytic streptococci by API 20 Strep with identification by 16S rRNA gene sequencing was only 26% by species and 63% by streptococcal group. Rapid ID 32 Strep system is a more recent system and allows faster identification. The aim of this study was to evaluate the reliability of Rapid ID 32 Strep for isolates of α-hemolytic streptococci from human dental plaque to the species and group levels. Rapid ID 32 Strep test was carried out according to the manufacturers' instructions. The 16S rRNA gene was amplified and 16S rRNA gene sequences were subjected to BLAST analysis. The Rapid ID 32 Strep correctly identified 80% by species and 87% by streptococcal group. This led to better success with identification at the group level than at the species level. Rapid ID 32 Strep was failing to correctly identify any of the three *S. cristatus*, one *S. australis* and one *S. tigurinus* isolates.
[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 (2010-0025532)]

D024

Effect of Sub-Minimal Inhibitory Concentration Antibiotics on Morphology of Oral Gram Positive Bacteria

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Sub-minimal inhibitory concentration (MIC) of antibiotics have been reported to affect bacterial morphology. We examined the morphological change of oral gram positive bacteria after treatment with sub-MIC antibiotic. *Actinomyces naeslundii, Actinomyces odontolyticus, Streptococcus gordonii, Streptococcus mutans* and *Lactobacillus acidophilus* were used in this study. *A. naeslundii* was observed change length with penicillin and amoxicillin. *A. odontolyticus* showed decreased length with doxycycline and tetracycline. *S. gordonii* was observed increased length with Penicillin and amoxicillin. *S. mutans* was observed increased length with penicillin and amoxicillin but observed increased chain of bacteria with doxycycline and tetracycline. *L. acidophilus* was observed decreased length and thick after incubation with amoxicillin and penicillin whereas, observed decreased length and thin with doxycycline and tetracycline.
[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 (2010-0025532).]

D025

Effect of Sub-Minimal Inhibitory Concentration Antibiotics on Morphology of Oral Periodontal Pathogens

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Minimal inhibitory concentration (MIC) is the lowest concentration of an antibiotic that inhibits the visible growth of a microorganism. Sub-MIC of antibiotics may result in morphological alterations, biochemical and physiological changes in bacteria. We examined morphological changes of oral periodontal pathogens after treatment with sub-MIC antibiotics. *Fusobacterium nucleatum*, *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans* were used in this study. The MIC for amoxicillin, penicillin, doxycycline, tetracycline and metronidazole were determined by broth dilution method. The length of *P. gingivalis* and *A. actinomycetemcomitans* were increased after incubation with penicillin, amoxicillin and metronidazole. *F. nucleatum* showed increased length after incubation with all of sub-MIC antibiotics used in this study. In this study, we observed that sub-MIC antibiotics can affect the morphology of oral periodontal pathogens.
[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 (2010-0025532).]

D026

Complete Genome Sequence of Viral Hemorrhagic Septicemia Virus (VHSV) Isolated from an Olive Flounder in Korea

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Viral hemorrhagic septicemia virus (VHSV) is a seriously problematic pathogen in olive flounder (*Paralichthy olivaceus*) aquaculture farms in Korea. The entire genome size of VHSV isolate FYeosu05 was 11,168 bp including all coding regions and intergenic sequences. The genome consisted of 6 ORFs arranged in the order 3'-N-P-M-G-NV-L-5', which is the same order found in other fish rhabdovirus. It showed over 96 % of identity with Genogroup I (99 % with JF00Ehil and KJ2008; 97 % with KRRV9822; 96 % with MI03GL) and 86 % with all other Genogroup strains. Among all the VHSV proteins, RNA Polymerase (L) protein was the highest conserved protein (over 96 % identities) while non-virion (NV) protein was the most divergent protein that showed 72 – 100 % identities. It shows a putative polyadenylation motif (AGAT(T/A) GAAAAAAA), which signals to generate poly (A) tail to the 3' end of mRNA and is followed by -GGCAC- nucleotide which is a putative transcription start signal. The 5' and 3' untranslated regions (UTR) are 54 and 101 nucleotides, respectively. This genome sequence will be useful for virus diagnostic and comparative analysis with other genotype virus.

D027

Genetic Positioning of Aquabirnavirus Isolates from Cultured Japanese Eel *Anguilla japonica* in Korea

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Aquabirnavirus is an epidemic virus in Japanese eel *Anguilla japonica* farms in Korea, although its origin is unclear. In the present study, nucleotide sequences of the VP2/NS junction region of nine Korean aquabirnaviruses from cultured eel in various areas of Korea during 2000-2009 were analyzed to evaluate their genetic relatedness to worldwide isolates. The nucleotide sequences showed more than 94.2% identity among the nine Korean eel isolates, 71.2% identity among 16 Korean isolates from freshwater and marine fish, and 71.1% identity among 25 worldwide isolates. All nine isolates in this study were phylogenetically classified into genogroup II including isolates from Denmark, Spain, Taiwan and Japan, and were discrete from salmonid and marine fish isolates (genogroup I and VII) in Korea. These results suggest that the Korean eel isolates have most likely been introduced from outside the country and not from coastal areas of Korea.

D028

Conformation of CRISPR-associated Csn2 DNA-binding Ring Is Regulated by Milimolar Concentration of Ca^{2+} Ion

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CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) and *cas* (CRISPR-associated) genes form a microbial RNA-based immune defense against the invading nucleic acids. We previously reported the crystal structure of the CRISPR-associated Csn2 protein, an essential protein for new spacer acquisition in Type II-A CRISPR system, and showed that Csn2 assembles into a tetrameric ring structure to slide ds-DNA nonspecifically in a Ca^{2+}-dependent manner. Here we show that of the two sets of Ca^{2+} binding sites in the *E. faeclias* Csn2 tetramer, the four Ca1 sites are occupied at nanomolar Ca^{2+} concentration, whereas the four Ca2 sites are occupied at ~1.5 mM range. We use crystal structures and small angle X-ray scatting methods to show that dissociation of Ca^{2+} from Ca2 sites alters the Csn2 ring conformation, rearranges the DNA-binding surface, and narrows the inner diameter of the ring, resulting in the loss of ds-DNA-binding activity. The influx of extracellular Ca^{2+} in turn switches on the DNA-binding function of Csn2 to assist the acquisition of new spacers into the CRISPR locus.

D029

Reversion of Mucoid *Pseudomonas aeruginosa* to Nonmucoid Form by Sulfate Ion

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Alginate overproducing mucoid *Pseudomonas aeruginosa*, responsible for chronic airway infections in cystic fibrosis (CF) patients, is resistant to antibiotics and immune clearance. Performing a Phenotype Microarray, sulfate was identified as a alginate suppressing molecule. When a mucoid strain CM21 and additional mucoid isolates were grown with 5 % sodium sulfate, decreased levels of alginate were produced. Alginate suppression was also induced by other sulfate salts. Furthermore, bacterial cell shape was altered in CM21, but not in PAO1, a wild type strain suggesting that sulfate-stimulated cell shape change is associated with suppression of the alginate operon. Finally, a CM21 *lpxC* mutant continued to produce alginate and maintained rod shape when grown with sulfate. These results suggest a potential involvement of LPS biosynthesis in the sulfate-induced reversion to nonmucoid phenotype. Together, this study proposes a novel strategy that can be potentially applied to treat infections of mucoid P. aeruginosa.
[Supported by grant from NRF, MEST (No. 2011-0016210) and Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, A110096]

D030

Effect of Low Water Temperature on Immune Response of Olive Flounder (*Paralichthys olivaceus*) Vaccine Containing Adjuvants, against Viral Haemorrhagic Septicaemia (VHS)

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Water temperature affects teleost fish (poikilotherms) immunity. It is difficult to develop specific immunity at low water temperature. In vaccine studies, it is important to know the optimum water temperature range that fish obtain protective immunity. We investigated the suitable water temperature that obtain protective immunity in VHS infected olive flounder using squalene (5%) and aluminum hydroxide (0.5%) containing inactivated VHS vaccine. Vaccinated fish reared at 10, 13, 15 and 20℃ and challenged with VHS virus (VHSV) ($10^{7.8}$TCID$_{50}$/fish) at 10, 20, 30 and 40 days post vaccination (dpv) at 15℃ and calculated the relative percent survival (RPS). Similar to 20℃, low water temperatures (10, 13 and 15℃) showed protective immunity. RPS for fish group vaccinated at 15℃ was 58, 93, 87 and 93% (10, 20, 30 and 40 dpv, respectively) and obtained high protection. Comparatively high protection (42/50, 64/79, 53/53 and 80%/87%) observed at 10/13℃ groups showing protective immunity. Results indicate that vaccinated fish can induce protection against VHS in various water temperatures (even low water temperature at 10-15℃), and can provide protection to control disease outbreaks.

D031

Inhibition Ability of *Solanum nigrum* L Extract for Viral Hemorrhagic Septicemia Virus (VHSV) Replication in Fathead Minnow (FHM) Cell Line

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Viral hemorrhagic septicemia (VHS) causes severe mass mortalities in aquaculture industry between 8-15℃. We assessed inhibition ability of *Solanum nigrum L* extract against VHSV replication in FHM cell line. FHM cell line incubated with media containing VHSV ($10^{2.8}$TCID$_{50}$/ml) at 15℃ for 6 h. At post incubation, 0.5, 1, and 2 μg/ml of the extract were treated to each well. Inhibition ability was calculated at 1d and 2d as follows; Inhibition rate (%) = {(pfu/ml in positive control – pfu/ml in treatment) ÷ pfu/ml in positive control} × 100. In the experiment, inhibition rate at 1 d and 2 d of the samples treated with *S. nigrum L* extract at 0.5, 1, and 2 μg/ml were 90.9/62.6%, 39.4/33.6% and 27.2/28.9%, respectively. Repeated experiment showed similar results and inhibition rates were 78.5/68.7%, 35.7/27.0% and 14.2/27.0%, respectively for the respective time points. The data indicates that 0.5 μg/ml could highly inhibit viral replication. Although, effect of the extract for virus replication in fish remains unclear, data clearly demonstrated the inhibition of VHSV replication by *S. nigrum* L extract *in vitro* and possibility in developing preventive measure against VHSV using the extract.

D032

LuxR Type Regulator AbaR is Essential for *Acinetobacter baumannii* Biofilm Formation and Motility

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Quorum sensing is a cell to cell communication system that coordinates gene expression in many bacterial species. *Acinetobacter baumannii* utilizes N-(3-hydroxydodecanoyl)-l-homoserine lactone (OH-dDHL), and a putative LuxR type receptor, AbaR, for quorum sensing. In the present study, functions of AbaR were assessed by the construction of an isogenic mutant and by evaluating its phenotype change in biofilm formation and motility. The disruption of *abaR* resulted in a significant decrease in biofilm formation. Especially, the *abaR* mutant was unable to form a biofilm at the air-liquid interface. Introduction of *abaR* in trans complemented the defects. Moreover, the diameter of the swimming area of the *abaR* mutant was substantially decreased compared to that of the wild type. Complementation of the *abaR* mutant by introduction of recombinant *abaR* recovered the reduced motility. These results indicated that AbaR plays important roles in *A. baumannii* biofilm formation and motility.

D033

Vibrio anguillarum Infection in Rainbow Trout (*Oncorhynchus mykiss*) during Seawater Adaption

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We examined the cause of a disease outbreak in rainbow trout (*Oncorhynchus mykiss*), which were adapting to a seawater in an aquaculture farm in Jeju on April, 2013. Most of the diseased fish showed an severe ulcer on the skin. Although no parasites, fungi or viruses were isolated from diseased fish, over 200 same type of bacterial colonies were isolated from spleen, kidney and liver. Nucleotide sequences of the 16S rDNA gene of the bacterium in our study showed 100% identity with *Vibrio anguillarum*. This study is the first report of rainbow trout disease during sea adaption in Korea.

D034

In Vitro System to Investigate the Gastrin Expression by *Helicobacter pylori*

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Helicobacter pylori is a major contributor for gastric cancers and *H. pylori* induced hypergastrenemia was postulated to be a major risk factor for the development of gastric cancer. As gastrin hormone regulates gastric acid secretion, it is important to find out the mechanism which *H. pylori* uses to regulate acid secretion. Our purpose is to establish an *in vitro* system to examine the gastrin promoter induction by *H. pylori*. Human gastrin promoter-luciferase reporter construct was made by cloning 240 bp of gastrin promoter into pGL3 basic vector. This construct was stably transfected into AGS cells with co-transfection of pcDNA3 to select with G418. After stimulate the stably transfected AGS cells with G27 *H. pylori* WT and its isogenic mutants, luciferase activity was measured. AGS cells stably transfected with Gastrin-Luciferase construct showed higher luciferase activity with wild type and CagA deficient *H. pylori* while *H. pylori* lacking entire *cag*PAI showed reduced promoter induction. Several stably transfected AGS cell clones were examined to exclude the clone specific effect. This system can be used to examine the mechanism of gastrin expression by *H. pylori*.

D035

Comparison of Immune Response Elicited by Gamma Irradiated and Chemically Killed Pneumococcal Whole Cell Vaccine

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Streptococcus pneumonia is one of the leading causes of death in humans. In spite of the high degree of effectiveness of pneumococcal conjugate vaccine, recent research have reported an increase in the rate of disease caused by non-vaccine serotypes. Pneumococcal whole cell vaccine (WCV) is expected to confer protection against a wide variety of serotypes. Thus, we investigated the efficacy of irradiated WCV as compared to chemical WCV. Pneumococcal WCV were prepared by treat gamma-irradiation or formalin and immunized intranasally to CD1 mice. Although irradiated WCV increased anti-streptococcal IgM in serum similar level to chemical WCV, it elicited significantly higher level of anti-streptococcal IgG in serum and IgA in BAL fluid than chemical WCV. Moreover, when vaccinated mice were inoculated intraperitoneal with a lethal dose of *S. pneumonia* TIGR4, 60% mice were survived after 48 hours by irradiated WCV vacination, but all mice were dead in the group of PBS or chemical WCV vaccination. These findings suggest that irradiated WCV elicit effective IgG antibody that protects pneumococcal infection and may be valuable to develop an ideal serotype independent vaccine.

D036

A Potential Trade-Off between Multi-Drug Resistance Phenotype and ROS Stress Response in *Pseudomonas aeruginosa*

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Bactericidal antibiotics were known to utilize different mechanisms to kill bacteria until 2007. The newly suggested idea was that different classes of antibiotics universally induce formation of hydroxyl radicals via Fenton reaction, damaging DNA, lipids and proteins, which results in cell death. While the idea is still controversial, it could be a possible explanation why bacteria rapidly become Multi-Drug Resistance (MDR) strains once starting to gain resistance. To see if each MDR *P. aeruginosa* strains have distinct ROS stress response abilities, initial screening of 8 clinical samples was performed. From the screening, 75% of samples showed varying levels of decreased ROS stress responses compare to that in PAO1. To increase the validity in our study, clinical sample size was raised to 38, including samples from the initial screening. Not all samples showed dampened ROS stress responses. Also, the level of ROS stress responses varied among the samples. While the results suggest some possibility of correlation between MDR phenotype and ROS stress responses, further investigation is required to firmly conclude.
[Supported by BK21 Plus project for Medical Science.]

D037

Nuclear Targeting of Urease Subunit A of *Helicobacter pylori* Induces Hummingbird Phenotype in Human Gastric Epithelial Cells

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Urease subunit A (UreA) of *Helicobacter pylori* targets in the nuclei of COS-7 cells through nuclear localization signals. This study was investigated whether UreA of *H. pylori* targeted in the nuclei of gastric epithelial cells and subsequently induced host cell pathology. Immunohistochemical analysis showed that UreA was detected in gastric epithelial cells of *H. pylori*-positive specimen. *H. pylori* secreted outer membrane vesicles (OMVs) and UreA was translocated into AGS cells treated with OMVs. Similar to nuclear targeting of GFP-tagged UreA in COS-7 cells, GFP-tagged UreA, rrUreA, and UreA in the OMVs could target in the nuclei of AGS cells. Nuclear targeting of rUreA did not induce cell death, but resulted in morphological changes of AGS cells, such as cellular spreading and elongation, so called hummingbird phenotype. Nevertheless, AGS cells treated with rUreAΔNLS proteins did not induce hummingbird phenotype. Nuclear targeting of UreA differentially regulated 102 morphogenesis-related genes. In conclusion, nuclear targeting of *H. pylori* UreA induces a morphological change and regulates morphogenesis-related genes in gastric epithelial cells.

D038

Loop-mediated Isothermal Amplification of *vanA* Gene Leads to Rapid and Naked-eye Detection of Vancomycin-Resistant Enterococcus Infection

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Vancomycin-resistant enterococcus (VRE) is one of the leading causes of nosocomial infection at intensive care unit(ICU). We studied a distinctive DNA amplification assay, Loop mediated isothermal amplification (LAMP) as rapid diagnosis tool for VRE. LAMP is a progressive diagnosis method notable for its technological simplicity, speed and the ease of detection. The use of Bst DNA polymerase allows the target gene amplification at a constant temperature. LAMP products can be confirmed with the turbidity of magnesium pyrophosphate, by-product of LAMP. We targeted the major and transposable vancomycin-resistant gene, vanA for the LAMP assay. VRE vanA-LAMP drew a great result on 62°C for 1 hr. At minimum detectable DNA concentration and reaction time test, VRE vanA-LAMP could detect 80pg and run even in 40min. The sensitivity and specificity of VRE vanA-LAMP were verified by testing 39 clinical specimens and comparing the data with phenotype test and PCR assay. We expect rapid diagnosis to prescribe suitable antibiotics for VRE diagnosis with LAMP.
[Supported by BK21 PLUS project for medical science]

D039

The Effect of Altered Gut Microbiota Composition on Defense against *Vibrio cholerae* Infection in Adult Mice

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Many attempts have been made to establish intestinal infection models using adult mouse, no clear explanation has been proposed why intestinal infection by *Vibrio cholerae* does not occur with human-like symptoms. The role of gut microbiota in successful defense against enteric infections has been long considered. In this study, we hypothesized that mice with altered gut microbiota population by various antibiotics may exhibit differential capabilities to respond to intestinal infections by *V. cholera*. A broad spectrum antibiotics, streptomycin and ampicillin, or narrow spectrum antibiotics, vancomycin and clindamycin, were orally administrated to each Balb/c mice to induce changes in gut microbiota population. The murine models were infected with *V. cholerae* N16961. The fecal samples of mice were collected before infection and the mice were sacrificed after 24hours later. Quantitative real-time PCR and gut microbiota community analyses were performed to provide a comprehensive view of how gut microbe ecosystem affects against the *V. cholerae* infection. Our study will elucidate a previously undescribed role of commensal gut microbes in defense against enteric infections.

D040

Microbiological Evaluation of Broiler Chickens from Slaughterhouses in South Korea

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The objective of present study was to evaluate the microbiological quality of broiler meat from slaughterhouses in South Korea. Between February 2014 and March 2014, a total of 120 whole chickens were collected from six slaughterhouses (20 samples each) located in different provinces. Chicken carcasses were rinsed with buffered peptone water with gentle shaking for 1 min to ensure even distribution. The rinsate solution (1 ml) and 10-fold serial dilutions were inoculated onto AC and EC petrifilm for the enumeration of total aerobic bacteria and *E. coli*, respectively. The identity of *Salmonella* colonies was biochemically confirmed by Vitek 2 assay. Enumeration of aerobic bacteria and *E. coli* from the 120 chicken carcasses showed growth ranging from 2.11 log CFU/ml to 4.56 log CFU/ml and 1.00 log CFU/ml to 3.62 log CFU/ml, respectively. A total of 11 *Salmonella*-positive samples from 120 carcass rinses were identified. The results of this nation-wide survey provide useful information of the microbiological safety of broiler chickens consumed in South Korea.
[Supported by Golden Seed Project, MAFRA]

D041

Inducement of Transient Mutant of *Acanthamoeba* Mannose-Binding Protein

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Acanthamoeba is a protozoan pathogen that can cause a blinding keratitis and a fatal granulomatous encephalitis, however the pathogenic mechanisms of these organisms remain incompletely understood. To obtain *Acanthamoeba* lacking MBP, carbohydrate selection method was employed. Briefly, *Acanthamoeba* trophozoites (2×10^7 amoebae) were treated with 10 ml of methyl-α-D-mannopyranoside (α-Man; 100 mM final conc.) and flasks incubated for an additional 24 h. *Acanthamoeba* were treated with mannose for 20 cycles. For the phagocytic analysis, following the co-incubation with amoebae and bacteria, the supernatants were removed and gentamicin was added to kill any remaining extracellular bacteria. Interestingly, MBP mutant exhibited significantly less phagocytic ability compared with wild type amoebae. And MBP mutant exhibited significantly decreased extracellular proteolytic activities compared with the wild type.

D042

Evaluation of Commercialized Contact Lens-Cleaner to Protozoan Infections *In Vitro*

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Acanthamoeba castellanii is a protozoan pathogen that can frequently cause a blinding keratitis in contact-lens users. The keratitis is accompanied with bacterial and fungal infections, and thus it is very difficult to treat the amoebial keratitis. Even broad spectrum of antibiotics may be required. Contact lens-users are randomly applying contact-lens cleaners by only their preference. This study is to prove amoebicidal effects of lens-cleaners and evaluate product values *in vitro*. At first, traditional counting method was better to analyze their cytotoxicity because lens-cleaners could prevent precise data from spectrophotometer assay using lactate dehydrogenase assay, as a biochemical assay. When the lens-clearing solutions were treated to the amoeba, the amoebae were changed into round form of no lightness under microscope. In particular, *A. castellanii* treated with lens-clearing solution of "Biotrue" at 0 h and 24 h were changed into cysts. Approximate 50% cytotoxicity of the lens-clearing solutions was also proved.

D043

Clinical Features and Prevalence of Adenovirus Infections in the Pediatric Patients with Respiratory Infections

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Adenovirus (ADV) cause acute respiratory tract infections and are often associated with increased fates of hospitalization and death, particularly in infants and young children. The aim of this study was to analyze the clinical features and molecular phylogeny of ADV isolated in Busan, from January 2010 to November 2013. Total of 3,230 specimens (throat swabs) were collected from influenza-like illness patients and patients with acute respiratory tract. Multiplex real-time RT-PCR (rRT-PCR) was performed to detect eight respiratory virus [rhinovirus, adenovirus, respiratory syncytial virus, human coronavirus, human metapneumovirus, human bocavirus, parainfluenza virus and influenza virus] and detected 1,485 (46.0%) cases. Among 1,485 positive specimens, 257 (8.0%) cases were ADV. Serotypic distributions of isolated ADV was analyzed by sequencing of hexon gene. ADV was identified seven different serotypes (1~6, 8), revealing a high similarity among the isolates (>97%). The predominant types were type 1 in 2011, type 3 and 4 in 2012, type 3 in 2013, respectively. ADV type 3 was major causative type during outbreaks in 2013.
[Supported by grants from KNIH]

D044

Detection, Purification and Characterization of Diketopiperazines (DKPs) a Quorum-Sensing Signal Molecule from *Escherichia coli* SE15 Isolated from Indwelling Catheter

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Quorum-sensing (QS) is a process of bacterial cell-to-cell communication involving the signaling molecules called autoinducers. Bacteria can utilize QS systems to monitor their own population density via exchange of signal molecules and then activate the expression of virulence genes after reaching a threshold value of concentration. There are several different types of signal cues, which include Acyl-homoserine lactones (AHLs), autoinducing oligopeptides (AIPs), cyclic dipeptides, such as 2,5-diketopiperazines (DKPs), cholera autoinducer-1 (CAI-1), furanosyl diesters (AI-2) and diffusible signaling factors (DSFs).
In this study, we performed the purification and identification of family of diketopiperazines (DKPs) from cell-free supernatants of *E. coli* SE15 isolated from indwelling catheter. Based on the analysis of TLC and GC/MS, we confirmed one of the DKPs (cyclo-(Leu-Pro) in supernatant of *E. coli* SE15. Our results showed that *E. coli* SE15 could produce cyclo-(Leu-Pro) as QS signal molecule. Furthermore, are analysis how this molecule modulate AHL-mediated quorum sensing in *E. coli* SE15 and also with other quorum sensing bacteria.

D045

Evaluation of Protective Immunity and Immunogenicity of a Killed Whole-cell Cholera Vaccine Containing Cholera Toxin B Subunit in a Murine Pneumonia Model

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Although oral vaccines against cholera have been licensed, the assessment of protective immunity has been hindered due to the lack of appropriate animal models. In this study, we demonstrated a murine pneumonia model induced by intranasal administration with *Vibrio cholerae*. Bacterial components of *V. cholerae*, but not cholera toxin, induced lethal and acute pneumonia with massive inflammation. Intranasal immunization with Dukoral™, a commercial cholera vaccine comprised of killed whole bacteria and recombinant cholera B subunit (rCTB), increased both mucosal and systemic antibody responses as well as protection against the infection. Although rCTB-free Dukoral and rCTB alone partially protected against the infection, reconstitution of rCTB-free Dukoral with rCTB restored full protection. Parenteral immunization with Dukoral™ provoked strong systemic antibody responses, but not mucosal antibody responses as well as protection against the infection. Taken together, anti-bacterial and anti-toxic immunities are required for the protection against *V. cholerae*-induced pneumonia and this murine pulmonary model is useful for pre-clinical assessment of candidate cholera vaccines.

D046

Anthranilate and Indole have Influence on Enhancement of Biofilm Formation in *P. aeruginosa*

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Indole has been reported to enhance the biofilm formation of *P. aeruginosa*. The enhancement of biofilm formation by indole was QS-independent and none of QS regulators were activated by indole. Instead, a QS-related regulator, AntR was significantly activated by indole. We investigated the anthranilate effect on the biofilm formation of *P. aeruginosa*. Interestingly, anthranilate enhanced the biofilm formation at early stage of biofilm development by augmenting the initial attachment of cells, but it destabilized the biofilm structure at later stage, like flat biofilm. Distinctively, indole structured robust biofilm and accelerated the biofilm development, advancing the dispersion. Co-treatment of anthranilate and indole activated AntR additively and also enhanced the biofilm formation additively at early stage. But at later stage, the biofilm enhancement by indole was dampened by anthranilate effect. The anthranilate effect on the biofilm formation was QS-independent, since the QS mutant still showed the enhanced attachment at early stage and flattening the biofilm structure at later stage in the presence of anthranilate.

D047

Development of a Reverse Transcription-PCR Assay for Specific Detection of Maedi-Visna Virus

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Although many kinds of sheep are sensitive to Maedi-Visna Virus (MVV), there is no official case of infection so far in South Korea. Recently a sheep-breeding rises rapidly as sheep receive attention as a health supplement food. Although the threat for MVV is increasing, there is no commercial detection kit for MVV in Korea. Therefor it is necessary to develop a specific detection kit for MVV. MVV is a small ruminant lentivirus belonging to the Retroviridae family. MVV affects the lungs, the central nervous system and other organs and is usually transmitted via pulmonary aerosols colostrum or semen. We developed a reverse transcription-PCR assay to detect MVV. Spevifivity, Sensitivity, limit of detection (LOD), and robustness of the method were validated according to European Directorate for the Quality of Medicine (EDQM) and International Conference of Harmonisation (ICH) guidelines. The established conventional PCR assay was validated to be very specific to MVV, reproducible, and robust.

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D048

Development of a Reverse Transcription-PCR Assay for Specific Detection of Parainfluenza Virus Type 5

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Parainfluenza virus 5 (PIV5) is an important cause of acute respiratory infection and predominantly affect infants and young children. PIV5 have been associated with a variety of upper and lower respiratory syndromes including rhinitis, otitis, laryngotracheobronchitis or croup, bronchiolitis, and pnemonia as well as asymptomatic infections. Therefore rapid and accurate diagnostic method with a high degree of specificity is very important. PIV type 5 is a member of the Paramyxoviridae and have a single stranded negative sense RNA genome. We developed a reverse transcription-PCR method to detect PIV5. Specificity, sensitivity, limit of detection (LOD), and robustness of the method were validated according to European Directorate for the Quality of Medicine (EDQM) and International Conference of Harmonisation (ICH) guidelines. The established conventional PCR assay was validated to be very specific to PIV5, reproducible, and robust.

[This work is financially supported by the Ministry of Education through the fostering project of Capstone Design and Industry-University Fusion Laboratory]

D049

Bacterial Lipoproteins-mediated Bone Destruction through the Induction of Osteoclast Differentiation and Activation

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Bacterial components can cause inflammatory bone diseases accompanied by the bone destruction due to excess generation of osteoclasts. In this study, we investigated the role of lipoproteins in bacteria-induced bone destruction using *Staphylococcus aureus* and synthetic lipopeptides. Formaldehyde-inactivated *S. aureus* or the synthetic lipopeptides induced severe bone loss in the femurs of mice after i.p. administration and in a calvarial bone implantation model, while the lipoprotein-deficient *S. aureus* did not show such effects. Mechanism studies further identified three action mechanisms for the lipopeptide-induced osteoclast differentiation and bone resorption via (i) enhancement of osteoclast differentiation through Toll-like receptor 2 and MyD88-dependent signaling pathways, (ii) induction of pro-inflammatory cytokines, TNF-α and IL-6, and (iii) up-regulation of RANKL expression with down-regulation of osteoprotegerin expression in osteoblasts. Taken together, these results suggest that lipoprotein might be an important bacterial component responsible for bone destruction during bacterial infections through augmentation of osteoclast differentiation and activation.

D050

Caspase-1 Dependent IL-1β Secretion in Macrophages by *Enterococcus faecalis*

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Enterococcus faecalis is a Gram-positive bacterium and causes various diseases using its virulence factors. Inflammasome is a component of innate immune system. It triggers caspase-1 activation, which induces maturation of proinflammatory cytokines such as interleukin-1 beta (IL-1β) and IL-18, and a proinflammatory cell death pyroptosis. Inflammasome can be activated by pathogen-associated molecular patterns and damage-associated molecular patterns. In this study, we investigated inflammasome activation in macrophages after infection of *E. faecalis*. We found by using immunoblotting, real-time RT-PCR and ELISA that *E. faecalis* efficiently induced both IL-1β transcription and caspase-1 activation. We performed LDH-cytotoxicity assay and PI staining to determine cell death. LDH was released from the *E. faecalis*-infected macrophages to the extracellular space. Which was inhibited by caspase-1 inhibitors. *E. faecalis*-infected macrophages dose-dependently released ATP. Based on these results, we conclude that *E. faecalis* induced inflammasome activation in THP-1 macrophages, possibly via NLRP3.

D051

Inhibitory Effect of *Lactobacillus plantarum* Lipoteichoic Acid on *Staphylococcus aureus* Biofilm Formation

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Biofilm is an aggregate of microorganisms in which cells adhere to biological or non-biological surfaces and is responsible for various infectious diseases. Although many *Lactobacillus* strains have been known to inhibit biofilm formation of pathogenic bacteria, the molecular mechanisms by which lactobacilli inhibit the formation of bacterial biofilm are not clearly understood. Here, we demonstrate that *L. plantarum* lipoteichoic acid (Lp.LTA) inhibits the biofilm formation of various pathogenic bacteria including *Staphylococcus aureus* without affecting bacterial growth. Lp.LTA inhibited expression of *ica*-operon responsible for the production of poly-N-acetylglucosamine (PNAG), which is required for the biofilm development of *S. aureus*. Furthermore, we found that D-alanine is an essential component of Lp.LTA to inhibit the biofilm formation of *S. aureus*. These findings indicate that Lp.LTA is critical molecule in *L. plantarum* to inhibit biofilm formation of pathogens.

D052

Loop-mediated Isothermal Amplification for Detecting *Neisseria meningitidis* in Cerebrospinal Fluid

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A novel nucleic acid amplification technique, loop-mediated isothermal amplification (LAMP), was studied to assess its suitability for detecting *Neisseria meningitides (N.m)* in CSF. The LAMP assay was evaluated using a set of 269 randomly selected CSF specimens from children with suspected meningitis collected between 1998 and 2002 in Vietnam, China and Korea. The primer specificity was validated using 14 *N.m* (serogroup A, B, C, D, 29-E, W-135, X, Y and Z) strains and 15 non-*N.m* species. The LAMP method proved to be more sensitive than previously described polymerase chain reaction (PCR) methods when using CSF samples. Within 60 minutes, the assay could detect 10 or more copies of purified *N.m* DNA with a sensitivity ~ 1,000 times greater than conventional PCR. In this set of tested CSF specimens, PCR showed a sensitivity of 85.7% and a specificity of 100% relative to the LAMP assay. These results suggest that LAMP is a sensitive and accurate means of diagnosing *N.m* infection in CSF. Prospective clinical-epidemiologic studies are now in development to evaluate the utility of *N. m* LAMP for the clinical diagnosis of invasive meningococcal disease in children and adults.

D053

Clinical Evaluation of a Loop-mediated Isothermal Amplification (LAMP) Assay for Detecting *Mycobacterium tuberculosis* in Sputum

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Tuberculosis (TB) is an infectious disease that is caused by *M. tuberculosis* complex and still remained one of the leading causes of death from infectious diseases in the world. Culture confirmation of *Mycobacterium tuberculosis* takes at least six weeks and other diagnostic methods often give false results, therefore, new diagnostic technique should be developed to control tuberculosis disease effectively. A sensitive and efficient loop-mediated isothermal amplification (LAMP) assay targeting *hspX* was established for diagnosis of *M. tuberculosis* more rapidly and accurately in this study. The sensitivity of TB *hspX* LAMP was 1,000 times better than that of TB *hspX* PCR. For the clinical evaluation, culture confirmation, PCR, and LAMP assay were applied to 303 sputum specimens. PCR assay targeting *hspX* showed no positive results among the sputum specimens. Therefore, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TB *hspX* LAMP were calculated 71.1%, 98.8%, 91.4% and 95.1%, respectively in comparison with TB culture as the gold standard for diagnosis of *M. tuberculosis*.

D054

CTX Phages in *Vibrio cholerae* O1

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A global population change in *Vibrio cholerae* O1 in the last century, from the classical biotype to El Tor biotype was recognized as an unusual characteristic of *V. cholerae*. Another population change is ongoing within the El Tor biotype strains. The prototype El Tor strains that produced biotype-specific cholera toxin are now being replaced by atypical El Tor variant harboring classical cholera toxin. The binding subunit of the CT (CTB) is encoded by *ctxB* and the CTBs of the two phages vary by two amino acids (39^{th} and 68^{th}) out of the full 125 amino acid protein. Atypical El Tor variants, El Tor strains producing classical cholera toxin were first recognized in 2006 and a number of atypical El Tor variants have since been reported. In this study, we analyzed full sequence of CTX phages of *V. cholerae* O1 strains, including the classical biotype strains, prototype El Tor biotype strains, Atypical El Tor variants, and US Gulf Coast Strains. The DNA sequence analysis shows that the CTX phages in the atypical El Tor variants are mosaic of pre-existing phages.

D055

Isolation and Identification of Carbapenem-resistant *Enterobacteriaceae* Isolates from Korean Carriers

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A total of 300 individual carrier samples were collected in October 2013. The isolation and identification of carbapenem-resistant *Enterobacteriaceae* (CRE) were performed by two-step process. First, the carrier samples are plated on LB agar medium in the presence of imipenem 4 mg/L, and then suspected colonies are plated on MacConkey agar selective medium in the presence of imipenem 4 mg/L for selection of gram-negative organisms. The isolated CRE were identified by 16S rRNA gene analysis. *In vitro* antimicrobial susceptibility testing was performed, and PCR assay was done for detection of metallo-β-lactamase (MBL) genes. As a result, twelve CRE isolates were identified; *Stenotrophomonas maltophilia* (4 isolates), *Morganella morganii* (2 isolates), *Halomonas hamiltonii* (2 isolates), *Proteus mirabilis* (2 isolates), *Proteus vulgaris* (1 isolate), *Enterobacter ludwigii* (1 isolate), and *Pseudomonas aeruginosa* (1 isolate). Most of CRE isolates were resistant to polymyxins, cephalosporins, and aminoglycosides. No MBL genes were detected. In this study, we identified CRE in Korean carrier samples. Diverse species of CRE isolates was notable.

D056

Molecular Epidemiological Characterization of Enterovirus 71 Isolated in Busan

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Enterovirus 71 (EV-71) is responsible for frequent large-scale outbreaks of hand, foot, and mouth disease (HFMD), herpangina, and severe neurological complications such as encephalitis, aspectic meningitis, and even death. This study was performed to investigate epidemiological characteristics and diversities of EV-71 isolated from 2011 to 2013 in Busan. A total of 2,978 specimens were collected from children and screened for the isolation of enterovirus by cell culture and realtime RT-PCR. 458 positive specimens (15.4%) were identified with enterovirus infections and 50 positive isolates out of them were EV-71. Most of EV-71 isolates were from children's specimen under 10 years old and 26 isolates (52%) were 01 years old. The positive rates of EV-71 were 1.7% and 2.0% in CSF and stool samples, respectively. Aseptic meningitis was the most common clinical manifestation (46%); herpangina (4%), and HFMD disease (4%). The 22 VP1 gene sequences of EV-71 were 98--100% homologous with that of the Human Enterovirus 71 (HEV71) isolate H11-23-KOR. The result of phylogenetic analysis of EV-71 isolates based on VP1 gene showed that most of them were classified into subgenotype C4a.

D057

Cholera Toxin Production During Anaerobic Trimethylamine N-oxide Respiration is Mediated by Stringent Response in *Vibrio cholerae*

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Production of cholera toxin (CT), a major virulence factor of *Vibrio cholerae*, is highly promoted during anaerobic growth using trimethylamine N-oxide (TMAO) as an alternative electron acceptor. Here, we investigated the molecular mechanisms of TMAO-stimulated CT production and uncovered the crucial involvement of stringent response (SR) in this process. *V. cholera* strain N16961 produced a significantly elevated level of ppGpp, the bacterial SR alarmone, during anaerobic TMAO respiration. A *ΔrelA ΔspoT* ppGpp overproducer strain produced enhanced level of CT, while anaerobic growth via TMAO respiration was severely inhibited. In contrast, a $ppGpp^0$ strain (*ΔrelA ΔspoT ΔrelV*) grew substantially better, but produced no CT. In deletion mutant of *dksA* gene, which encodes a protein that works cooperatively with ppGpp, the capability to produce CT was completely lost and SR growth inhibition was alleviated. *In vivo* virulence of $ppGpp^0$ or *ΔdksA* mutants were significantly attenuated. Together, our results reveal that SR is activated during anaerobic TMAO respiration and it regulates CT production in a growth-dependent manner in *V. cholerae*.
[This work was supported by grants from KRF.]

D058

Prevalence and Characterization of Food-borne Bacteria Isolated from Diarrhea Patients in Busan

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We investigated food-borne bacteria of ten from diarrhea patients for EnterNet surveillance project. The diarrhea stool specimens were collected from diarrhea patients in cooperation five hospitals of Busan.
We isolated 165 causing bacteria from 1,197 stool and the prevalence of isolation was 13.8%. A total 165 strains were *Staphylococcus aureus* 59 strain (35.8%), pathogenic *E. coli* 44 strains (26.7%), *Clostridium perfringens* 40 strains (24.3%), *Bacillus cereus* 14 strains (8.5%) and *Salmonella* sp. 8 strains (4.9%). *S. aureus,* pathogenic *E. coli* and *Clostridium perfringens* were showed frequently isolated diarrhea-causing bacteria during an annual. By antimicrobial susceptibility tests, 21 strains (47.7%) of pathogenic *E. coli* isolates were susceptible to all of the 17 antimicrobial agent used in this study, and 22 strains (54.5%) were resistant to two or more antimicrobial agent. All (Eight stains of *Salmonella* isolates) of *Salmonella* spp isolates were resistant to six or more agent, showing multi drug resistance and the isolates resistant to the 11 antibiotics were 1 strain.
[Supported by grants from KNIH]

D059

Isolation and Proteomic Analysis of Extracellular Membrane Vesicles of *Streptococcus pneumoniae*

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Bacteria constitutively release extracellular membrane vesicles (MVs) to communicate with the environment in natural conditions. MVs are spherical structures that contain various native bacterial components, which are delivered to the environment where they fulfill various roles. A wide range of Gram-negative bacteria secrete MVs, which are known as outer membrane vesicles (OMVs). However, the extracellular MVs from Gram-positive bacteria have been studied much less than OMVs from Gram-negative bacteria. Here, we report the isolation of extracellular MVs from Gram-positive bacteria Streptococcus pneumonia BAA-255. Proteomic analysis reveled that a total of 104 protein components are included in *S. pneumoniae* derived MVs. Among them, extracellular proteins and membrane proteins take major portion of the identified proteins, suggesting that there may be a specific sorting mechanism for vesicular proteins. In addition, *S. pneunoniae* MVs can induce immunity to bacterial infection in mice without cytotoxic effect. This strongly suggests that MVs of Gram-positive bacteria also can be used for acellular vaccine.
[Supported by grants from KBSI(T32414) and SMBA(PCC231)]

D060

Characterization of 38 *Acinetobacter baumannii* Isolates from a Single Patient Died by Hemophagocytic Lymphohistiocytosis

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We characterized 38 *A. baumannii* isolates that had been isolated consecutively from blood, skin swab, and tracheal aspirate from a single patient who died by hemophagocytic lymphohistiocytosis. Genotypes of *A. baumannii* isolates were determined using MLST and PFGE. *In vitro* antimicrobial susceptibility testing was performed. The 38 isolates showed the same ST138 in MLST analysis. PFGE analysis showed similar but different genotypes among them. All isolates were resistant to imipenem, cefepime, ciprofloxacin, and piperacillin-tazobactam. And 32 isolate were resistant to amikacin, 6 to polymyxin B and colistin, and 3 to tigecycline. The 6 colistin-resistant isolates exhibited an amino acid substitution in *pmrB* gene. No inhibitory effects on tigecycline MICs by efflux pump inhibitors were observed. It was found that the 38 *A. baumannii* isolates had the same AbaR-type resistance island structure. Isolates from blood showed significantly higher survival rate against human serum than the other isolates. Our study shows that the patient was suffered from a few different strains of *A. baumannii*. It is noteworthy that the isolates from blood showed high virulence phenotype.

D061

Characteristics of Non-typeable (NT) *Streptococcus pneumoniae* Isolates from Asian Countries

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We aimed to investigated the presence of *pspK* gene in NT *S. pneumoniae* isolates from Asian countries and their characteristics was also examined. 120 NT *S. pneumoniae* isolates were collected from 1998 to 2009 from 8 Asian countries. The presence of *pspK* gene was assayed by PCR. Genotype was confirmed by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). Antimicrobial susceptibility testing was also performed. 21 NT isolates (17.5%) were identified to contain *pspK* gene: 2 isolates from Korea, 4 from Vietnam, 4 from Hong Kong, 8 from Thailand, and each one from Taiwan, the Philippines, and Saudi Arabia. 7 isolates from Korea, Vietnam, and Taiwan showed ST1106, and 1or 2 isolates belonged to ST310, ST393, ST1439, ST2754, and ST4136. 6 new STs were also identified in 7 NT isolates. PFGE patterns were different among ST1106 NT isolates except for Vietnam. All but one ST1106 NT isolates showed non-susceptibility to penicillin, and all isolates were resistant to erythromycin, clindamycin, and cefuroxime. Our results suggested that NT *S. pneumonia* isolates with *pspK* gene distributed in several Asian countries and might have emerged independently.

D062

Stimulation of Gammaherpesviral Production by Genipin

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Genipin is an aglycone derived from an iridoid glycoside called geniposide. Genipin is an excellent natural cross-linker for protein, collagen, gelatin, and chitosan cross-linking. We investigated if genipin would be one of anti-gammaherpesviral agents using *in vitro* iSLK-BAC16 and SNU719 infection systems. Genipin increased not only intracellular and extracellular genome copy numbers of KSHV by 2-fold and 6-fold but also those of EBV by 2-fold and 3-fold, respectively. Genipin increased production of transcripts from EBV lytic genes by minimum 0.5-fold to maximum 37-fold. These results indicated that genipin is stimulating production of EBV and KSHV progenies. In addition to effects on gammaherpesvirus, genipin showed cytotoxic effects on host cells EBV and KSHV infected. Genipin arrested cell cycles of both cell lines by delaying G2-M/G1 transition with expanded S phase. Furthermore, Genipin inhibited early apoptosis in iSLK-BAC16 whereas induced early apoptosis in SNU719. Taken together, these findings suggest that genipin would be one of the appropriate anti-viral candidates in anti-viral therapies of gammahespesvirus.

D063

Induction of Gammaherpesvirus Lytic Replication by Chalcone

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Isoliquiritigenin is a licorice-derived chalcone which plays a role of potent positive allosteric modulator for GABA-A benodiapine receptor. The ionotropic GABA-A receptor protein complex is also the molecular target of the benzodiazepine class of tranquilizer drugs. Isoliquiritigenin decrease gammaherpesvirus extracellular copy number by 2-fold to EBV. Whereas it increase KSHV extracellular copy number by seven fold to KSHV. On the other hand, qucertin gently decrease both gammaherpesvirus intracellular copy number, whereas it increase EBV extracellular copy number by two fold yet decrease KSHV extracellular copy number. RT-qPCR assay revealed that the isoliquiritigenin vigorously activated EBV and KSHV lytic gene induction while it moderately induced the gammaherpesviral latent gene expression. In addition, we investigated physiological effects of isoliquiritigenin and quercetin on iSLK-BAC16 and SNU-719 cells. Taken above results together, these studies proposed that chalcone compounds including isoliquiritigenin and quercetin have specificity in antiviral activities against EBV and KSHV, respectively.

D064

Investigation Result of *Heamaphysalis longicornis* Collected in Busan, 2013

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To investigate the infection of Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) in Busan 16 areas and domesticated animal. We collected 104 ticks in the mountain and two dogs that 102 ticks (98.1%) were the *Haemaphysalis longicornis*. the remaing two (1.9%) were *Haemaphysalis flava*. Classification of 102 *H. longicornis* were 40 larvae, 30 nymphs, 32 adults. Collection area were 6 out of 16 regions, mostly low mountains and cattle farms but crop field in rural areas were not collected. Examination of SFTS virus was not detected 13 pools from one to 30 ticks.

E001

Transcriptome Analysis of Pikromycin-producing Strain *Streptomyces venezuelae* ATCC15439

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Streptomyces are well known organisms as producers of antibiotics and have a complex cell cycle. Secondary metabolite production shares components with that of the sporulation process. Unlike *Streptomyces coelicolor*, *S. venezuelae* is a useful system for secondary metabolite engineering and differentiation study by having benefits from rapid growth rate and sporulation in liquid culture. We focus on the role of sigma factors and their regulation of secondary metabolite biosynthesis in *S. venezuelae*. We analyzed transcriptome using RNA sequencing in *S. venezuelae* and *S. coelicolor* in various growth phases under liquid culture condition. The expression of genes related in bioactive compounds production and differentiation in *S. venezuelae* was correlated with growth phase. *S. coelicolor* shows no differentiation-related gene expression, however, the secondary metabolite gene clusters are activated under this condition. In stationary phase, sigma factors and sporulation factors of *S. venezuelae* were differently expressed in comparison to those of *S. coelicolor* cultured in liquid media, especially *sigF* and *sigG* which are related to sporulation.
[Supported by ISBC of Global Frontier Project]

E002

Differential Roles of Isa1 and Isa2 in Fe-S Cluster Assembly and Iron Regulation in *Schizosaccharomyces pombe*

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The process of forming Fe-S clusters involves scaffold proteins that form transient Fe-S cluster and carrier proteins that transfer Fe-S to specific apo-targets. The role of carrier proteins (Isa1 and Isa2) that are homologues of IscA, an A-type carrier in *E. coli*, was examined in *S. pombe*. Unlike in *S. cerevisiae*, each of the $isa1^+$ and $isa2^+$ gene is essential for the growth of *S. pombe*. Repression of *isa1* or *isa2* expression caused growth defect in EMM media. Although Isa1 and Isa2 are similar in sequence, multi-copy *isa2* did not restore growth defect of *isa1* mutant and *vice versa*. In both conditional mutants, the activity of aconitase and succinate dehydrogenase was decreased under aerobic growth condition. Under anaerobic condition however, only *isa1* mutant showed defect in succinate dehydrogenase activity. Additionally, the expression of an iron uptake gene ($fio1^+$) was induced in *isa1* mutant. Even though the activities of mitochondrial Fe-S enzymes were affected in both mutants, only Isa1 was involved in iron homeostatic regulation. These results demonstrate that Isa1 and Isa2 perform differentiated roles in supporting Fe-S enzymes and iron-homeostatic gene regulation.

E003

Crystal Structure of C-terminal Effector Domain of VncR

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VncR is the response regulator component of the VncRS two-component signal transduction system (TCS) of infectious pathogen *Streptococcus pneumoniae.* VncRS associates with regulation of bacterial autolysis and antibiotic resistance such as vancomycin. VncR contains two different functional domains, *N*-terminal receiver domain and C-terminal effector domain. Here, we investigated VncR DNA binding domain ($VncR_b$) structure using crystallization approach. Crystallization was performed using the micro-batch method. The crystals diffracted to a 1.964 Å resolution and belonged to space group $P2_12_12_1$. The crystal unit-cell parameters a=25.71, b=52.97, c=60.61 Å. The structure of $VncR_b$ has helix-turn-helix motif, it is similar to the other response regulator proteins, for example, PhoB of *E. coli*. Determination of the VncR structure will provide insights into the mechanisms of VncR binding to the target DNA.

E004

Molecular Analysis of the Medium-Chain-Length Polyhydroxyalkanoate Depolymerase Gene from *Variovorax* sp. DSH and Characterization of the Gene Product

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An extracellular medium-chain-length polyhydroxyalkanoate (MCL-PHA) depolymerase gene ($phaZ_{DSH}$) was cloned from the genomic DNA of *Variovorax* sp. DSH. The $phaZ_{DSH}$ gene was found to be 837 bp with a deduced protein of 278 amino acids. The amino acid sequence had at least 68% homology to the known MCL-PHA depolymerases from *Pseudomonas* strains and consist of three domains in the sequential order; signal peptide, an N-terminal substrate binding domain, and a catalytic domain. The $phaZ_{DSH}$ gene was expressed in *Escherichia coli* and the gene product was purified and biochemically characterized. The enzyme consisted of a monomeric subunit having a molecular mass of 27.9 kDa as determined by SDS-PAGE. The maximum activity of the enzyme was observed at pH 8.5 and 45^0C. Its hydrolyzing activity was significantly sensitive to PMSF, EDTA, *N*-bromosuccinimide, and non-ionic detergents. The highly significant homology of the deduced amino acid sequence of $PhaZ_{DSH}$ with those of the known *Pseudomonas* MCL-PHA depolymerases and several characteristics that are common among these enzymes strongly suggest the possibility of horizontal transfer of the MCL-PHA depolymerase gene in bacterial strains.

E005

A Role of Cps35/Swd2 in the Regulation of COMPASS' Activity for the Trimethylation of Histone H3K4 Depending on Ubiquitinated Histone H2B

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The histones in the eukaryotic chromatin are highly modified with many diverse post-translational modifications. These modifications can influence each other to regulate transcription and other processes. One of the best-known histone crosstalks is that H2BK123 should be monoubiquitinated for higher level of H3K4 trimethylation (me3). Cps35/Swd2 within Set1 complex (called COMPASS) was originally thought to connect H2B ubiquitination (H2Bub) and H3K4 methylation. Recently, one group reported that a truncated form of Set1 (762-Set1) can methylate histone H3K4, even though there is no interaction of Cps35/Swd2. However, we reported the mislocalization of the H3K4me3 by 762-Set1. We used ChIP-seq approach and then concluded that the H3K4me3 by 762-Set1 occurred not in promoter-proximal region correctly but also in gene bodies and intergenic region. This result suggests that Cps35/Swd2 interacting H2Bub machinery could play a role in focusing H3K4me3 to the correct location. Our coimmunoprecipitation data also showed Cps35/Swd2 interacts with Rad6 and some components of Paf1 complex. So we suggest a model in which the H2Bub machinery could function as a prerequisite for H3K4me3.

E006

Butyryl-CoA Dehydrogenase Has an Electron Bifurcating Role for Energy Conservation in Acetogen, *Eubacterium limosum* KIST612

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Eubacterium limosum KIST612 is a strict anaerobic, Gram-positive, acetogen that uses synthesis gas as carbon and energy source *via* the WL pathway. The products are acetate and butyrate. Butyrate production is via acetoacetyl-, hydroxybutyryl- and crotonyl-CoA. Crononyl-CoA reduction in Clostridia was recently shown to be energy conserving via coupled ferredoxin (Fd) reduction and subsequent Na+/H+-motive Fd oxidation. Genome mining of KIST612 showed the presence of two genes encoding electron transferring flavopotein (EtfAB) next to butyryl-CoA dehydrogenase (Bcd). It was assumed that Bcd of KIST612 may use electron bifurcation to drive Fd reduction. To the test, the Bcd was partially purified. The enzyme catalyzed crotonyl-CoA reduction, but only in the presence of NADH and Fd. Most important, NADH oxidation and Fd reduction occured simultaneously. The ratio between ferredoxin reduction and NADH oxidation was approximately 1 to 2. It is finally concluded that KIST612 has a Bcd-EtfAB complex which catalyzes electron bifurcation for Fd reduction at the expense of NADH oxidation with crotonyl-CoA reduction, and this mechanism is contribute to energy metabolism of KIST612.

E007

Antioxidant Effect of Lactic Acid Bacteria-Fermented Ginseng Extracts (FGE)

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The effect of lactic acid bacteria-fermented ginseng extract (FGE) on physiological activities was evaluated. Ginseng extract (GE) were inoculated with five strain of LAB (1.0×10^6 CFU/ml) and incubated at 37℃ for 72 h. Among 5 kinds of LAB, two substrains of *Lactobacillus salivarius* E4191, *Lactobacillus johnsonii* OCS41 were selected based on their dose dependent stimulation of the growth of LAB in the presence of ginseng and changes in pH, acidity and viable cell counts during fermentation was examined. E4191 specifically was found to show the best growth on 7% GE and reached nearly 10.4×10^8 CFU/ml after 24 h of fermentation and pH was significantly lowered from 7.00 to 4.31. Antioxidant activity of GE and FGE was also analyzed by DPPH radical scavenging activity assay. E4191 - FGE showed an 82.6% inhibition of DPPH radical at a concentration of 3.00%. GE showed a 63% inhibition of DPPH radical at the same concentration. These results suggest that FGE could be used as an active ingredient for health functional food.

E008

Inhibitory Activity of Lactobacilli on Calcineurin mRNA Expression of Human Fibroblast–like Synovial Cells

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Calcineurin is a calcium-dependent serine-threonine phosphatase. It stimulates the growth and differentiation of T cells in immune system. When calcium level in cytoplasma is increased, overexpressed Calcineurin induces the production of other cytokines and it relates to autoimmune. Lactobacilli are known to have nutritional functions and positive effects to human health. Some Lactobacillus species have shown effects on modulating inflammatory cytokines. In this study, inhibitions of Calcineurin levels using Lactobacilli in the MH7A rheumatoid synovial cell were demonstrated. 20 strains of Heat-killed Lactobacillus were pre-treated to MH7A Cells, then Polyinosinic-polycytidylic acid (PolyI:C) and Lipopolysaccharide (LPS) were added to cells to induce Calcineurin. mRNA levels of Calcineurin were expressed by using RT-PCR. *Lactobacillus brevis* KY21 and *Lactobacillus rhamnosus* GG from infant feces have an effect on enhancing the inhibition of PolyI:C-induced Calcineurin mRNA expression in MH7A Cells. These results suggest that *Lactobacillus brevis* KY21 and *Lactobacillus rhamnosus* GG have the potential in further researches in treatment for overexpressed Calcineurin.

E009

Inhibitory Effect of *Gleditsia sinensis* Thorn Extract and Fermentation on Lipopolysaccaride-induced Interleukin-8 in Intestinal Epithelial Cells

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Probiotics are live microorganisms, which have been shown to exert beneficial effects on human health, including inflammatory bowel disease. Recently, probiotic bacteria are incorporated into beverages in the hope of producing functional beverages. *Gleditsia sinensis* is one of the most common medicinal herbs in Asia. Its thorns have demonstrated their pharmacological activity, particularly in anti-inflammatory properties. The objective of this study was to determine the inhibitory effect of probiotic bacteria and *Gledistia sinensis* thorn (GST) extract on lipopolysaccharide (LPS)-induced pro-inflammatory cytokines in the human intestinal epithelial cell line HT-29. We expected that Synergistic effect of probiotic bacteria and GST extract on LPS-induced IL-8 in HT-29. Some strains were able to grow in modified MRS broth containing 100 ug/ml of GST extract. We found that GST extract and fermentation showed inhibitory effect on LPS-induced IL-8 mRNA expression and protein from HT-29 cells. Results from the present study demonstrated that GST extract and fermentation containing *Lactobacillus brevis* KY 21 could contribute to protective effect on human intestinal epithelial cells.

E010

Antibiotics Stress Response Mediated by SigR in *Streptomyces coelicolor*

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Antibiotics are secondary metabolites produced by microorganisms in nature. At high concentration, antibiotics kill or inhibit the growth of bacteria. At low concentration, however, antibiotics induce physiological responses that cause bacteria to have antibiotic resistance. Antibiotics producing bacteria are good for studying intrinsic resistance in that they have various intrinsic resistance mechanisms to antibiotics. Among them Streptomycetes could be strong model systems which produce two thirds of all clinically relevant secondary metabolites. In *Streptomyces coelicolor*, we found that σ^R is involved in intrinsic resistance. σ^R is induced by subinhibitory concentrations of antibiotics (chrolamphenicol, hygromycin, and tetracycline) and SigR-RsrA null mutants are hypersusceptible to diverse antibiotics. In addition, using RNA-seq we analysed the transcriptome of *S. coelicolor* under antibiotic stress. The physiological meaning and the mechanism behind this deserves systematic studies.
[Supported by grants from NRF]

E011

Leucine Aminopeptidase (LAP) of *Aspergillus sojae* Isolated from Fermentation Products

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Leucine aminopeptidase (LAP) is exopeptidase, which removes the N-terminal L-leucine from peptide substrates. LAP is one of the most interesting enzymes in fermentation industry because it plays an important role in the flavor development of soybean-fermented foods. We collected total 34 *A. sojae* isolated from various fermentation products and analyzed their LAP activity profile for the further studies on the regulation of LAP activity. Because *Aspergillus* section *Flavi* is often misidentified due to their phylogenetic similarity, we re-identify them at morphological and molecular genetic levels. About 9 strains were reclassified to other species. The LAP activities were measured at various levels ranging from 0.02 to 0.2 U/g biomass and 0.2 to 2.0 U/mg crude proteins. These *A. sojae* strains showing various LAP activities will be further analyzed to understand the underling mechanism on the differences in LAP activities.
[Supported by grants from "World class 300 project (Project No. A2013-0391)" of the Minisry of Trade, Industry and Energy, Republic of Korea.]

E012

Functional Characterization of Putative UDP-Glucose 4-Epimerase (TM0509) from the Hyperthermophilic Eubacterium *Thermotoga maritima*

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UDP-glucose 4-epimerase (GalE) catalyzes the interconversion of UDP-glucose (UDP-Glc) and UDP-galactose (UDP-Gal), which is a pivotal step in the Leloir pathway for galactose metabolism. Although GalEs are widely distributed in Bacteria and Eukaryotes, there is little information on hyperthermophilic GalE. Herein we cloned and overexpressed the TM0509 gene from *Thermotoga maritima*. The recombinant protein was purified to homogeneity by heat precipitation, Ni^{2+} affinity chromatography followed by size-exclusion chromatography. The recombinant TMGalE could reversibly catalyze the epimerization of UDP-Gal and UDP-Glu in the presence of NAD^+ at elevated temperatures. The apparent optimal temperature and pH for epimerization activity were 85°C and pH 7.0, respectively. In addition, we determined not only the crystal structure of TMGalE at 1.9 Å resolution, but also the co-crystal structure of TMGalE with UDP-Glu at 2.0 Å resolution. These biochemical and structural data showed that TM0509 is an UDP-galactose 4-epimerase involved in galactose metabolism, which is the first detailed characterization of a thermostable GalE from hyperthermophilic bacterium. [Supported by a grant from IPET]

E013

The Stability of pVHL is Regulated by the Interaction between Hepatitis B Virus X Protein and VBP1

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Von Hippel-Lindau protein (pVHL) is a tumor suppressor protein which is associated multiple benign and malignant tumors. pVHL regulates proteasomal degradation of the hypoxia-inducible factor-1α (HIF-1α). HIF-1 plays several roles in oxygen homeostasis and tumorigenesis. It has been reported that von Hippel-Lindau binding protein 1 (VBP1) interacts with pVHL. It is possible that VBP1 may influence the stability of pVHL, since VBP1 is known as a molecular chaperone. Hepatitis B virus (HBV) is one of the most important human pathogen for various liver disease. Among HBV proteins, hepatitis B virus X protein (HBx) is a viral oncoprotein associated with liver carcinogenesis. By using yeast two-hybrid system, we selected several possible cellular partners of HBx including VBP1. Here we tested the regulatory effect of HBx on the stability of pVHL through its interaction with VBP1. We found that VBP1 stabilizes pVHL by suppressing the ubiquitination of pVHL. In addition, HBx decreases the level of pVHL by blocking the stabilizing activity of VBP1 on pVHL. Taken together, HBx regulates a tumor suppressor pVHL through VBP1 suggesting a new possible mechanism for tumorigenesis by HBx.

E014

The Effect of Oxygen in Nitric Oxide-mediated Branched-chain Amino Acid Auxotrophy of *Salmonella*

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Nitric oxide has been known to cause amino acid auxotrophy in *Salmonella* Typhimurium. Branched-chain amino acids (BCAA) play major roles in bacterial recovery from amino acid auxotrophy under nitrosative stress, implying that some enzymes for BCAA biosynthesis are vulnerable to NO. LeuCD and IlvD are essential enzymes for BCAA biosynthesis and possess NO-targetable Fe-S clusters, so that their inactivation has been implicated as a cause of NO-mediated BCAA auxotrophy. In this study, we examined their roles in NO resistance under different oxygen tensions by using *leuCD* and *ilvD* mutants constructed in *hmp* mutant *Salmonella* deficient in NO-detoxifying enzyme flavohemoglobin. The NO-caused growth arrest of mutants *leuCD hmp*, *ilvD hmp,* and *leuCD ilvD hmp* was relieved by supplementation with the combination of BCAA under aerobic cultures, whereas it was not under semiaerobic or anaerobic cultures. Data suggest that NO inactivates key enzymes of BCAA biosynthesis whose damage can be overcome by supplying end-products, but it can further inactivate enzymes functioning beyond their known roles in BCAA biosynthesis under oxygen-limited conditions.
[Supported by NRF grant (2008-0062283)]

E015

Structural Insights into Conserved L-Arabinose Metabolic Enzymes Reveal the Substrate Binding Site of a Thermophilic L-Arabinose Isomerase

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Structural genomics demonstrates that despite low levels of structural similarity of proteins comprising a metabolic pathway, their substrate binding regions are likely to be conserved. Herein based on the 3D-structures of the α/β-fold proteins involved in the *ara* operon, we attempted to predict the substrate binding residues of thermophilic *Geobacillus stearothermophilus* L-arabinose isomerase (GSAI) with no 3D-structure available. Comparison of the structures of L-arabinose catabolic enzymes revealed a conserved feature to form the substrate-binding modules, which can be extended to predict the substrate binding site of GSAI (i.e., D195, E261 and E333). Moreover, these data implicated that proteins in the L-arabinose metabolic pathway might retain their substrate binding niches as the modular structure through conserved molecular evolution even with totally different structural scaffolds.
[This work was supported by a grant 311042-05-1-HD120 from the Korea Institute of Planning & Evaluation for Technology (iPET) funded by the Ministry for Food, Agriculture, Forestry and Fisheries to Dr. Dong-Woo Lee, and by research funds from Chosun University (2008) to Dr. Sung Haeng Lee.]

E016

Biochemical and Structural Characterization of a Thermophilc L-Arabinose Isomerase from *Geobacillus kaustophilus*

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Thermophilic L-Arabinose isomerase (AI), that catalyzes the interconversion of L-arabinose to L-ribulose, can also isomerize D-galactose to D-tagatose as a natural sugar substitute. Unlike mesophilic AIs, thermophilic AIs showed the distinct metal dependence for catalytic activity and thermostability at elevated temperatures. However, it still remains unclear how thermophilic AIs showed different substrate preferences and metal requirements at molecular levels. Herein we presented the first crystal structures of the apo and holo forms of a thermophilic AI from *Geobacillus kaustophilus* (GKAI) by X-ray crystallography to 2.40 and 2.30 Å, respectively. In comparison with the crystal structures of *Escherichia coli* AI as a mesophilic counterpart, the GKAI structures revealed conserved structural features for substrate and metal binding, except for subtle interactions of a few polar residues with water molecules near the substrate binding region. Our comparative analysis provides a versatile strategy to engineer the promiscuity of substrate specificity for sugar isomerases as well as thermostability for mechanistic studies and industrial applications.
[Supported by a grant from iPET]

E017

Vibrio vulnificus HPr Stimulates Pyruvate Kinase A Activity to Protect Cells against H_2O_2 Stress

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The bacterial phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS) consists of two general energy-coupling proteins (enzyme I and HPr) and several sugar-specific enzyme IIs. In addition to the phosphorylation-coupled transport of sugars, the PTS components participate in many physiological processes. In this study, we have identified pyruvate kinase A (PykA) as a binding partner of HPr in *V. vulnificus*, which is an opportunistic human pathogen. The interaction between HPr and PykA was strictly dependent on the presence of inorganic phosphate and only unphosphorylated HPr interacted with PykA. Domain swapping experiments between PykA and its *E. coli* ortholog revealed a requirement for the C-terminal domain of PykA for specific interaction with HPr. Unphosphorylated, but not phosphorylated, HPr decreased the Km of PykA for PEP about 4 fold without affecting Vmax. A *pykA* mutant became more susceptible to H_2O_2 than wild-type *V. vulnificus* and this sensitivity was completely rescued by the addition of pyruvate to the culture medium. Based on these data, our data suggest that PykA plays an important role in H_2O_2 stress response in the presence of PTS sugars.

E018

Regulation of Flagellar Motility by the PTS in *Vibrio vulnificus*

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Vibrio vulnificus is an opportunistic human pathogen that causes food-borne diseases such as gastroenteritis and primary septicemia, and its single polar flagellum-based motility is one of the potential virulence factors. Notably, it has been reported that glucose prevents the synthesis of flagella and hence swimming motility in some bacteria. Glucose is transported through the phosphoenolpyruvate: sugar phosphotransferase system (PTS) in most bacteria. The components of the PTS have multiple physiological roles as well as catalysis of the transport and accompanying phosphorylation of numerous PTS sugars. Here, we show that the dephosphorylated form of enzyme IIA of the glucose PTS, but not its phospho-form, interacts with a hypothetical protein (henceforth called Protein X) in *V. vulnificus*. A deletion mutation in protein X resulted in loss of flagellum synthesis and reduced expression of several flagellar genes, indicating that Protein X is essential for the flagellar motility. Taken together, we suggest that the interaction between enzyme IIA^{Glc} and Protein X regulates the flagellar motility by sensing glucose in *V. vulnificus*.

E019

Hypocholesterolemic and Anti-thrombotic Effect of Fermented Mailard Reaction Products (MRPs) by Probiotic *Lactobacillus* Strains

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The Mailard reaction is a complex reaction that occurs between carbonyl and amine groups of milk protein and lactose and its product have been called Mailard reaction products (MRPs). Probiotic *Lactobacillus* can readily utilize and hydrolyze MRPs via proteolysis. In this study, hypocholesterolemic and anti-thrombotic effect of MRPs, fermented by LAB, has been measured. Using selected strains, mailard reaction products (MRPs) was fermented and then focused on evaluating the protective effect against cardiovascular diseases (CVD), especially atherosclerosis. Cholsterol reduction activity of probiotic strains and fermented MRPs were done by colorimetric assay. Cholesterol reduction rate of fermented cMRPs (casein derivated MRPs) by *Lactobacillus gasseri* MF27 and *Lactobacillus fermentum* MB50 will have been measured. And anti-thrombotic effects of fermented wMRPs (whey protein concentrate (WPC) derivated MRPs) using spectrophotometric assay. For identify the effect of hydrolysis of MRPs during fermentation, degree of hydrolysis were measured using OPA-method.

F001

Integration Helper Plasmid Mediated One-Step Inactivation in *Escherichia coli*

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We developed an integrated helper plasmid-based gene manipulation system for more efficient and rapid engineering of *Escherichia coli*. The integrated helper plasmid, pCW611, contains two recombinases which are expressed in reverse direction by two independent inducible systems. The main advantage of this system is that the time and effort required can be significantly reduced 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 (*adhE*, *sfcA*, *frdABCD*, and *ackA*) and two genes simultaneously for two cases (*adhE-aspA* and *sfcA-aspA*). Also, sequential deletion of four target genes (*fumB*, *iclR, fumA*, and *fumC*) was successfully performed for the construction of fumaric acid producing strain.
[Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A2026556) funded by the Ministry of Education, Science and Technology]

F002

Roles of the VeA-dependent Proteins Identified by Proteome Analysis in *Aspergillus nidulans*

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In *Aspergillus nidulans*, VeA is a key regulator involved in light-sensitive control of differentiation. Our previous proteomic analysis using VeA-deletion mutant revealed 12 VeA-dependent proteins, VdpA~VdpL, of which expression level was affected by VeA. To examine the function, genes for Vdps were individually disrupted. Phenotypic analysis was performed in vegetative growth, asexual and sexual development. Among the *vdp*-deletion mutants tested, the strains lacking VdpA or VdpJ revealed various developmental defects. The VdpA has a survival factor-like domain of yeast. Strain lacking the VdpA showed defective phenotypes: reduced radial growth, small mycelia balls with hyper-branching, frequent septation, reduced spore production and abnormality in asexual and sexual organ. The VdpJ is known as a hypothetical protein. Strain lacking the VdpJ showed reduced growth on minimal medium and arginine auxotrophy. Since sexual development is affected by the amount and type of carbon source, we are examining sexual development of the mutant on variable media, including 3% glucose, 2% Lactose and 2% glycerol as carbon source
[Supported by grants from NRF]

F003

Molecular Characterization of Hydrogen Peroxide-Sensing OxyR Regulon in *Acinetobacter oleivorans* DR1

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A Diesel-degrading *Acinetobacter oleivorans* DR1 has annotated OxyR homolog (AOLE_14380) which has two conserved cysteine residues. Four catalases are present in the genome of DR1. Alignment of OxyR-binding regions from *P. aeruginosa* and *E. coli* with promoters of 4 catalases identified putative OxyR-binding site upstream of two catalases. qRT-PCR analysis under H_2O_2 demonstrated that expression of those catalases (AOLE_09800 and AOLE_11770) were 7 and 5 fold increased, respectively. Proteomics was conducted to investigate the effect of H_2O_2 on whole protein expression level. Our result has shown that 54 proteins were differentially expressed after 1hr in response to 1 mM H_2O_2. Among them, up-regulated 18 proteins were identified by MALDI-TOF MS. Functional classification of these proteins showed a relationship with oxidative stress, energy production and conversion, nucleotide transport and metabolism. Interestingly, the most overexpressed protein was peroxiredoxin (Prx) which belongs to typical 2-Cys Prx class. Currently, characterization of differently expressed proteins along with EMSAs using purified OxyR is under investigation.

F004

Isolation of Genes Involved in mRNA Export by Complementation of Synthetic Lethal Mutants in Fission Yeast

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The Nab2 is known as a poly(A)$^+$ RNA binding protein that modulates poly-A tail length. The Nab2 interacts with Yra1, Sub2 and Mex67 which function as mRNA export and thus, it also support mRNA export process by shuttling between nucleus and cytoplasm. In fission yeast *Schizosaccharomyces pombe*, the nab2 null mutant is not essential for cell growth and mRNA export but over-expression causes a severe mRNA accumulation in the nucleus. In order to further study, we isolated mutants that show synthetic lethality with the Δnab2 (SLnab2). By using these mutants, we have searched for genes that could potentially have overlapping or complementary function with *nab2*. From SLnab2 transformants with DNA library, we selected plasmids that rescue the growth defect in the presence of thiamine. Isolation and sequence analysis of the plasmids revealed 4 genes, *nab2*, *rmn1*, *uap56* and SPCC1442.04c. From among these, we focused on SPCC1442.04c gene. The SPCC1442.04c null mutant shows no growth defect but wild type strains overexpressing SPCC1442.04c gene show severe growth retardation and poly(A)+ RNA accumulation phenotype in the nucleus.
[Supported by grants from NRF]

F005

LAMMER Kinase-mediated Regulation of MBF Activity for Cell Cycle Progression in Fission Yeast

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Our previous study with LAMMER-kinase (Lkh1) deletion mutant displays several phenotypes, which may be related the cell size control and cell-cycle progression. Microarray analysis of the *lkh1*$^+$ deletion mutant revealed that the expression of various classes of genes were affected by the *lkh1*$^+$ deletion. Among those up-regulated genes by the *lkh1*$^+$ deletion, only four were identified as the genes related to the cell cycle. Interestingly, these genes are regulated by MBF (*Mlu*1 cell cycle box binding factor), a transcription factor that regulates cell-cycle genes in G1/S phase. Quantitative RT-PCR of transcripts revealed that Lkh1 may be associated with negative feedback regulators of MBF because MBF-dependent genes are up-regulated in *lkh1*$^+$ deletion mutant. Pull-down assay, kinase assay and PMF suggested that Lkh1 phosphorylates threonine 40/41 residues in Yox1 of negative feedback regulator of MBF. Experiments to see the effects of Lkh1-dependent Yox1 phosphorylation on the assembly MBF components are under investigation and the results will be discussed.

F006

The Unique Roles of Ire1 in Bisexual and Unisexual Mating in *Cryptoocccus neoformans*

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Mating is an essential biological process for producing genetically diverse offspring, which contributes to the increased fitness of certain species in its environmental niches. Previously we have shown that the unfolded protein response pathway, comprising the Ire1 kinase/endonuclease and its downstream transcription factor Hxl1, governs ER stress response and virulence of *C. neoformans*. Here we present a series of evidence showing that Ire1 modulates bisexual and unisexual mating responses of *C. neoformans* without involvement of Hxl1. In bilateral crosses, *ire1*Δ mutant was decreased in the formation of mating filaments in serotype A and D strains. The impairment of *IRE1* caused defective in cell-cell fusion and pheromone-mediated conjugation tubes. However, Ire1 negatively regulates expression of the mating pheromone gene (*MFα1*) in a Cpk1 MAPK-independent manner. Moreover, the deletion of *IRE1* results defective in unisexual mating response. In conclusion, the Ire1 regulates both bisexual and unisexual mating of *C. neoformans* in Hxl1-independent manners.
[This research was supported by National Research Foundation of Korea grants (2008-0061963 and 2010-0029117) from MEST]

F007

The Antifungal Mode of Action of a 13-(4-isopropylbenzyl)berberine Derivative, KR-72

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Although many antifungal drugs have been developed, a limited drugs are clinically available because of the similarity between fungi and mammalian cells. Therefore, Amphotericin B and the azole compounds remain the mainstream antifungal drugs despite their serious side effects and high efficacy. Recently a 13-(4-isopropylbenzyl)berberine derivative (named KR-72) was synthesized and examined for antifungal activities against various human pathogenic fungi. The synthesized compound exhibited remarkably enhanced antifungal activity than berberine and berberrubine.
Regardless of the potent antifungal activity of KR-72, its mode of action and the physiological impacts of the drug on fungal metabolism remain elusive. In this study, we performed the DNA microarray-based transcriptome analysis to identify KR-72 responsive genes and employed reverse genetics approaches to characterize their functions in *Cryptococcus neoformans*, which causes fatal meningoencephalitis in humans.
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F008

Actinomycetes Cyclosporin A Hydroxylase: Which Site Determines Its Regio-selectivity?

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The cytochrome P450 (CYP) is an important industrial enzyme involved in biosynthesis and bioconversion of various natural products. The catalytic abilities of CYPs are highly attractive due to their superior regio-selectivities. Previously, we identified two actinomycetes CYPs (CYP-sb21 from *Sebekia benihana*, CYP-pa1 from *Pseudonocardia autotrophica*) that catalyze the regio-specific hydroxylation of the immunosuppressive agent cyclosporin A (CsA). The CYP-sb21 exhibited a preferential hydroxylation activity at the position of 4th N-methyl leucine of CsA, whereas the CYP-pa1 did at the position of 9th N-methyl leucine of CsA. These genes were isolated and characterized by gene disruption, complementation. To further identify the regioselectivity-determining regions, several candidates were selected through a sequence alignment between two CYPs, followed by a site-to-site domain swapping. The mutated gene was introduced into each CYP knock-out mutant (ΔCYP-sb21, ΔCYP-pa1) strain for CsA bioconversion assay. The more detailed results will be further discussed.
[This work was supported through the Global Frontier Program for Intelligent Synthetic Biology (20110031961)]

F009

Regulation of an Acidic Laccase Promoter of *Coprinellus congregatus* in Two Different Fungi

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When *Coprinellus congregatus* was transferred to acidic pH (pH 4.0-4.5), a laccase (acidic laccase) was synthesized and secreted into the culture supernatant. We have cloned the genomic and cDNA genes and confirmed its expression under acidic conditions. However the regulation of expression has not determined yet. In order to analyze its regulation mechanism, a reporter gene should be used instead of the laccase gene itself. We have constructed an expression vectors of the acidic laccase promoter (*lac2* promoter) of with GFP using pBARGEM7-1 and pPICZB vectors, and these were introduced into *C. congregatus* and *P. pastoris* in order to determine the regulation mechanism of the *lac2* promoter. We will report the expression pattern of the *lac2* promoter under acidic and oxidative conditions in two different fungi.

F010

Ultramicroscopic Structural Changes of Plant Pathogenic Fungi by Fungal Chitinase Treatment

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Two different chitinases, Chi1 and Chi2, are expressed during the whole life cycle and mushroom autolyzing phase in *Coprinellus congregatus*, respectively. We have cloned two cDNA genes and constructed the expression vectors using the pPICZ vector. These vectors were introduced into *Pichia pastoris* and both Chi1 and Chi2 were successfully expressed by methanol induction. We have determined their biochemical characteristics of both Chi1 and Chi2.We have found that these chitinases had good inhibition activities against human pathogenic yeasts (*Candida albicans* and *Cryptococcus neoformans*) and plant pathogenic fungi (*Alternaria alternata*, *Fusarium graminearum* and *Trichoderma harzianum*).
We will report their hydrolyzing patterns (endochitinase or exochitinase) against colloidal chitin. We will also examine the growth inhibition mechanism against several plant pathogenic fungi, such as *F. graminearum* and *T. harzianum* using the TEM and SEM.

F011

Nuclear Localization of NsdD is Affected by Various Genetic and Environmental Factors in *Aspergillus nidulans*

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NsdD, the GATA type transcription factor, which controls sexual development of *Aspergillus nidulans* has a nuclear localization sequence (NLS) just upstream of C4 zinc finger domain. The sGFP gene fused onto N-terminus of *nsdD* ORF was used to transform the *nsdD* deletion mutant and the green fluorescence was traced by confocal laser microscope. The NsdD was found to be localized in nuclei 16 h after germination. Missense mutations in NLS inhibited the NsdD from entering nuclei indicating that the NLS play a critical role in nuclear localization of NsdD. The mutation could not complement the *nsdD* deletion mutation and also could not repress the *nrsA* transcription, which suggested that nuclear localization of NsdD is important for the regulatory function of NsdD in development. The IndB and IndD known to bind to zinc finger of NsdD also inhibited NsdD from entering nuclei when they were over-expressed. Zn finger mutation to which Inds could not bind did not affect the entrance of NsdD to nuclei. The result implies that the binding of IndB and IndD to NsdD zinc finger inactivates the NLS function, which results in the failure of decision of sexual development.

F012

Genetic Incorporation of *p*-Azido-*L*-phenylalanine in *Escherichia coli*

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Site-specific incorporation of unnatural amino acids (SSIUA) into proteins is a promising method to generate novel proteins with expanded biological, chemical, or physical properties and can be achieved *in vivo* by coexpression of an orthogonal pair of suppressor tRNA and engineered aminoacyl-tRNA synthetase (ARS) that specifically ligates an unnatural amino acid to the suppressor tRNA. Using an orthogonal pair of *Saccharomyce cerevisiea* Tyrosyl-tRNA synthetase (*Sc* TyrRS) and a variant of *E. coli* initiator tRNA $tRNA_2^{fMet}$ (*fMam* $tRNA_{CUA}$), we have evolved ARS-tRNA pairs capable of incorporating *p*-Azido-*L*-phenylalanine (AzPhe) with an expanded genetic code in *E.coli.* All the clones showed higher chloramphenicol resistance and β-galactosidase activity in the presence of AzPhe that were generated by amber suppression of *CAT* amber and *lacZ* amber genes, respectively. Incorporation of AzPhe was also analysed by an immunoblot assay and used for protein modification of site-specific labeling of a fluorophore. This additional genetic incorporation of AzPhe can be used in diverse modifications of protein.
[Supported by GRRC grant]

F013

Identification and Characterization of the High Temperature Response Genes by Comparative Transcriptome Analysis Using *sch9*Δ Mutant in *Cryptococcus neoformans*

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Adaptation to the host physiological temperature is one of the crucial virulence factors for *Cryptococcus neoformans*. The Sch9 protein kinase plays negative roles in thermotolerence of *C. neoformans*, but its regulatory mechanism remains elusive. In this study we performed comparative transcriptome analysis between the wild type and *sch9*Δ mutant during temperature shifting from 25°C to 37°C or 40°C. A global scale of remodeling in gene expression profiles was observed in the wild type strain during temperature shifting from 25°C to 37°C. The expression levels of the genes encoding chaperones, heat shock proteins, or the ergosterol biosynthesis proteins were differentially regulated by temperature shifting. Notably, expression of *HSF1* (Heat Shock Factor1) was reduced during temperature adaptation in wild type, whereas reduction of *HSF1* was delayed in *sch9*Δ mutant. This study elucidates the regulatory mechanism of Sch9 in thermotolerence as well as provides further insight into the regulatory mechanism of thermotolerance in *C. neoformans* through a genome-scale identification of the Sch9-dependent genes.
[Supported by grants from NRF]

F014

Identification of Catalase in a Gamma-radiation Resistant Bacterium *Hymenobacter swuensis*

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Hymenobacter swuensis, a gamma-radiation resistant bacterium, is isolated from mountain soil in South Korea and newly classified as a novel species within genus *Hymenobacter*. The complete genome sequence indicated that *H. swuensis* consists of one chromosome with three plasmids (pHsw1, pHsw2, and pHsw3). The genome sequence indicated that *H. swuensis* includes a unique 2'-hydroxy-carotenoid (2'-hydroxyflexixanthin) and four catalase genes, which protect cells from damage caused by oxidative stress. Catalase assay using native polyacrylamide gel electrophoresis from the cell extracts resulted in three catalase activity bands, which were designated Cat1 to Cat3. Of these, Cat2 showed higher levels of catalase activity. Furthermore, cell survival assay showed that *H. swuensis* is highly resistance to H_2O_2 compared with *E. coli*. These data suggest that a unique carotenoid and strong catalase activity contribute to the oxidative stress resistance of *H. swuensis*.

F015

Genome-Wide Synthetic Lethal Screen of NatB N-Terminal Acetylase in *Saccharomyces cerevisiae*

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The N-terminal acetylase NatB complex is composed of catalytic subunit Naa20 (Nat3) and an auxiliary subunit Naa25 (Mdm20) in *Saccharomyces cerevisiae*. For defining cellular function and basic mechanism of the NatB, we used a Synthetic Genetic Array (SGA) and screened genes that are essential for cell growth in the absence of *NAA20*. Through Genome-wide synthetic lethal screen of Naa20, we found that Vps15 (a serine/threonine protein kinase for vacuolar protein sortin), Gas5 (1,3-beta-glucanosyltransferase for cell wall maintenance) and Gga1 (a Golgi-localized protein for trafficking) are related to NatB. The present study indicated that the NatB is involved in the vacuolar protein sorting, cell wall maintenance and Golgi protein trafficking.
[This work was supported by grants from the Korea Healthcare technology R&D Project, Ministry of Health & Welfare (HI11C1279).]

F016

Signal Transduction by ArcB Sensor Kinase without PAS Domain

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The ArcB/A (anoxic redox control) two-component signal transduction system of *E. coli* modulates the expressions of numerous genes depending on the redox conditions of growth. Here, we characterized the ArcB/A two-component system of the rumen bacterium *M. succiniciproducens*. MsArcB sensor kinase lacks the PAS domain, which contains the two redox-active cysteine residues critical for redox signaling by the *E. coli* ArcB protein. Moreover, it has been shown that unlike the *E. coli* ArcB, the *in vitro* kinase activity of ArcB of *M. succiniciproducens* is not affected by quinone compounds or by anaerobic metabolites. According to transcriptome profiles of ArcA overexpressed strain, the majority of 79 repressed genes were involved in energy metabolism and carbohydrate transport and metabolism, while the majority of 82 induced genes were involved in hypothetical or unknown functions. Our results taken together indicate that the Arc system of *M. succiniciproducens* is involved in adaptive redox dependent gene regulation in response to signals other than quinone compounds.
[Supported by the Intelligent Synthetic Biology Global Frontier Program, and the Next-Generation BioGreen 21 Program.]

F017

Identification and Characterization of the QseBC Two-Component System of a Rumen Bacterium

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Two-component signal transduction systems, which consist of a membrane bound sensor kinase and a response regulator, are highly conserved in nature and mediate adaptive responses to a variety of environmental changes. In this study, we identified and characterized the quorum sensing QseBC two-component signal transduction system of *M. succiniciproducens*. The purified N-terminally truncated QseC which was deleted for its transmembrane domain was able to autophosphorylate and transphosphorylate QseB, demonstrating that these two proteins are a functional sensor kinase and a response regulator, respectively. In an attempt to identify the target operons regulated by QseBC system, we investigated the genome-wide transcriptome profiles of *M. succinici-producens* in response to overexpression of QseB response regulator by using a whole genome DNA microarray. The up- or down-regulated genes by at least two-fold upon overexpression of QseB were chosen as the putative targets and further analyzed by electrophoretic mobility shift assay and reporter gene fusion expression.
[Supported by the Intelligent Synthetic Biology Global Frontier Program, and the Next-Generation BioGreen 21 Program.]

F018

Regulation of Stress Responses by Two-Component System in *H. polymorpha*

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The thermotolerant methylotrophic yeast *Hansenula polymorpha* is an attractive model organism for diverse fundamental studies, such as the genetic control of methanol metabolism, peroxisome biogenesis, nitrate assimilation, and resistance to heavy metals and oxidative stress. Here, to understand the regulatory mechanisms governing the osmotic or oxidative stress responses of *H. polymorpha*, we investigated roles of representative signaling and regulatory proteins. The hybrid histidine sensor kinases Sln1 and Nik1, a histidine-containing phosphotransfer protein Ypd1, response regulator proteins Skn7 and Ssk1, high osmolarity glycerol pathway regulator Hog1, and oxidative stress response regulator Yap1 were functionally characterized by mutant construction, growth phenotype comparison, protein overexpression, *in vitro* protein phosphorylation, and comparative transcriptome analysis. Our results indicate that the Skn7/NiK1-Ypd1-Skn7/Ssk2 two-component signal transduction pathway plays critical role in oxidative, osmotic, and cell wall stress responses in *H. polymorpha*.
[Supported by the Intelligent Synthetic Biology Global Frontier Program, and the Next-Generation BioGreen 21 Program.]

G001

Overexpression of Antisense RNA against the Phosphotransbutyrylase Gene in *Clostridium beijerinckii* NCIMB 8052

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Clostridium beijerinckii NCIMB 8052 is capable of producing 1-butanol through its acetone-butanol-ethanol fermentation. 1-Butanol is a chemical of interest as an advanced biofuel, and for industrial production this organism should be engineered to possess increased butanol yield and titer. Since organic acids and pH are key factors for 1-butanol biosynthesis, it is needed to study the physiology of *C. beijerinckii* strains in which the acid biosynthetic fluxes are altered. In the present study, we designed various types of antisense RNA for *in vivo* downregulation of C. beijerinckii phosphotransbutyrylase (*ptb*) gene and examined the knock-down efficiencies.
[This work was supported by the Advanced Biomass R&D Center of Korea (ABC-2011-0028386) through the Global Frontier Research Program of the Ministry of Science, Ict and Future Planning (MSIP). Further supports by BioFuelChem, EEWS program of KAIST, and the World Class University program (R32-2008-000-10142-0) of the MEST are appreciated.]

G002

Rational Design of Metabolic Pathway for the Production of Fumaric Acid

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In this study, *Escherichia coli* was metabolically engineered for the production of fumaric acid under aerobic condition. Firstly, the *iclR* and three known fumarase genes (*fumA*, *fumB* and *fumC*) were deleted to accumulate fumaric acid. The resulting strain was able to produce 1.45 g/L of fumaric acid from 15 g/L of glucose in flask culture. This base strain was further engineered by plasmid-based overexpression of the native *ppc* gene, encoding phosphoenolpyruvate carboxylase (PPC), based on *in-silico* aided prediction strategy, which resulted in the production of 4.09 g/L of fumaric acid. And then, the *arcA*, *ptsG*, and *aspA* genes were sequentially deleted to reinforce the flux to fumaric acid. The native promoter of the *galP* gene was replaced with the strong *trc* promoter to increase glucose uptake rate and fumaric acid productivity. Finally, 28.2 g/L of fumaric acid was produced by fed-batch fermentation.
[Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A2026556) funded by the Ministry of Education, Science and Technology]

G003

Gamma-butyrolactone Production by Chemical and Biological Method

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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 in succinic acid overproducing *M. succiniciproducens* LPK7 by the introduction of heterologous genes that encode succinyl-CoA synthetase, CoA-dependent succinate semialdehyde dehydrogenase, and either 4-hydroxybutyrate dehydrogenase in LPK7 (p3S4CD) or succinate semialdehyde reductase in LPK7 (p3SYCD). Fed-batch cultures of LPK7 (p3S4CD) and LPK7 (p3SYCD) 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 can be produced by the metabolically engineered *M. succiniciproducens*.
[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 and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556).]

G004

Identification of Flux-coupled Genes for the Improved Simulation Accuracy of Flux Balance Analysis

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A competitive advantage of flux balance analysis (FBA) using genome-scale metabolic network models is to use various numerical constraints to improve its simulation accuracy. In this regard, namely flux-coupled genes (FCGs) were searched: genes with expression levels changing in accordance with their flux values as the environmental condition changes. Seven most consistent FCGs (i.e., *gnd*, *pfkB*, *rpe*, *sdhB*, *sdhD*, *sucA*, and *zwf*) were identified from the comparative analysis of transcriptome and ^{13}C-flux data of *Escherichia coli* at five different dilution rates during its chemostat cultivations. Accuracy of FBA with FCGs was then compared with conventional simulation approaches (e.g. FBA without FCGs and MOMA). FBA with FCGs is straightforward to operate due to the relative easiness of obtaining transcriptional information.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012-C1AAA001-2012M1A2A2026556) and by the Bio & Medical Technology Development Program (2012048758) from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea.]

G005

Metabolic Engineering of *Clostridium acetobutylicum* for Enhanced Production of Butyric Acid by Switching to Acidogenic from Biphasic Fermentation

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C. acetobutylicum has been considered an attractive platform-host for biorefinery due to its metabolic diversity. Considering its capability to overproduce butanol through butyrate, it was thought that butyric acid can also be efficiently produced by this bacterium through metabolic engineering. In this study, *pta* and *ctfB* genes were knocked-out to block acetic acid production with protection of butyric acid re-uptake in *C. acetobutylicum*. *pta-ctfB* deficient *C. acetobutylicum* CEKW was assessed for its potential as a butyric acid-producer in fermentations with four controlled pH-values at 5.0, 5.5, 6.0, and 6.4. Furthermore, the CEKW strain was further engineered by knocking-out *adhE1* to prevent solvent-production. The simultaneous deletion of *pta-ctfB-adhE1* in *C. acetobutylicum* resulted in metabolic switch from biphasic to acidogenic fermentation, which enhanced butyric acid-production.
[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 and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556).]

G006

Metabolic Pathway Analysis for Efficient Succinic Acid Production

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Mannheimia succiniciproducens has intensively been studied due to its native capabilities to produce a substantial amount of succinic acid. We reconstructed a small scale model of *M. succiniciproducens* that consists of central metabolic reactions including biomass equation. Among the thousands of EMs, we designed a efficient approach, pathway clustering analysis, by clustering optimal EMs that have the higher succinic acid production without loss of growth rates to identify metabolic network. Pathway clustering analysis could present linear relationships with biomass or succinic acid in both *M. succiniciproducens* and *E. coli* metabolic network. In this proof-of-concept study, the biochemical network of *M. succiniciproducens* was rewired to improve succinate production by overexpressing *zwf* gene and *mdh* gene in LPK7 strain.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012-C1AAA001-2012M1A2A2026556) and by the Bio & Medical Technology Development Program (2012048758) from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea.]

G007

Biosynthesis of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by Metabolic Engineering

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Polyhydroxyalkanoates (PHAs) are bio-based polyesters accumulated in many bacteria. Among PHA copolymers, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] is one of the important copolymers. So far, for the production of P(3HB-co-3HV), adding of second auxiliary carbon source was needed. However, due to the toxicity of auxiliary carbon source, it is hard to maintain the balance between cell growth and P(3HB-co-3HV) production. Thus, we developed the *E. coli* can stably synthesize 3HB-CoA and 3HV-CoA in controlled ratio from glucose without feeding of exogenous auxiliary carbon source by metabolic engineering.
[This work was supported by the Technology Development Program to Solve Climate Changes from National Research Foundation of Korea (Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A2026556) and Intelligent Synthetic Biology Center (2011-0031963) of Korea through the Global Frontier Research Program of the Ministry of Education, Science and Technology (MEST). Further supports by the World Class University program (R32-2008-000-10142-0) of the MEST are appreciated]

G008

Altered Membrane Fluidities and Their Effects in Recombinant *Clostridium acetobutylicum* Strains

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C. acetobutylicum is a model organism of acetone-butanol-ethanol fermentation. However, butanol toxicity has been considered as the most limiting factor for achieving high butanol titer. To achieve high butanol tolerance, Cyclopropane fatty acid (CFA) synthase under control of acetoacetate decarboxylase promoter was introduced into *C. acetobutylicum*. The effect of CFA was investigated in flask and pH controlled fermentation and the membrane composition of cells was analyzed by MIDI analyzer. We observed that overexpression of CFA affects cell's physiology that leads higher butanol tolerance, cell rigidity and butanol productivity than control strain.
[This work was supported by the Technology Development Program to Solve Climate Changes from National Research Foundation of Korea (Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A2026556) and Advanced Biomass R&D Center of Korea (ABC-2011-0028386) through the Global Frontier Research Program funded by the Ministry of Education, Science and Technology (MEST)]

G009

Production of Polyhydroxyalkanoates (PHAs) Containing 2-Hydroxybutyrate (2HB) by Metabolic Engineered *Escherichia coli*

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PHAs are polyesters that have the properties of biodegradability, biocompatibility. *E. coli* strain was metabolically engineered to synthesize PHAs containing 2-hydroxybutyrate (2HB) monomer from glucose. PHAs consisted of 2HB, 3HB and a small fraction of lactate were synthesized. Also, heterologous metabolic pathway supplying 2HB from glucose was constructed via the citramalate pathway. Recombinant *E. coli*expressing the *phaC1437, pct540, cimA3.7, leuBCD* genes with the *L. lactis* II 1403 *panE* gene produced PHAs consisting of 2HB, 3HB, and a small fraction of lactate by varying the 3HB concentration in MR medium.
[This work was supported by the Technology Development Program to Solve Climate Changes (systems metabolic engineering for biorefineries) from the Ministry of Education, Science and Technology (MEST) through the National Research Foundation of Korea (NRF-2012-C1AAA001-2012M1A2A2026556) and by the Intelligent Synthetic Biology Center (2011-0031963) through the Global Frontier Research Program of the MEST. Further supports by the World Class University program (R32-2008-000-10142-0) of the MEST and by the R&D Program of MKE/KEIT (10032001) and KRICT are appreciated.]

G010

Au Particle-on-Wire SERS Sensor for the Identification of Multiple Pathogenic Bacteria

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Pathogenic bacterial infections are life-threatening, with high morbidity and mortality, but diagnostic methods for these pathogens still remain a challenge for simple, sensitive, specific and multiplex detection. Surface-enhanced Raman scattering (SERS) has been considered as an attractive method for label-free multiplex DNA detection because of its single molecule level sensitivity, molecular specificity, and insensitivity to quenching. Here, we developed a SERS-based Au particle-on-wire system for the identification of pathogenic bacteria. The system operates by the self-assembly of Au NPs onto Au nanowire in the presence of target DNAs, providing reproducible SERS signals. A pattern formed by multiple Au nanowire sensors provides positional address and identification for each sensor. By using this system, multiplex sensing of target DNAs was possible in a quantitative manner with a detection limit of 10 pM. Target DNAs from reference bacteria and clinical isolates were successfully identified by this sensor system.
[Supported by grant from World Class University Program of MEST]

G011

Synthetic Regulatory Small RNA for Fine-Tuning Gene Expression in *Escherichia coli*

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Optimized modulation of metabolic fluxes through the control of gene expression is one of the key challenges in metabolic engineering. Here, we developed rational design principles for synthetic regulatory small RNAs (sRNAs) for adjustable expression control. We then expanded our method to create a system utilizing synthetic sRNAs as a portable and conditional chromosomal gene controller, and engineered *Escherichia coli* to produce tyrosine and cadaverine as a model. An engineered *E. coli* strain capable of producing 21.9 g/L of tyrosine was developed by combinatorial knockdown experiments on various candidate genes in 14 different strains using respective synthetic sRNAs. As another example, this strategy was applied to an already metabolically engineered strain producing cadaverine by applying a library of 130 synthetic sRNAs. The feasibility of using synthetic sRNAs to modulate gene expression holds great promise in next-generation metabolic engineering and synthetic biology applications.
[Supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from MSIP through NRF of Korea]

G012

Bacillus licheniformis* Isolated from Korean Traditional Food Resources Induces the Host Immune Response in *Caenorhabditis elegans

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Here, we investigated whether probiotic bacteria *B. licheniformis* strains isolated from Korean traditional food resources in the Institute of Sunchang Fermented Soybean Products, influence the immune response of *C. elegans* as surrogate host model. Initially, we found that twenty-four probiotic *Bacillus licheniformis* strongly produced antimicrobial bacteriocins against to various foodborne pathogens including *B. cereus* and *Staphylococcus aureus*. Next we explored if bacteriocin-producing *B. licheniformis* can augment the *C. elegans* defense response to *S. aureus*. First, we evaluated that *B. licheniformis* strains are not harmful to *C. elegans in vivo* host. And then, worms were pre-conditioned by transferring young adult worms to *B. licheniformis* lawns for 24 h and then transferring to *S. aureus* RN6630 using solid killing assays with *fer-15;fem-1* worms.

G013

Screening of an Enantioselective Epoxide Hydrolase from Marine Microorganisms

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Gyeongbuk Institute for Marine Bio-Industry (GIMB)

Enantiopure epoxides and vicinal diols are versatile synthetic intermediates for the preparation of enantiopure bioactive compounds. One of the most promising ways for preparing such chiral synthons under environmentally gentle conditions is the enantioselective hydrolysis of racemic epoxides using cofactor-independent epoxide hydrolase (EHase; EC 3.3.2.3). EHases are ubiquitous enzymes that have been isolated from a wide variety of sources such bacteria, yeast, fungi, insect, plant and mammalian. To screen strains producing an EHase which hydrolyzes (*R*) or (*S*)-epoxide preferentially, 4 strains of bacteria isolated from marine sediments and seaweed primarily by the capability of living on styrene oxide were tested for EHase activity using gas chromatography (GC). Among those, one strain was selected by enantioselective hydrolysis of styrene oxide, confirmed by GC. The EHase from one strain preferentially hydrolysed the (*R*)-epoxide of styrene oxide, with a value of 99.9% ee (enantiomeric excess).
[This work was supported by GIMB in-House R&D Program and the bioresources in this study were provided from Marine & Extreme Bioresources Collection.]

G014

Production and Characterization of a Sialoglycoconjugate Binding Lectin Derived from a Mushroom

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Sialic acids are a family of nine carbon α-keto aldonic acids, which are occupied at the non-reducing end of glycoconjugate, but the diversity of sialoglycoconjugate is one of huddle to detect the carbohydrate moiety in glycoconjugates. Lectins with carbohydrate binding specificity are powerful tools to detect glycoconjugates. To study the functional analysis of a sialic acid specific binding lectin from a mushroom, the lectin-coding gene was expressed in the methylotrophic yeast *Pichia pastoris* and in *Escherichia coli*. Approximately a few milligram of recombinant lectin was purified per liter medium. Lectin blot analysis with ConA-lectin and endoglycosidase treatment revealed that the yeast produced lectin was partly *N*-glycosylated and the lectin polypeptide contains N-glycans which is the most yeast abundant high-mannose structures. Glycan binding analysis showed that the recombinant lectin interacts with fetuin containing both N-/O-linked sialoglycoconjugates. A mushroom lectin will be a used as a powerful tool to detect sialic acid binding specificity compared with other glycan-binding lectins.
[Supported by the grant (No. PJ009783), Rural Development Administration, KOREA]

G015

Isolation of Biogenic Amines Non-producing *Saccharomyces cerevisiae* for Manufacturing Mulberry Wine

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Sunchang Research Center for Fermentation Microbes (SRCM)

Mulberry fruit, commonly called Oddi in Korea, recently has been used for manufacturing wine, fruit juice, and jam. According to traditional Oriental medicine, mulberry fruits can protect against liver and kidney damage, strengthen the joints, improve eyesight, premature graying of hair, nourish the yin and blood, and have anti-aging effects. In the present study, we isolated the *S. cerevisiae* BA33 as biogenic amines non-producing strain for manufacturing the mulberry wine from mulberry fruit juice, and then investigated the morphological characteristics, biogenic amine producing ability, and alcohol fermenting ability, and resistance of alcohol, glucose and sulfur dioxide. We isolated biogenic amine non-producing yeast BA33. Using the 18S rRNA sequencing and API kit, BA33 was confirmed S*accharomyces cerevisiae.* Nextly, BA33 was produced alcohol of 12.333%, and confirmed resistance of alcohol, glucose and sulfur dioxide. Finally, BA33 strain was confirmed to be the useful yeast which can be used for the manufacturing the mulberry wine.
[Supported by grants from "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009990032014)" RDA.]

G016

Statistical Optimization of Culture Medium Biogenic Amines Non-producing *Saccharomyces cerevisiae* for Manufacturing Mulberry Wine

Hee-Jong Yang, Su-Ji Jeong, Seong-Yeop Jeong, Ju-Hee Heo, and Do-Youn Jeong*
Sunchang Research Center for Fermentation Microbes (SRCM)

Mulberry fruit, commonly called Oddi in Korea, recently has been used for manufacturing wine, fruit juice, and jam. Based on preliminary study, we investigated cell growth, and optimization of culture medium compositions for improving the dried cell weight in *S. cerevisiae* BA33 using response surface methodology as statistically method. RSM was used central composite design, and molasses having ability of industrial application was used as carbon source. Through the statistically analysis, we obtained the optimum values were: molasses 20.0% (w/v), peptone 3% (w/v), and yeast extract 2.17% (w/v). Result of model verification, we confirmed about three-fold improvement of the dried cell weight from 7.81±0.1217 g/L to 22.1033±0.2915 when compared to the dried cell weight using basal YPD medium. Finally, we were manufactured mulberry wine using the selected strains BA33, and produced 17.73% alcohol. BA33 strain was confirmed to be the useful yeast which can be used for the manufacturing the mulberry wine.
[Supported by grants from "Cooperative Research Program for Agriculture Science &Technology Development (Project No. PJ009990032014)" RDA.]

G017

Isolation of Biogenic Amines Non-producing *Saccharomyces cerevisiae* for Manufacturing *Rubus coreanus* (Black raspberries) Wine

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Sunchang Research Center for Fermentation Microbes (SRCM)

Rubus coreanus are known as Korean black raspberry, native to Korea, Japan, and China. Preliminary studies to evaluate their benefit for cancer treatment in mammalian test systems are ongoing. Especially, black raspberries are of significant interest because they contain high levels of anthocyanins. Anthocyanins in black raspberries are important because of their potential health benefits as dietary antioxidants, anti-inflammatory compounds, and/or chemopreventive agents. In the present study, we isolated more than 300 yeast, and selected biogenic amine non-producing yeast BA29 in manufacturing mulberry wine. Using the 18S rRNA sequencing and API kit, BA29 was confirmed S*accharomyces cerevisiae.* Nextly, BA29 was produced alcohol of 13.696%, and confirmed resistance of alcohol, glucose and sulfur dioxide. Finally, BA29 strain was confirmed to be the useful yeast which can be used for the manufacturing the black raspberry wine.
[Supported by grants from "Cooperative Research Program for Agriculture Science &Technology Development (Project No. PJ009990032014)" RDA.]

G018

Statistical Optimization of Culture medium Biogenic Amines Non-producing *Saccharomyces cerevisiae* for Manufacturing *Rubus coreanus* (Black raspberry) Wine

Su-Ji Jeong, Hee-Jong Yang, Seong-Yeop Jeong, Ju-Hee Heo, and Do-Youn Jeong*
Sunchang Research Center for Fermentation Microbes (SRCM)

Black raspberry are of significant interest because they contain high levels of anthocyanins. Based on preliminary study, we investigated cell growth, and optimization of culture medium compositions for improving the dried cell weight in *S. cerevisiae* BA29 using response surface methodology as statistically method. RSM was used central composite design, and molasses having ability of industrial application was used as carbon source. Through the statistically analysis, we obtained the optimum values were: molasses 20.0% (w/v), peptone 3% (w/v), and yeast extract 4% (w/v). Result of model verification, we confirmed about three-fold improvement of the dried cell weight from 8.6±0.0808 g/L to 20.9167±0.7925 when compared to the dried cell weight using basal YPD medium. Finally, we were manufactured mulberry wine using the selected strains BA33, and produced 20.33% alcohol. BA29 strain was confirmed to be the useful yeast which can be used for the manufacturing the black raspberry wine.
[Supported by grants from "Cooperative Research Program for Agriculture Science &Technology Development (Project No. PJ009990032014)" RDA.]

G019

Determination of Anti-inflammatory Peptides in Chungkookjang

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Chungkookjang, Korean traditional fermented soybean, contains isoflavones and diverse peptides which are produced during fermentation. Since Chungkookjang is known to prevent inflammation, we tried to find anti-inflammatory peptides among its diverse peptides. Yellow soybean was fermented with *Bacillus licheniformis* B1. Peptide 1(4mer) and peptide 2(7mer) were purified with HPLC. Peptide 1 and peptde 2 suppressed the expression of inflammatory cytokines iNOS, TNFα and IL6. Daidzein in Chungkookjang also suppressed the expression of iNOS. Since anti-inflammatory peptides were found in our Chungkookjang, it can be developed as a functional food.
[Supported by grants from The Research Institute for Basic Science, Hoseo University]

G020

Preliminary Study on the Dextran Production by Psychrotrophic *Leuconostoc mesenteroides* CS-5 Growing at a Low Temperature

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Characterization of *Leuconostoc mesenteroides* strains growing at a low temperature ranged from 14°C to 30°C was attempted in this study. Among isolates from kimchi, a strain named as CS-5 showed a narrow optimal temperature that ranged from 24°C to 28°C and the strain was characterized as *Leuconostoc mesenteroides*. Interestingly the CS-5 was turned out to be a dextran producing strain. It could grow in the media containing 6% NaCl. From the disaccharide fermentation test, salt tolerance of the CS-5 was increased in media containing sucrose or maltose but its ability was decreased in media containing trehalose, melibiose or cellobiose. In order to improve dextran production of the CS-5, viscosity of the media was measured by a Reometer system. Higher values of viscosity (0.153 $Pa.s^n$) were obtained in media containing 30% sucrose (w/v) comparing to the control (0.01 $Pa.s^n$). Optimum temperature for dextran production was 22°C rather than 25°C. When 1.5% of skim milk was added to the media, its consistency index was enhanced by twofold (0.3 $Pa.s^n$). From a time course study, the maximum production of dextran was obtained as 0.57 $Pa.s^n$ at 48 h incubation rather than 72 h incubation.

G021

Shotgun Membrane Phosphoproteomic Analysis of Cyanobacteria

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In the present study, we attempted to enrich the cyanobacterial membrane and determine the phosphorylation sites of the membrane proteins. Total cyanobacterial membranes were separated on SDS-PAGE gel and phosphoprotein-stained gel bands were subjected to in-gel trypsin digestion. Phosphorylation sites of resultant peptides were determined by assigning the neutral loss of [M-H_3PO_4] on serine, threonine, and tyrosine residues by using 7T FT-ICR MS. As an initial application, 111 proteins and 33 phosphoproteins were identified in which contained 11 integral membrane proteins. Four unknown phosphoproteins with transmembrane helices were proposed to be membrane migration or transporter on the basis of functional information by BLASTP search. Overall neighboring hydrophobic amino acids were rich around pSer and pThr whereas hydrophilic amino acids were the highest at -1 position around pTyr. The global membrane phosphoproteomic analysis will provide the insight of fundamental regulation process and the comprehensive understanding for the functional phosphoprotein network of cyanobacteria.
[This work was supported by the grants from Korea Basic Science Institute (D34403).]

G022

Engineered *Pichia pastoris* for Bioethanol Production by Synergistic Lignocellulosic Degradation Activity by Xylanase and Cellulase

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The utilization of lignocellulosic feedstocks for production of ethanol demand on cellulose and hemicellulos. In this study, *Pichia pasotoris* is used for ethanol production by degrading of xylan and cellulose. *P. pastoris* was engineered for expression of recombinant scaffolding protein (mCbpA), xylanase (XynB) and chimeric endoglucanase (cCelE). The mCbpA, XynB and cCelE with gene coding for the secretion signal sequence of the α-mating factor were highly expressed in the yeast *P. pastoris* under the control of the ADH2-Promoter, which is activated under O_2 limitation. The enzyme complexes via assembled cohesin-dokerin interaction increase the activity against the biomass substrate compared the corresponding wild type *P. pastoris*. Ethanol production of recombinant *P. pastoris* was 1.4-folds higher than that of wild type X-33. This means is what we successfully synergistic produce ehtnaol from cellulose and xylan by enzyme complexes. Based on these results, this recombinant *P. pastoris* is suitable for next generation biofuel production systems.
[This work was supported by the New & Renewable Energy Technology Development Program of KETEP grant funded by the MKE (No.20113010090040)]

G023

Why We Focus on NPP-specific Polyene Glycosyltransferase in *Pseudonocardia autotrophica*?

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Polyene macrolide antibiotics such as nystatin and amphotericin B produced by actinomycetes are clinically potent antifungal drugs to treat systematic fungal infection, yet limited in application due to severe toxicity and low solubility. In this reason, we need to develop new polyene-based antibiotics with improved properties. Previously, we discovered a novel polyene compound named NPP (Nystatin-like Pseudonocardia Polyene) that is consist of aglycone identical to nystatin and unique di-sugar moiety, mycosaminyl-(α1-4)-*N*-acetyl-glucosamine in rare actinomycetes, *Pseudonocardia autotrophica*. Compared with nystatin which bears a mono sugar moiety, NPP has 300-fold higher water solubility and 10-fold reduced toxicity to human. These results were caused by second sugar of NPP and that is why we focus on NPP-specific glycosyltransferase. To identify the glycosylation mechanism of NPP biosynthesis, we analyzed its whole genome sequence and found the putative NPP-unique glycosyltransferase genes. Furthermore we performed the targeted gene disruption, complementation of the target genes. The more detailed results will be discussed.
[This work is supported by grants PJ009522 from RDA.]

G024

Biosynthesis of *cis,cis*-Muconic Acid in *E. coli* through the Codon-optimized Synthetic Gene Expressions

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Adipic acid is primarily used as a precursor for the synthesis of nylon, lubricants, and plastic. It is mainly produced in chemical processes from petrochemicals like benzene. Because of depleting petroleum reserve, recurring crisis and heavy environmental impact, it is necessary to develop various biotechnological production processes which would provide alternative approaches. Here we describe the engineered *Escherichia coli* strain harboring the synthetic foreign pathways involved in *cis,cis*-muconic acid (CCM) biosynthesis, which can be chemically dehydrogenated to adipic acid. The synthetic pathway consists of three *E. coli* codon-optimized genes encoding heterologous microbial enzymes such as 3-dehydoshikimate dehydratase, protocatechuic acid decarboxylase, and catechol 1,2-dioxygenase. The engineered *E. coli* strain produced a significant amount of CCM in the liquid culture, implying that this strategy paves the way for the microbial synthetic biotechnology for the industrial production of CCM.
[This work was supported by the Technology Innovation Program (10043985, Development of bio-muconic acid production process) funded By the Ministry of Trade, industry & Energy (MI, Korea)]

G025

Immobilization of Hyperthermophilic Archaeon *Thermococcus onnurineus* NA1 on Amine-coated Silica Material for H_2 Production

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Hyperthermophilic archaeon, *Thermococcus onnurineus* NA1 has high potential for H_2 production on carbon monoxide, sodium formate and starch. For H_2 production using immobilization of *T. onnurineus* NA1, celite and porous silica beads as supporting materials were investigated to adsorb cells of this strain. Experimental results of adsorption test and scanning electron microscopy showed that amine-coated porous silica beads are favorable for adsorbing cells of *T. onnurineus* NA1. In repeated batch on sodium formate, immobilized *T. onnurineus* NA1 on the support showed the stability and reproducibility for H_2 production. From this study, it was demonstrated that *T. onnurineus* NA1 can be simply immobilized by adsorption using amine-coated porous silica beads and H_2 production using immobilized *T. onnurineus* NA1was feasible.
[Supported by grants from KIOST in-house program (PE99212) and the Development of Biohydrogen Production Technology Using the Hyperthermophilic Archaea program of the Ministry of Oceans and Fisheries in the Republic of South Korea.]

G026

High-throughput Retrieval of Sequence-verified DNA for Genome-scale Engineering

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De novo gene synthesis has provided powerful tools to study novel genes in microbiology and utilize them for producing valuable bio-functional materials. Moreover, recent progresses in gene synthesis, for example, chemically synthesized genome that controls heterologous bacterial host, pave the way to efficiently engineer molecular pathway in genome scale. Generally, the *de novo* construction of gene consists of two steps; high-fidelity DNA oligonucleotide (oligo) synthesis followed by assembly of oligos. In spite of matured technologies for assembling oligos or larger gene fragments, methods for obtaining high-fidelity oligos rest on 1990's technologies – solid-phase DNA synthesis on controlled-pore glass substrate, cloning in microbe and Sanger sequencing. Thus, the high cost precursor preparation process hinders genome-scale *de novo* gene construction. Here, we present high-throughput sequence-verified DNA synthesis technology for next-generation gene and genome synthesis. By using microarray-derived oligos and massively parallel sequencing, we prescreen sub-million molecular clones at once. After identifying error-free clones, we retrieve them with laser-based DNA extraction system, which enables the retrieval of 2,000 error-free clones in a single day. As demonstration of its scalability, we synthesize 4,345 high-fidelity 120 bp DNA that can be assembled to 200 protein-coding genes at a single run.
[This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (NRF-2012-0009555).]

G027

Isolation and Identification of Probiotic Bacterial Species from Korean Yellow Loess Agricultural Soils

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In the present study, we isolated an aerobic probiotic bacterial species from Korea Agricultural Yellow Loess Soil. A serially diluted soil samples were spread on M17 agar plate and incubated at 30℃ under aerobic condition for 2 days. After incubation, one bacterial isolates was found to be the aerobic probiotic bacteria and identified through Gram positive, and catalase negative. The 16S rRNA sequence was performed to construct phylogenetic tree. From the phylogenetic relations the isolate was identified as *Lactobacillus murinus*. This bacterial isolate was used to find out antibacterial and antifungal activity phytopathogens. From experimental results, the L. *murinus* showed highest antibacterial activity against MRSA, *E. coli*, *S. aureus*, *Listeria monocytogenes*. On the other hand, the antifungal activity was also showed high activity against C. *glaeaspariaides*, *F. culmorum, A. alternata, F. oxysporumand* and *S. sclerotiorum*. Therefore, from this study it was revealed the probiotic bacterial isolate has potent antibacterial and antifungal activity and also this isolate have beneficial aspects in agricultural sector.

G028

Isolation and Identification of Novel Chitinase Producing Bacterial Species from Yellow Loesses Agricultural Soils

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Recently, bacterial chitinase has received much attention towards agriculture sector for biocontrol of phytopathogenic fungal species. In the present study, 40 bacterial isolates were screened for chitinolytic activity and on the basis of chitin hydrolysis zone. Initially, isolation of chitinase producing bacterial sps., was performed using 0.5% swollen chitin enriched media. The chitinase producing bacteria was screened with clear zone appearance in chitin agar plates. Among bacterial isolates, 6 bacteria were found to be the highest zone of clearance (>30 mm) was selected for further studies. The bacterial species were identified by biochemical tests and 16S rRNA, eubacterial and chiA. From the experimental results, we found 4 *Bacillus* sp., and 2 *Serratia* sp. The selected bacterial isolates were used to find out the antifungal activity against various phytopathogenic fungi. In the concluding remarks, all 6 selected isolates showed effective antifungal activity against plant pathogenic fungal sps. Therefore, it may be applicable to field condition against plant pathogenic fungi which is the major problem for agricultural food production.

G029

Characterization of Bacteriocin Produced by *Lactobacillus plantarum* SY222 Isolated from *Jeotgal*

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LAB having antibacterial activity against *Bacillus cereus* KCTC 3624 was isolated from *Jeotgal* using a MRS selective plate. Strain SY222 finally selected was identified as *Lactobacillus plantarum* based on sugar fermentation pattern test using API 50 CHL system. Also, the 16S rDNA sequence of strain SY222 showed 99% identity to those of reference strain of *L. plantarum*. The bacteriocin exhibited inhibitory activity against the food pathogen, *Escherichia coli* CFT073 ATCC 700928, *Bacillus cereus* KCTC 3624, *Staphylococcus aureus* KCTC 1928, *Salmonela Enterica* KCTC 1925, *Salmonela Enterica* KCTC 1926, *Shigella flexneri* KCTC 2517, *Shigella sonnei* KCTC 2518 and *Staphylococcus epidermidis* KCTC 3958. Antibacterial activity of the bacteriocin was completely disappeared by proteinase K, which indicates its proteinous nature. The bacteriocin was inactivated by protease such as trypsin, chymotrysin, subtilisin, α-amylase, pepsin. The bacteriocin was fully stable at 121°C for 60 min. Solvents such as chloroform, ethanol, acetone, acetonitrile, hexane, isopropanol did not effect on the activity. The molecular weight of bacteriocin was estimated to be about 1.5 kDa by Tricine-SDS-PAGE.

G030

Characterization of a Bacteriocin Produced by *Leuconostoc mesenteroides* SY111 Isolated from Vegetable *Kimchi*

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Bacteriocin-producing Lactic acid bacterium having antagonistic activity against *Bacillus cereus,* was isolated from *Kimchi,* was identified as *Leuconostoc mesenteroides* on the basis of transmission electron microscope, carbohydrate fermentation reactions were recorded by using API 50 CHL test, 16S rDNA sequencing analysis and a phylogenetic tree and named as *Leu. mesenteroides* SY111. The activities of various enzymes were investigated using the API 20 ZYM system, was observed in Leucine arylamidase, Valine arylamidase, Crystine arylamidase, β-Glucuronidase, β-glucosidase and N-acetyl-β-glucosaminidase. The bacteriocin also showed a relatively broad spectrum of activity against non-pathogenic and bacteriocin exhibited inhibitory activity against the food pathogen, *Bacillus cereus, Salmonella. enterica*, and *Micrococcus luteus*. The antimicrobial substance retained activity after exposure to 121 for 30 min or pH 3.0–12.0. Solvents such as acetone, chloroform, ethanol, isopropanal, acetonitrile, and methanol had little effect on bacteriocin activity. The molecular weight of bacteriocin was estimated to be about 3.2 kDa by Tricine-SDS-PAGE.

G031

Evaluation of a Highly Efficient Reporting System Employing a Directed-Evolved β-Glucosidase

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As a part of synthetic biology, reporter genes can be widely used to detect or probe the expression of a gene of interest, especially when the target gene is poorly expressed and thus produced proteins that could not detect obviously by a typical technique. The second and more valuable aspects of employing reporter systems are able to quantify the level of expression of a gene by comparing with a readily identified product. Thus, a lot of reporter systems have been developed and expected to be further proliferating. Typically, the successfully implemented reporting techniques are closely linked with fluorescent sensors mainly derived from GFP. However, it needs the time-consuming maturation step of fluorophore and prerequisites for exposing the samples or clones to toxic UV to excite the fluorophore. Here, we present a highly efficient reporting system using a versatile β-glucosidase. This system produces a sensitive and reproducible colored or fluorescent signal according to the incubated substrate supplemented, allowing it to be detected and quantified. The fusion ability of this reporter could broaden the range of application more practically.
[Supported by grants from NRF]

G032

A Constructed Genetic Circuit Using Antisense RNA for the Screening of AHL Degradase

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As recognized generally, bacterial resistances to antibiotics have become a serious medical problems and thus many efforts are being made to develop antibiotic alternatives. A possible alternative for the treatment of bacterial infections is a kind of chemical reagents or enzymes blocking bacterial communication. In fact, it has been recently reported that quorum quenching enzymes like AiiA significantly attenuate the infectivity of bacterial pathogens and biofilm formation. Therefore, an efficient method to screen these quorum-quenching enzymes from biological resources is promising and thus highly needed. Here, we suggest an artificial genetic circuit as a screening tool for the detection of active clone expressing AiiA gene. The genetic circuit, pScreening, is constructed by the combination of quorum sensing and ansens RNA-reporter module, which shows reporter signals depending on AHL-degrading activity. This screening circuit enables access rapidly and quantitatively to the positive mutant AiiA among clones in a highly reproducible manner. By using this circuit, we could screen the mutant AiiA with improved activity from directed-mutagenesis pool.

G033

Displaying Specific Affinity Ligand on the Surface of Minicells Using Anchoring Proteins

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In recent decades the idea that a drug delivery system using bacterial minicells or similar nano-carriers for tumor targeting have been attempted and evaluated further potential for practical applications. In most cases of these studies, cancer cell-specific antibody was used as a targeting ligand by conjugation to the surface of delivery vehicles. These systems, however, the tedious process of coupling antibody with some specific receptor on the surface of minicells is prerequisite for targeting cancer. There is also a problem that the targeting efficiency was highly fluctuated depending on the conjugation process. Here we developed bioengineered minicells that could target cancer cells by functional display of anti-HER2 affibody and anti-EGFR repebody on the bacterial surface. Among the various anchoring systems, we used an autotransporter proteins (OmpC) and ice nucleation proteins (INP). The surface display and localization of both OmpC-affibody (repebody) and InaV-affibody (repebody) was demonstrated by western blot analysis. Further analyses of targeting effects using FACS provided some promising results for practical applications when compared to that of minicells (NC).

G034

A Genetic Circuit Designed to Express Continuously the Gene Encoding a Drug Protein *In Vivo* by Positive Feedback Loop

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The rational and model-guided construction of biological parts enables us pave a new way to develop therapeutic platforms. Here we present a novel genetic circuit strategy that is designed to induce the continuous expression of anti-leukemia drug by using the ligand-independent activity of a transcription factor. For this purpose, we first constructed an artificial, but functional, transcription factor ($AraC^c$) by rational design, which was no longer needed an inducer to bind to its own promoter P_{BAD}. Then we established a novel circuit that operated by self-positive feedback activity of $AraC^c$. This system could generate target gene transcription persistently when the initial expression of $AraC^c$ is triggered. Additionally, directed evolution of $AraC^c$ increased the sensitivity of the feedback loops, providing a more promising system that was protractedly activated during repeated batch of cell culture. This type of a synthetic circuit could facilitate the delivery of drugs into the target cells in favorable condition because it eventually set the stage for expression of drugs from memorizing cells by auto-activating transcription factor.
[Supported by grants from NRF]

G035

Fermentation and Quality Characteristis of *Cheonggukjang* Fermented with *Bacillus* Strains

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The object of this study was to improve the quality of Cheonggukjang with new starter, *Bacillus* stains. 9 kinds of strains were prepared by applying the Chungkookjang fermentation was for 48 h at 37 degrees. Soybean produced by each strain component analysis of the general quality (Moisture content, pH, Salinty, nitrogn content, free amino acid) attributes such as flavor and enzyme activity were examined. The enzymatic activities were analyzed with clear zone test. Antioxidant activity was also measured and compared. Using strain exhibited very different characteristics.

G036

Comparative Genomics and Parallel Evolution Reveals Toxicity-Circumventing Mechanisms from Membrane Protein Overexpression

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Membrane proteins constitute up to 30% of the total proteins in a cell, and play important roles in metabolism. Though overexpression of membrane proteins is pivotal for the studies of biochemical, structural, and functional aspects, it is considered a major bottleneck due to their toxicity to the host cell and other complications. To uncover the genetic background of toxicity-escape mechanism of *Escherichia coli* C41(DE3) and C43(DE3) derived from the common protein expression host BL21(DE3), we sequenced their genomes. Comparative genome analysis with their ancestral strain revealed various genetic changes. In other efforts, a series of evolved *E. coli* mutants producing membrane proteins were generated and mutation hotspots were identified. Through these combinatorial approaches, we found genetic changes linked to the reduction of cell toxicity caused by overexpression of membrane proteins. These results shed light on understanding the factors that contribute to overcoming the toxicity during membrane-protein overexpression, and could be applied to the development of a better expression system.
[Financial support from the Global Frontier Program for Intelligent Synthetic Biology]

G037

In Silico Structural and Functional Characterization of Cellulases

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Cellulose is an abundant renewable organic resource with a great deal of potential for high-value products useful in biotech industry and agriculture. Cellulases belong to glycoside hydrolases (GHs), which hydrolyze the glycosidic bond between two saccharide groups or between a carbohydrate moiety and a noncarbogydrate one. Pervious classification schemes have been based on substrate specificities of an enzyme, but the same protein family fold may harbor several types of specificities. In this study, we identified and characterized 9,095 cellulases from 715 species (Eukaryota: 258, Bacteria: 432, Archaea: 24, and Virus: 1) by a robust computational approach. Enzymatic function of GHs usually was found to occupy more than half of the length of a cellulase sequence, located at the N-linked region. Some of them have been shown in many of the carbohydrate binding modules and other protein domains, such as dockerin, on O-linked glycosylated residues. Phylogenetic analyses indicated the non-monophyletic distribution of cellulases and that many GH domains may have undergone horizontal gene transfer. The Ω loop on the surface of cellulases has shown to be highly conserved at its base.

G038

A Rapid Antimicrobial Susceptibility Test based on Single Cell Morphological Analysis

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The rapid antibiotic susceptibility test (RAST) is desperately needed in clinical area for fast and proper antibiotic administration. The traditional AST cannot cope with urgent cases of bacterial infection and antibiotic resistance owing to relatively long test time. Even though many new methods have been tried, they are not practiced in the clinic. As a significant breakthrough, we suggest a novel AST method called Single Cell Morphological Analysis (SCMA) to determine antimicrobial susceptibility by analyzing and categorizing morphological changes of single cell in various antimicrobial conditions. When four CLSI standard strains and 189 clinical samples including Extended-spectrum β-lactamase (ESBL) E. coli and K. pneumoniae, IRPA, MRSA and VRE from hospitals were tested with SCMA, the AST results were obtained only in 3~4 hours showing 93.3% categorical agreement and 6.2% minor, 0.4% major and 0.5% very major discrepancies to satisfy the recommendation of FDA. SCMA offers a rapid and accurate AST results as an available method.

G039

Pharmacodynamics of Marbofloxacin against _Actinobacillus pleuropneumoniae_

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The purpose of this study was to investigate *in vitro* the pharmacodynamics of marbofloxacin against 20 *Actinobacillus pleuropneumoniae* (APP) strains. Broth microdilution testing was used to determine the Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) and multi-step resistance selection range were 0.00391-32 μg/ml. For time-kill experiments, colony counts were determined by plating each diluted sample onto plate count agar and an integrated pharmacokinetic/pharmacodynamics area measure (log ratio area) was applied to all cfu data. The range of MIC and MBC were 0.03152-1 and 0.0625-8 μg/ml, respectively. Furthermore, we will discuss in terms of *ex vivo* time killing study against APP strains in detail.

H001

Establishment of Washing Method for Accurate Transduction Assay and a Novel Transduction Peptide from a DNA-binding Protein in *Blumeria graminis*

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Recently, research on peptide drugs as anticancer therapy agents or vaccine components has been increasing importance. Concomitantly, intracellular delivery using cell-penetrating peptides (CPPs) has received major attention as a novel function. Over twenty years, hundreds of different peptide sequences have been described within the CPP classification. However, since the strong tendency of cationic CPPs to associate with the plasma membrane leads to an over-estimation of cellular uptake, establishment of the washing method is important for the accurate assessment. In this study, we set up the washing method for measurement of the transduction and found a novel CPP from a DNA binding protein in *Blumeria gramis*, which is a fungus that causes powdery mildew on grasses, including cereals such as barley powdery mildew and corn mildew. The basic residue rich peptide from the partial region of the protein had the potential to have cell-penetrating ability. The details will be presented in the meeting.
[Supported by grants from NRF]

H002

Genome Sequences of Vancomycin-producing Strains of *Amycolatopsis orientalis*

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Vancomycin, a natural glycopeptide antibiotic produced by the soil bacterium *Amycolaptosis orientalis*, is world-widely used for the treatment of serious infections by Gram-positive bacteria that are resistant to other antibiotics. Recent synthetic biology approaches combined with genome engineering will enable us to obtain high yields of the antibiotic production. Toward this end, we determined the genome sequences of four *Amycolaptosis* sp. strains (DSM 40040, DSM 43388, DSM 46075, and KCTC 9412) using the Next-Generation Sequencing (NGS). The comparative genome analysis of these four strains reveals several glycopeptide antibiotic synthetic modules and host-resistant genes, reflecting their distinct cellular physiologies and different production yields for vancomycin. In addition, average nucleotide identity and whole-genome alignment analyses based on these genome sequences provide evolutionary relationships in *Amycolatopsis* strains. Therefore, this study provides us the molecular basis of the biosynthesis of antibiotics, which is of great importance for the large-scale industrial production of vancomycin.
[This study was supported by the Small and Medium Business Administration]

H003

Growth Phase-Dependent Roles of Sir2 in Oxidative Stress Resistance and Chronological Lifespan in Yeast

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Silent Information Regulator 2 (Sir2), a conserved NAD^+-dependent histone deacetylase, has been implicated as one of the key factors in regulating stress response and longevity. Here, we report that the role of Sir2 in oxidative stress resistance and chronological lifespan is dependent on growth phase in yeast. In exponential phase, *sir2*Δ cells were more resistant to H_2O_2 stress and had a longer chronological lifespan than wild type. By contrast, in post-diauxic phase, *sir2*Δ cells were less resistant to H_2O_2 stress and had a shorter chronological lifespan than wild type cells. Similarly, the expression of antioxidant genes, which are essential to cope with oxidative stress, was regulated by Sir2 in a growth phase-dependent manner. Collectively, our findings highlight the importance of the metabolic state of the cell in determining whether Sir2 can protect against or accelerate cellular aging of yeast.
[Supported by grants from Korean Government (NRF-2010-0013086) and Ministry of Trade, Industry and Energy (R0001989)]

H004

Mortality Due to *Megalocytivirus* in Rock Bream (*Oplegnathus fasciatus*) can be Controlled by Regulation of Water Temperature and Survivors Obtain Protective Immune

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Repeated outbreak of rock bream iridovirus (RBIV) disease in summer causes huge losses to aquaculture industry especially to rock bream (*Oplegnathus fasciatus*). Rock bream injected with RBIV at fixed water temperatures (29, 26, 23 and 20°C) had 100% mortality. However, all fish survived at 17°C until 100 days post infection (dpi). In water temperature shifting experiment, mortality rates of rock bream exposed to virus for 2 d, 4 d and 7 dpi at 23/26°C before reduced to 17°C were 26.6/73.2%, 66.6/100% and 93.4/100%, respectively until 100 dpi. Survived fish transferred to 26°C at 100 dpi did not show any disease signs with low virus copy (below 10^3). In other experimental set shifting of water temperature, by lowering the water temperature from 23°C to 17°C at 4 dpi, all fish were survived. High survival rates of fish on re-infection of RBIV indicating that protective immunity and exhibit possibility in developing a long term preventive measure against RBIV. In this study, we confirmed that RBIV mortality highly correlates with water temperature in rock bream.

H005

Analysis of the Status of Resources for Characterization of Pathogens and Standardization in Informative Database Registered in Bio-bank Network

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The purpose of this study is to suggest the plan for the efficient registration and management of pathogen resources by establishing the information standards and code system and to seek to customize the regional branch of National Culture Collection of Pathogens (NCCP) for collecting the variety of resources, with the results of the analysis to resources status in each regional branch. We have performed an analysis for estimating a status of pathogen resources registered in NCCP and its regionals and made codes for specimens, resources and antimicrobial agents. We have identified the status of pathogen resources which we had in all biobanks and analysed the antimicrobial pattern of major pathogens, the sequence information of 16S rRNA of them, so recognised the intraspecific diversity between regions. And also, we made codes for specimens, pathogens, and antimicrobials. The collecting system with 3 regional banks should be maintained as well as present. 3 groups including Gram-positive cocci, Gram-negative Enterobacteriaceae, and Gram-negative non-fermenters is the best categorization for characterizing the collecting system to get and manage a variety of resources.

H006

Protective Efficacy of a Human Endogenous Retrovirus Envelope-Coated, Nonreplicable, Baculovirus-Based Hemagglutin DNA Baculoviral Vector Expressing H1N1 HA DNA Transgene Vaccine against Pandemic Influenza H1N1 2009

Yongdae Gwon[1], Jeongki Kim[2], Jaeyoo Choi[1], Yeondong Cho[1], Yoonki Heo[1], Kihoon Park[1], Yuyeon Jang[1], Haryoung Poo[2], and Yongbong Kim[1*]
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Influenza viruses cause respiratory disease in humans and animals with high morbidity and mortality rates. In this study, we constructed novel baculovirusbased vaccine (AcHERV-sH1N1 HA) using baculovirus expression system, a recombinant baculovirus bearing HA gene of A/Influenza H1N1 2009. For vaccine efficacy test, C57BL/6 mice and BALB/C mice were injected intramuscularly with 2×10^7 particles of the AcHERV-SH1N1 HA, with two boosts at 2-week intervals. Whole killed influenza vaccine (Greenflu® 2 μg, GREENCROSS) was used as a control. AcHERV-sH1N1 HA immune group induced high level of humoral immune response (IgG) with Greenflu®. And for challenge test, mice were intranasal challenged with pandemic H1N1 virus (A/California/04). Day 6 post-challenge, 100% of BALB/C mice immunized with the commercial vaccine or AcHERV-sH1N1 HA survived. In contrast, C57BL/6 mice immunized with AcHERV-sH1N1 HA or the commercial vaccine showed 60% and 70% survival respectively.
[This study supports that AcHERV-sH1H1 HA vaccine can be a potential efficient prophylactic influenza vaccine. Supported by grants from KRIBB and iPET and Konkuk University.]

H007

The Tumor Protection of Human Papillomavirus E6/E7 DNA Vaccine

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Previously, we developed human endogenous retrovirus envelope protein-coated non-replicating recombinant baculovirus (AcHERV) for a human papillomavirus (HPV) 16L1 DNA vaccine nanocarrier. Because HPV E6 and E7 are promising tumor antigens in HPV related cervical cancer, we constructed AcHERV-E6/E7. To improve the immunogenicity of E6/E7 gene product, E6/E7 gene was synthesized with codon optimization and fused with sorting signal of the lysosomal-associated membrane protein LAMP-1. AcHERV-(opti)E6/E7, and AcHERV-(opti)E6/E7LAMP-1. C57BL/6 mice were injected I.M with 2×10^7 particles of each DNA vaccine on day 0, day 7 and day 14. After 1 week from first immunization, 1×10^5 TC-1 tumor cells were subcutaneously transplanted into right flank leg. Tumor growth was monitored weekly for 7 weeks. Compare to control group, AcHERV-(opti)E6/E7 treated group showed retardation of tumor growth. AcHERV-(opti)E6/E7LAMP-1 treated group showed the anti-tumor effect. These DNA vaccine generate the highest number cytokine secreting of E6 or E7 specific splenocytes. These results indicate that fusion of LAMP-1 to an E6/E7 gene enhance the potency of HPV DNA vaccine against cervical cancer.

H008

Trivalent Human Papillomavirus (16,18,58) DNA Vaccines Encapsidated in Single Non-replicable Baculovirus Nano-carriers

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We constructed a multivalent human papillomavirus (HPV 16, 18, and 58) DNA Vaccines. We compared vaccine efficacy of the bivalent AcHERV vaccines with AcHERV-HPV16/18L1 and AcHERV-HPV18/16L1. Regardless of the sequential orders, the bivalent AcHERV DNA vaccines retained the immunogenicity of monovalent AcHERV DNA vaccines. Compared with monovalent AcHERV-HPV16L1 and AcHERV-HPV18L1, bivalent AcHERV vaccines showed similar level of immunity against HPV-16/18. Bivalent AcHERV-HPV16/18L1 and AcHERV-HPV18/16L1 showed similarly high levels of humoral and cellular immunity. In challenge test, both bivalent vaccines showed perfect protection against HPV16 and 18 pseudotyped viruses. Based on the retained immunogenicity of bivalent AcHERV vaccines, we constructed a trivalent AcHERV DNA vaccine encoding HPV 16/18/58L1 genes (AcHERV-HPV16/18/58L1). Trivalent AcHERV vaccine also showed high levels of humoral and cellular immunity and sterile protection against HPV16, 18, and 58 PVs challenge. Therefore, trivalent AcHERV-HPV16/18/58L1 is expected as a potential prophylactic and therapeutic DNA vaccine against HPV16, 18, and 58.

H009

Sublingual Immunization of Trivalent Human Papillomavirus DNA Encapsidated in Nonreplicable Baculoviral Vaccine

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We developed human endogenous retrovirus envelope protein-coated non-replicating recombinant baculovirus (AcHERV) for a multivalent human papillomavirus (HPV) DNA nanocarrier. In this study, we generated an AcHERV-based trivalent DNA vaccine against HPV type16, 18, and 58 (AcHERV-Trivalent) and tested vaccine efficacy of HPV 16, 18, and 58 following intramuscular or sublingual immunization without adjuvant. The both Immunization of trivalent vaccine induced IgG and IgA antibodies, neutralizing antibodies, IL-4, IFN-γ, and protection against challenge of HPV pseudoviruses. Serum IgG, IL-4, and neutralizing antibody responses of sublingual immunization were similar in intramuscular immunization. Vaginal IgA responses of sublingual immunization were superior to intramuscular immunization. Furthermore, all vaccinated groups by intramuscular or sublingual with trivalent vaccine showed perfect protection against genital challenge with HPV16, HPV18, and HPV58 pseudoviruses. These results suggest that vaccine against HPV trivalent vaccine and the potential of sublingual immunization as an efficient vaccination strategy for inducing mucosal immune responses.
[Supported by grants from KHT]

H010

Anti-Hypercholesterolemic Effects of *Lactobacillus plantarum* JB PML-16

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Probiotics are viable microorganisms that exhibit beneficial effects on the health of the host when they are ingested. In this study, lactic acid bacteria were isolated from fermented soy bean sauce and showed the high capacity for Ornithine synthesis and Conjugated linoleic acid (CLA) conversion. As a result of these screening, 5 isolates were selected and we investigated their probiotics activities, such as anti-hypercholesterolemic effects. JB PML-16 identified as *Lactobacillus plantarum* showed bile salt hydrolase (BSH) activity and the cholesterol-lowering activity. It was able to deconjugate bile salts and remove cholesterol *in vitro.* Also, It was a potent inhibitor in HMG-Co A reductase inhibition assay. These results indicated an effect of reducing cholesterol, as well as inhibiting cholesterol biosynthesis. The above results suggest that *Lactobacillus plantarum* JB PML-16 may be a good probiotics and can be developed functional food materials for reducing cholesterol.
[This research was support by the Regional Specialized Technology Convergence R&D Program funded by the Ministry of Trade, Industry and Energy.]

H011

Granulocyte Macrophage Colony Stimulating Factor-*Salmonella typhimurium* Flagellin 2 Fusion Adjuvant for Foot-and-mouth Disease Vaccine

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GM-CSF is produced by a variety of cell types including T cells, macrophages, endothelial cells upon receiving immune stimuli. *Salmonella typhimurium* Flagellin 2 (STF2) activates TLR5-mediated innate immune signaling pathways and induces inflammatory responses through the APCs. In this study, we investigated the effects of 2A-linked GMCSF-STF2 (GMCSF-STF2) as an adjuvant on the immune responses of commercial FMD vaccine (O Manisa, A Malaysia97, Asia 1 shamir strain). We constructed a recombinant baculovirus based GMCSF-STF2 fusion encoding adjuvant (Ac-ieI-PERVB-GMCSF-STF2). BLAB/c Mice were immunized two times with 2×10^7 FFU of Ac-ieI-PERVB-GMCSF-STF2 at 2-week intervals. Immunized mice sera were collected and the immunological effects of the Ac-ieI-PERVB-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 useful for potential adjuvant for FMD vaccine.
[Supported by grants from MAFRA]

H012

AcHERV-mGMCSF as an Effective Molecular Adjuvant for Influenza Vaccine

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Egg based killed influenza vaccine, Greenflu®, showed an important role for prevention of pandemic transmission. To enhance the immune efficacy, we constructed murine GM-CSF gene delivering recombinant baculovirus (AcHERV-mGMCSF) as an adjuvant. To evaluate GM-CSF effect in influenza vaccine, mice were vaccinated by single intramuscular injection with 1×107 FFU of AcHERV-mGMCSF with various doses of Greenflu®. Mouse serum was tested for the production of antibody by ELISA and hemagglutination inhibition (HAI) assay for humoral immunity. We also tested interferon elispot assay and CTL assay for cellular immune response. The group which was immunized by 0.2 μg of Greenflu® with AcHERV-mGMCSF showed higher level of IgG titers than that of only immunized with 0.2 μg of Greenflu®. Also, in HAI assay showed same patterns. Mice were challenged with influenza A/California/04/2009 virus at 3 weeks after vaccination. In the AcHERV-mGMCSF group, it showed significant protection against lethality and weight loss than control group. These data demonstrate that AcHERV-mGMCSF can be effective molecular adjuvant for influenza vaccine. [Supported by influenza vaccine from GREEN CROSS, Korea]

H013

Growth Suppression of a Gingivitis Pathogen *Propionibacterium acnes* by Organic Plant Extracts

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Propionibacterium acnes is an anaerobic Gram-positive strains found mainly on the skin as an acne-causing bacteria. In addition, *P. acnes* was also closely related to the acne causing gingivitis inflammation and bleeding or inflammation of the blood vessels, and consequently led to a serious heart valve disease. In recent years, the evolution of microorganisms such as *P. acnes* that became resistant to many antibiotics has emerged as a major clinical problem due to the widespread use of antimicrobial drugs in the treatment of infectious diseases. Thus, screening of more potential drugs or chemicals composed of complex ingredients rather than a pure chemical is highly required to avoid these serious problems. In these context, this study was aimed to test the inhibition activity of plant extracts against a gingivitis pathogen *P. acnes*. Resultantly, several extracts showed a considerable extent in the inhibition of cell growth. We will discuss here about the potential and practical applications of these extracts in more details.
[Supported by a research grant from Vericom Com.]

H014

Analysis of Food Components and Microorganisms in *Nelumbo nucifera* Leaf Sugar Broth

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Lotus farming in Korea has a long history, and recent studies of lotus leaf (*Nelumbo nucifera*) have shown various beneficial effects on human health. In order to identify food components and microorganisms associated with lotus leaf sugar broth, Lotus leaves were fermented in 57° Brix sugar broth over a period of 6 months. As results of analysis, the contents of crude protein, crude fat, ash, moisture, soluble sugars and amino acids indicated that the lotus leaves fermented broth contained rich sources of moisture 69.91%, crude protein 15.93%, crude fat 0.98% and ash 2.24%. The sucrose concentrations were changed from 2.5% to 0.07%, while concentrations of glucose and fructose were changed from 0.4% to 1.32% and 1.39%, respectively. For the amino acids, alanin 27.81 μg/ml, valin 13.22 μg/ml and γ-amino-n-butyric acid 25.85 μg/ml were analyzed in lotus leaf sugar broth. For the microbial population, two species of yeast were isolated using appropriate isolation medium. Based on API kit and 18S rRNA sequencing, *Saccharomyces cerevisiae* and *Candida sphaerica* were identified from initial stage of fermentation, and *Saccharomyces cerevisiae* was dominated at the end of fermentation

H015

Characterization of a Thermostable β-Gucosidase from the Hyperthermophilic Archaeon *Thermococcus pacificus*

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Hyperthermophilc archaeon, *Thermococcus pacificus* (T_{opt} of 80-88°C) could grow on various substrates such as peptides, starch, and so on. Based on the genomic analysis of the strain, we identified a novel GH1 β-glucosidase encoding gene (PAC_orf01364), which was located in glycoside hydrolase gene clusters consisting of cellulose-, laminarin-, and agarose-degrading enzymes. The gene revealed an ORF of 1,464 bp encoding 487 amino acid residues, and the deduced amino acid sequence showed 77% identity with *Pyrococcus furiosus* β-glucosidase (accession no. NP_577802). The gene was cloned and expressed in *Escherchia coli* system. The recombinant protein was purified by metal affinity chromatography and characterized. The purified enzyme shows optimum activity at pH 5.5 and 75°C, and thermostability with a half life of 6 h at 90°C. Interestingly, the enzyme also exhibits laminarinase activity and β-xylosidase activity.
[Supported by grants from KIOST in-house Program (PE99212) and NRF]

H016

Novel Glycoside Hydrolase Gene Clusters and Hydrolysis of Various β-Linked Polysaccharides in Hperthermophilic Achea

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Based on the comparative genomic analysis of 12 *Thermococcus species*; *T. aegaeus*, *Thermococcus* sp. CH5, *T. celericrescens*, *T. fumicolans*, *T. guaymasensis*, *T. litoralis*, *T. marinus*, *T. pacificus*, *T. profundus*, *T. barossii*, *T. stetteri*, we identified novel glycoside hydrolase gene clusters from 3 strains; *T. guaymasensis*, *T. pacificus*, and *T. waiotapuensis*. The clusters contain the two modular structures including glycoside hydrolases consisting of cellulose-, laminarin-, and agarose-degrading enzymes, and cellobiose/β-glucoside ABC transporter for transport of β-glucan oligomer into the cell. The strains could not be only grow and H_2 production on each carboxymethyl cellulose, laminarin, and agarose-containing medium as a sole carbon sources, but also on seaweed-containing medium, such as green (*Ulva*), red (*Gracilaria*), and brown (*Laminaria*). These results show that hyperthermophiles or enzymes from them could be applied to the bioconversion of macro algae (seaweed).
[Supported by grants from KIOST in-house Program (PE99212) and NRF]

H017

Imported Cases of *Theileria equi* Infection in Quarantine Facilities in South Korea

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Two equine herds originating from the United States (US) and China were held in quarantine facilities in South Korea. In the serodiagnosis of equine piroplasmosis, four horses showed positive reactivity. Subsequent analyses resulted in diagnosis of the apicomplexan protozoan, *Theileria equi*, infection. Confirmatory tests included a complement fixation test, an indirect fluorescent assay, *T. equi*-specific nested PCR, and analysis of the target sequence in the 18S rRNA gene. A common phylogenetic origin was discovered between the parasites identified in each case, and known isolates of *T. equi* circulating in the US and China. The infected horses were euthanized according to the relevant quarantine regulations.

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H018

Multiplex PCR for Species-Specific Detection of the Opportunistic Human Fungal Pathogens

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The human pathogenic fungi *A. fumigatus*, *C. albicans*, and *C. neoformans* cause various disease including aspergillosis and meningitis especially in immunocompromised patients. For rapid detection of these pathogens with PCR, species-specific DNA primers were generated by performing all-to-all blast with the program Blastn and the public primer design program Eprimer3 for identifying specific regions at each genome of ten species including 7 *Aspergillus*, 1 *Candida,* and 2 *Cryptococcus*. PCR was performed with selected primer pairs on genomic DNA from 13 species of *Aspergillus*, 2 species of *Trichoderma*, *Penicillium chrysogenum*, *Candida albicans*, and *Cryptococcus neoformans.* The AFSF1-AFSR1, CASF1-CASR1, and CNSF1-CNSR1 primer pairs in PCR exhibited species-specific DNA fragment for *A. fumigatus*, *C. albicans*, and *C. neoformans*, respectively. Furthermore, Multiplex PCR by these primer sets within a single tube produced amplicons of 0.7, 0.5, 0.2 kb specific to *A. fumigatus*, *C. albicans*, and *C. neoformans,* respectively.

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H019

Development of RT-PCR and Nested PCR Assay for the Detection of Non-reported Five Seed-transmitted Viruses in Quarantine

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Seed-transmitted viruses regarding quarantine are the most problematic plant diseases in crop seed industry. In this study, non-reported five seed-transmitted viruses [*Andean potato latent virus* (APLV), *Cherry rasp leaf virus* (CRLV), *Pelargonium zonate spot virus* (PZSV), *Spinach latent virus* (SpLV) and *White clover mosaic virus* (WClMV)] that have not previously been studied for PCR diagnostic system in Korean quarantine were targeted for the detection. For successful virus detection, we employed a diagnostic technique based on reverse transcription polymerase chain reaction (RT-PCR) and nested polymerase chain reaction (nested PCR) methods. Two RT-PCR primer sets for each virus were finally selected for the diagnosis. Nested primer sets developed in the present study were shown to be highly sensitive in detection and verification of the target viruses. Overall, RT-PCR and nested PCR were proven to be a useful diagnostic technique for the detection of APLV, CRLV, PZSV, SpLV and WClMV in quarantine.

H020

Survey of Microbial Contamination of Tomatoes at Farms in Korea

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This study investigated and evaluated contamination levels of bacteria on tomatoes at farms stage to evaluate potential hazards associated with fresh tomatoes. A total of 170 samples, 90 samples from 5 sampling sites from 18 farms and 80 samples from 1 sampling site from 4 farms every month for four months, were analyzed to enumerate aerobic bacterial counts, coliforms, *E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Aerobic bacterial counts ranged from 0.48 to 6.15 Log CFU/g, with the lowest and the highest bacterial cell counts recorded for A site and E site, respectively. Thirty five percent of the samples from the E site contained more than 2 Log CFU/g. Six samples (6.6%) of 90 samples contained *B. cereus* less than 1 Log CFU/g. In addition, the contamination level of indicator bacteria and *B. cereus* in tomatoes were higher on March than on April, May and June ($P < 0.05$). *S. aureus, E. coli, E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* were not detected in the tomato samples.

H021

Distribution of Sanitary Indicator Bacteria and Food-Borne Pathogens in Korean-Leek Farms

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To evaluate microbiological safety of Korean leek and producing environment, a total of 153 samples were collected from 3 Korean leek farms located in Yangju, Gyeonggi province. The collected samples were analyzed on sanitary indicator microorganisms (Aerobic plate count, coliform count, *Escherichia coli*) and foodborne pathogens (*Escherichia coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*). According to results, the numbers of APC, coliform, and *B. cereus* of soil were 5.47~6.32 log CFU/g, 1.16~2.10 log CFU/g, and 4.17~4.23 log CFU/g, those of korean leek during cultivation were 5.79~6.59 log CFU/g, 1.43~3.69 log CFU/g, and 2.50~2.76 log CFU/g, respectively. The number of APC from packing table, knife, chapping board, and hands in good hygienic farm was lower than those in poor hygienic farm by 1.15~2.33 log CFU/100 cm^2. *E. coli* was detected from soil, water, korean leek and *S. aureus* was also detected from chapping board, hands, korean leek. However, *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* were not detected. These data suggested that risk management system should be introduce to the Koean leek farms.

H022

Prevalence of Sanitary Indicator Bacteria in Dropwort (*Oenanthe javanica*) Cultivation Farms in Korea

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This investigation was conducted to analyze microbial hazards of dropwort (*Oenanthe javanica*) farms. Samples including cultivation and postharvest environments, personal hygiene (hands) and plants (raw materials, after trimming, and after washing) were collected from three different locations (eight dropwort farms) in Korea. As a results, total aerobic bacteria in cultivation and postharvest environments were detected at the levels of 0.33~6.50, 0.44~5.90, and 1.40~3.97 log CFU/100 cm^2, ml and coliform were detected at the levels of 0.33~3.37, 0.90~3.74, and 0.10~1.18 log CFU/100 cm^2, ml. *E. coli* were not detected by quantitative test, however, positive reaction in several environment factors (soil, water, and cutter) were detected by qualitative test. Especially, total aerobic count and coliform in hand showed higher than cultivation and postharvest environments. In plants, total aerobic bacteria and coliform in after washing showed similar to before processing in all farms. These results suggested that cultivation and postharvest environment and personal hygiene should be managed to reduce the microbial contamination in dropwort farms.

H023

Analysis of Pathogenic Bacteria from Dropwort (*Oenanthe javanica*) Cultivation Farms in Korea

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The aim of this study was to analyze pathogenic bacteria (*Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*) for cultivation and postharvest environments, personal hygiene (hands), and plants (raw material, after trimming, and after washing) from three different areas (eight dropwort farms) in Korea. In major pathogen, *B. cereus* were detected at the levels of 0.42~5.63, 0.33~3.25, and 0.33~1.26 log colony forming unit (CFU)/100 cm^2, ml in cultivation and postharvest environments. *B. cereus* contamination were detected at the higher levels in soil than other cultivation environment factors and this pathogenic bacteria were found in hands at the levels of 0.73~4.12, 2.12~3.43, and 3.16 log CFU/hand, respectively. In plants, the number of *B. cereus* in most final products was declined. *S. aureus* were not detected in all samples by quantitative test, however, positive reaction in plant (after washing) were detected by qualitative test in one farm. In this study, *E. coli* O157:H7, *L. monocytogenes* were not detected. These results indicated that need for management system of agricultural products and strict to personal hygiene compliance.

H024

Korea National Microorganisms Research Resource Center

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Kyonggi University

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 KNMRRC 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 KNMRRC is the followings: ①construction of standardized and integrated management system, ②construction of Core center and other organs network, ③Quality Control(QC) of microbial resources in the member banks, ④conservation of Resources in the member banks and the interrupted banks, ⑤education for professionals in the member banks, ⑥public Relations for raising people's awareness of the importance of microbiological resources.

H025

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: ① the collection and conservation of native environmental microorganisms and genetic resources, ② the construction of systematic management system for effective conservation and application of microbiological resources for environmental industries, ③ the provision fundamental data for ecosystem research and microbial classification, and ④ the development of biological treatment system for bioremedation 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.

H026

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 and their genes from the environmental samples have recently been achieved. Metagenomic approach that recovers the environmental DNA without the limitations of culture-dependent methods and constructs DNA libraries in suitable cloning vectors and host strains have been utilized for retrieving novel and useful genes. Korean Metagenome Bank (KMGB), a member of Korea National Research Resource center (KNRRC), has opened with the goal for the collection and distribution of metagenome (eDNA) and metagenomic library. The aims of the Korean Metagenome Bank are to contribute to the development of biotechnology by providing the metagenomic resources into various researches and to perform a national mission for maintaining the metagenomes as future biological resources

H027

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, *Magnaporthe 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. Also, CFGR seeks strategies for sustainable and scientific plant quarantine to better protect our ecosystem from invasive microorganisms.

H028

Bank of Waterborne Virus

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The purpose of this bank is collecting and storing various waterborne virus isolates provoking severe infections in animal and human. This bank collects research information on various viruses and provides this information when it is needed. We provide various waterborne viruses, genomes and host cells to hospitals, universities, research institutes, and government institutes in the country as well as abroad. We also provide the identification services of waterborne viruses and the research data through on-line. Finally, it contributes to progress biological science and to improve public health.

H029

Bacteriophagebank

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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 10^{30} to 10^{32} 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, Camphylobacter jejuni, Enterococcus faecium, Enterococcus faecalis, Cronobacter sakazakii, Seratia 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)

H030

Korea Collection for Oral Microbiology

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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.

H031

Korea Bank for Pathogenic Viruses

Ki-Joon Song*
Korea Bank for Pathogenic Viruses

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 etiologic agents of hemorrhagic fever with renal syndrome. To date, total of more than 43,000 materials(~100,000vials) from human and animal 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.knrrc.or.kr

H032

Plant Virus GenBank

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Plant Virus GenBank (PVGB) is a nonprofit semi-governmental organization, one of the Korea National Research Resources Collections (KNRRC) for special research materials Banks program financially supported by the Ministry of Education, Science & Technology (MEST) dedicated to collection, identification, characterization, preservation, research development, distribution and deposition of plant virus research biomaterials established since 1999. PVGB is one of substructure of Korea National Microbiological Research Resources Collections (KNMRRC). PVGB retains a number of accessions and a wide range of collections of Plant Virus Biomaterials useful for Plant Virology and Biotech-related research areas. PVGB has moved to its current status on November in 2000 and has modern facilities and infrastructures for supporting broad research fields as well as Plant Virology Community. PVGB has been recognized as a member of World Federation for Culture Collection & World Data Center for Microorganisms (WFCC-WDCM) and ISBER since April of 2001 and June of 2007, respectively. Main objectives and contents of PVGB can be categorized as 7 topics as follow ; collection and development of Plant Virus Research Biomaterials such as infectious plant virus culture, plant viral cDNA clone, plant virus antiserum, biologically active full-length cDNA clone, viral cDNA library, virus-induced plant cDNA library, and diagnostic primers, preservation of Plant Virus Research Biomaterials, Distribution of Plant Virus Research Biomaterials to worldwide researchers to support their research fields and Safe Deposit from virologists, Development of New Plant Virology Techniques, i.e., molecular taxonomy of plant viruses, infectious cDNA clones, molecular indexing of virus variation, screening of virus resistance, virus-resistant transgenic plants, and risk assessment for living modified (LM) virus and LM plant systems, collection and support of Research Information.

H033

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.

H034

Korea Marine Microalgae Culture Center

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Today microalgae are widely used in research and as educational materials. They are also commercialized in the industries of food, animal feed and environment. Microalgae exhibit a promising potential to be converted into pharmaceutical products and bio-fuel energy. For this reason, there are active, ongoing researches on microalgae with tremendous expectations of scientists. The Korea Marine Microalgae Culture Center (KMMCC) was established with a financial support from National Research Foundation of Korea in 1995. The collection of microalgae has been increasing continuously since 1995, and its number has reached to about 2,100 strains in 2013. The collection mainly consists of marine strains (80%) which are mostly isolated from Korean waters (96%). The major classes of the strains are Bacillariophyceae (54%), Chlorophyceae (18%), Dinophyceae (9%), Cyanophyceae (5%), Prasinophyceae (4%), Eustigmatophyceae (3%), Haptophyceae (2%), etc. With respect to identification of the strains, about 97% and 56% of them are identified at the level of species and genus, respectively. In addition, 3% are still unidentified, and about 51% of the strains are under axenic state. The culture strains of the KMMCC are introduced regarding information on sampling, culture, biological and chemical characteristics of each strain. The initial direction of the KMMCC focused on finding microalgal strains that had a good dietary value for larvae in aquaculture. Such strains used to be supplied to the hatchery. Our recent work, however, has shifted to collecting a wider range of diverse microalgae which are taxonomically different. We also pursue the effective preservation and quality control of the microalgae strains. In accordance with the KMMCC's progression on the strain collection, the demand for the strains in research fields has been expanding from the industry of aquaculture to biotechnology, environmental sciences, engineering, biological oceanography, etc. In Korea, recently, the annual domestic request for the microalgae from academic and commercial organizations has increased up to nearly 190 requests for as many as 400 strains. Making a deposit with newly isolated microalgal strains in the KMMCC by other investigators is always possible if they agree to be open about distributing their strains to other researchers as well. The final decision of the strains to be deposited belongs to the KMMCC. Even though assigning a correct taxonomical position to the strains has always been our primary concern, many difficulties still exist in identifying them.

H035

Culture Collection of Antimicrobial Resistant Microbes

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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 these areas are necessary. Since Culture Collection of Antimicrobial Resistant Microbes was established in 1999, CCARM has been 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 systemic 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. CCARM is now increasing the interaction and collaboration between culture collections through national and international network as a member of Clinical Laboratory Standards Institute since 2000, World Federation for Culture Collection & World Data Center for Microorganisms since 2003, International Society of Biological and Environmental Repositories since 2007, Korea National Research Resource Center since 2008, and Biological Repositories since 2009.

H036

Helicobacter pylori* Korean Type Culture Collection (HpKTCC) Collects and Distributes Clinical Isolates of *H. pylori

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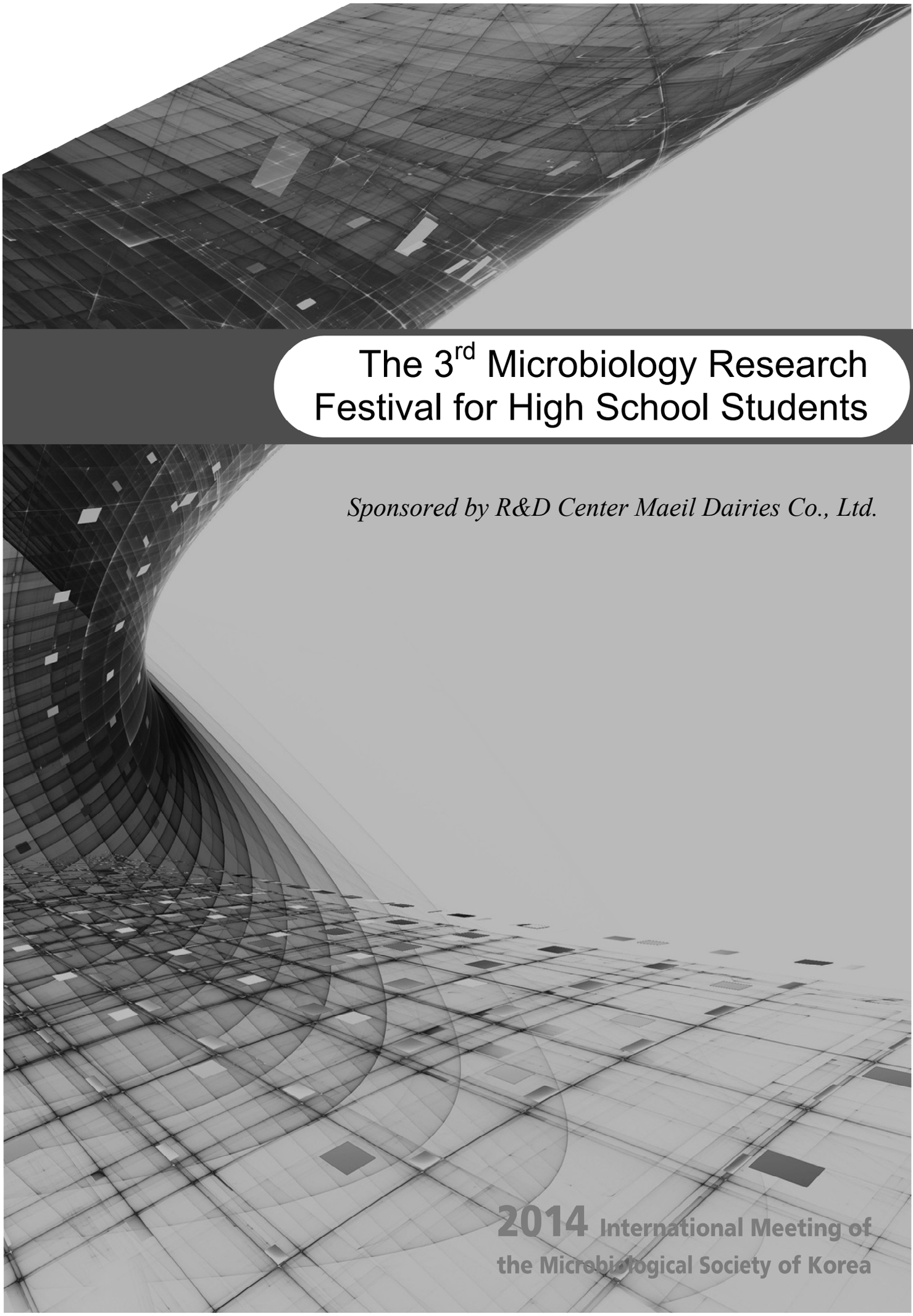
H. pylori that colonizes only in human gastric mucosa is one of the most common human pathogens and is the main cause of gastritis, peptic ulcer, and gastric cancer. Despite the clinical and commercial importance of *H. pylori*, many researchers have been blocked to investigate the diagnosis, treatment, and prevention of *H. pylori* infections because of difficulty in obtaining *H. pylori* isolates from patients. We have collected and characterized *H. pylori* isolates obtained from worldwide areas to allow researchers to access a variety of characterized *H. pylori* isolates. characterized *H. pylori* isolates. *H. pylori* KTCC contributes to promote the study for the diagnosis, treatment, and prevention of *H. pylori* infections by providing fundamental research materials to investigators.



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HS-1

철세균을 이용한 토양박테리아의 생장을 저해하는 박테리오파지의 억제효과에 대하여

양준용
중산고등학교
담당교사:이슬아

연수기와 하수구에서 철환원세균(iron-reducing bacteria, 철환원세균, 편의상 철세균으로 부르기로 함)인 철세균을 분리 동정하고 토양내 존재하는 토양박테리아를 파괴하는 용균성 박테리오파지를 토양으로부터 분리하였다. 토양박테리아가 분리된 용균성 박테리오파지에 의해 생장이 억제되는 반면 유해세균인 대장균의 경우에는 토양에서 용균성 박테리오파지에 의한 생장억제가 토양박테리아에 비해 상대적으로 낮고, 이로 인해 용균성 박테리오파지가 토양에 유입되면 토양박테리아가 사멸하고 그 결과 대장균이 빠르게 증식하게 된다. 박테리오파지는 철산화물이 부착된 토양박테리아에게는 접근을 하지 못하는 것으로 알려져 있는데, 철산화물의 경우 Fe^{3+}을 Fe^{2+}로 환원하는 철세균의 존재로 Fe^{2+}의 양이 증가하면 토양내 토양박테리아의 수가 용균성 박테리오파지에 의해 감소하는 것을 막을 수 있으며, 이로인해 발생하는 식물의 생장저하를 개선할 수 있다는 사실을 알게 되었다.

HS-2

기후 변화에 따른 토양의 온도변화가 항생제 내성 박테리아의 토양번식에 미치는 영향

장서현
세종과학고등학교
담당교사:김대준

토양을 15가지 온도변화 조건으로 8주간 온도변화를 실시하고 토양을 분석한 결과, 식물에게 공급하는 질소공급원의 제공자인 토양박테리아의 함량이 급격하게 감소한 것을 확인 할수 있었으며, 이로 인해 유기물의 함량이 감소하지 않으며 이것은 식물에게 공급할 질소공급원의 고갈을 의미한다. 또한 15가지 온도 변화 조건을 거친 토양에서는 항생제 hygromycin (20μg/ml)에 내성을 가지는 대장균의 경우 토양박테리아의 생장이 억제되는 대신 토양 내 번식이 증가하여 토양을 오염시키는 것을 확인하였다. 이를 해결하기 위해 실험을 통해 항동효과가 입증된 백년초 추출물을 토양에 투입하고 15가지 온도 변화 조건 중 가장 심각한 변화를 보였던 2,6,10의 온도변화조건을 실시한 결과, 항생제 내성 대장균의 성장이 억제되고 토양박테리아의 성장이 정상적으로 회복 되었으며, 유기물의 분해도 정상적으로 일어나 식물의 성장이 정상적으로 회복되는 것을 확인 할 수 있었다. 또한 온도 변화를 겪고 항생제 내성 박테리아로 오염된 토양에서 자란 식물은 항생제 내성 대장균으로 식물이 심각하게 오염되지만, 백년초추출물을 투여한 토양에서는 항생제 내성 대장균에 의한 오염이 거의 사라지는 것을 확인 할 수 있었다.

HS-3

효모를 이용한 돌외 및 10종 천연물의 수명 및 노화에 미치는 영향 및 관련 기전 분석

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수명 연장은 노화를 억제하거나 노화에 역행하도록 하여 수명을 늘리는 것으로 줄기 세포, 분자 수준의 세포 복구 기술 등이 연구되고 있으며, 또 다른 한편에서는 자연에 존재하는 다양한 천연물을 수명 연장에 활용하려는 연구가 있다. 이에 본 연구에서는 수명 연장의 가능성이 제기된 돌외 및 10종의 천연 추출물들을 진핵생물 중 단순한 효모를 이용하여 각각의 천연 추출물이 효모의 성장 및 수명 연장에 도움을 주는 지 알아보고, 각각 천연 추물물들이 열, 산화, UV, 영양분 부족 등 각종 스트레스에 대한 세포보호 기능을 통해 그 기전을 알아보고자 보고자 하였다. 연구 결과 백수오, 돌외 등 일부 천연 추출물은 효모의 수명 연장에 도움을 주었다. 또한 백수오는 산화 스트레스, 영양분 부족 환경에서 세포보호 효과가 있었던 반면에 돌외는 열 스트레스 환경에서 세포보호 효과가 있었다. 본 연구결과를 토대로 효모가 수명 연장 및 각종 스트레스를 통한 수명 연장 관련 연구에 유용한 모델임을 알 수 있었다. 또한 천연 추출물이 효모의 생명 연장에 대한 공통 기전을 규명할 수는 없었지만, 천연 추출물 별로 서로 다른 다양한 기전이 있을 가능성을 확인할 수 있었다. 향후 본 연구는 효모를 이용한 여러 가지 수명 및 노화 기전 연구 및 여러 가지 후보 물질에 대한 가능성 검증에 도움을 줄 수 있을 것으로 기대된다.

HS-4

유분 환경에 따른 *Houttuynia cordata*의 *Propionibacterium acnes* 생장 억제 효과 탐구

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Propionibacterium acnes (*P. acnes*)는 피부에 존재하는 미생물 중 하나로 혐기성이며, 여드름과 관련있는 것으로 알려진 박테리아이다. 어성초라고도 부르는 *Houttuynia cordata* (*H. cordata*)는 여드름 등의 발생 주원인인 *P. acnes*의 억제에 효과적으로 알려져 있다. 본 탐구에서는 이 사실들에 착안하여 일반 환경과 유분환경에서 *P. acnes*의 생장을 비교하고, 나아가 *H. cordata*의 *P. acnes* 생장 억제 효과를 비교해보고자 하였다. 실험은 배지에 균과 돈지(lard)를 섞어서 도말하는 방법과 배지를 만들 때 돈지를 첨가한 후 균을 배양하는 방법을 모두 시행하여 최대한 사람의 피지(sebum)와 비슷한 유분 환경을 조성하여 진행하였다. 또한 균을 돈지와 섞어서 도말한 경우와 균만 도말한 경우 모두 paper disc method를 사용해 *H. cordata*에 의한 생장 억제 효과를 비교하였다. 결과적으로 모든 균 농도에서 돈지가 없을 때에 비해 유분이 존재할 때 colony의 개수와 총 면적이 넓게 나타났고, 이로부터 *P. acnes*가 유분환경에서 잘 생장한다는 것을 확인할 수 있었다. paper disc method를 이용한 *H. cordata* 추출물의 *P. acnes*의 생장억제효과 실험에서는 배양 시 사용한 균주의 희석 비율에 관계없이 돈지의 사용 유무에 따라 각각 배지에서 생육 저지환의 크기가 비슷하게 나타났고, 돈지를 섞은 배지에서 생육 저지환의 크기가 더 작게 나타나는 것으로 나타났다.

HS-5

후추의 피페리딘 성분의 박테리아의 면역 저하 반응유도에 의한 유해 박테리아(항생제 내성 대장균)의 억제 방안

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담당교사:정영일

후추에 들어 있는 여러 성분 중, 피페리딘의 경우는 전체 함량의 0.2~0.7%를 차지하는 물질이다. 후추가 가지고 있는 대표적인 항균물질인 피페린이며 피페린의 항균효과에 대하여서는 많은 연구 결과들이 발표 되었다. 그러나 본 실험은 피페린 다음으로 후추에 존재하는 성분인 피페리딘에 대하여 주목하였는데, 항생제에 내성을 가지는 대장균의 경우에는 칼슘과 철이 결핍되면 생장이 억제된다는 결과를 얻을 수 있었고 이를 바탕으로 실험을 실시한 결과 검은 후추의 낱알을 40°C로 중탕 가열하여 추출한 피페리딘의 투여는 항생제 내성 대장균의 토양내 증식을 억제 할 수 있으며, 그 이유는 항생제 내성의 근간이 되는 plasmid DNA의 구조적 변화를 일으켜 분열 증식한 항생제 내성 박테리아가 점점 항생제 내성을 잃어가게 되는 것을 확인 할 수 있었다. 이는 plasmid DNA를 제한효소로 절단하는 실험을 통해 확인 할 수 있었으며, 마지막으로 항생제 내성 대장균을 피페리딘 추출물로 처리한 결과 처리 횟수가 증가할수록 항생제에 대한 내성이 감소하는 것으로 보아 피페리딘은 항생제 내성 세균이 항생제 내성을 감소시키고 항생제내성의 습득을 억제하는 효과가 있음을 확인 할 수 있었다.

HS-6

폐식용유가 토양에 존재하는 토양박테리아와 유해세균의 증식에 미치는 영향에 대하여

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폐식용유의 탄화수소계열의 물질은 생물학적 독성을 가지는 것뿐만 아니라 토양에 유입된 경우 토양의 유기물을 분해하고 물질 순환을 촉진하는 토양박테리아의 성장을 억제하며, 토양에 존재하는 미생물인 효모와 유산균의 생자에도 악영향을 끼친다는 사실을 확인 할 수 있었다. 구체적으로 살펴보면 가정에서 일반적으로 사용하는 콩기름과 올리브유의 경우 가열 횟수가 증가하면 벤조피렌의 함량이 급격하게 증가하며, 이는 식당에서 사용한 폐식용유의 벤조피렌의 농도와 비슷한 수준임을 알 수 있다. 또한 산도도 급격하게 증가하며, 과산화물의 증가도 벤조피렌의 함량의 증가와 비슷한 수준의 증가를 보인다. 이로 인해 발생하는 문제점은 토양에 존재하는 유익한 미생물군(토양박테리아, 효모, 유산균)의 성장은 유해물질의 증가와 더불어 그 수가 감소하지만, 유해세균인 대장균의 경우 생장의 억제가 유익한 미생물군보다 심각하지 않으며, 오히려 항생제에 노출된 경우 항생제에 내성을 가지는 속도가 빨라지는 것을 확인 할 수 있었다. 토양내 유산균의 경우 토양에 존재하는 유산균은 토양의 산성도를 조절하는 등 필수적인 역할을 하는 박테리아다. 토양 유산균은 정상적인 토양에서 상당히 많은 양이 존재하고 있음을 알 수 있다 그러나 식당에서 사용한 폐식용유를 토양에 투입한 결과 유산균의 함량이 급격하게 줄었음을 확인 할 수 있었다. 또한 토양에 유입된 폐식용유는 환경학적 스트레스를 유발하여 식물의 생장을 억제는 현상을 확인 할 수 있었다. 폐식용유가 토양에 유출되면, 토양박테리아를 비롯한 유익한 미생물군의 성장을 억제하고, 이로 인해 식물이 생장이 억제된다는 사실과, 항생제 내성균의 증가와 결합하면, 식용작물의 항생제 내성균의 오염이 발생할 가능성이 높아진다는 추론이 가능한 실험결과를 얻을 수 있었다.

HS-7

세포벽을 손상시킨 토양박테리아를 이용한 토양의 방사성 스트론륨의 제거방안

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스트론튬(Sr-88)이 90mM의 농도로 토양이나 식물의 성장이 가능한 0.7% 아가로즈젤에 투여되면 식물이 스트론튬(Sr-88)을 흡수해 성장이 억제되고 뿌리의 발달이 저해되어 결국 식물이 말라 죽게 된다. 또한 토양 내의 토양박테리아의 수도 급감하게 되어 토양에서의 경우 식물의 성장이 억제되는 것을 촉진 한다. 스트론튬(Sr-88)이 포함된 토양에 시화호토양에서 채취된 토양박테리아 5,6을 competent cell' 제조 방법을 이용하여 세포벽에 구조적 변화를 일으켜 투여하면 세포벽의 표면에 스트론튬(Sr-88)이 흡착되어 식물에 흡수되는 것을 억제하여 결과적으로 스트론튬(Sr-88)의 독성을 제거할 수 있다. 이는 토양에 존재하는 다른 토양박테리아의 경우에도 같은 효과를 보이는 것을 확인 할 수 있었다. 분자유전학적 고찰이 가능한 애기장대를 이용하여 애기장대에 중금속이 흡수될 경우 발현이 증가하는 HMA4 유전자의 검사를 통해 competent cell' 제조 방법으로 처리된 시화호토양박테리아 5,6번의 투입이 식물이 스트론튬(Sr-88)의 흡수를 억제 할 수 있음을 확인 할 수 있었다. 또한 토양에 스트론튬(Sr-88)이 포함되어 있는지에 대한 여부를 확인하기 위해 스트론튬(Sr-88)이 고농도로 존재하는 토양에서 생장 하는 토양박테리아를 분리 동정하였고 이를 이용하여 토양 내 스트론튬(Sr-88)의 존재여부를 파악할 수 있는 방안을 마련하였다.

HS-8

저염 환경에 적합한 유산균 선발 및 이를 이용한 저염 김치 생산

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본 연구는 이미 발효된 김치의 유산균을 이용하여 부작용 없는 안전한 저염 김치의 개발을 위해 진행 되어졌다. 여섯 개의 컵에 발효 전 김치를 나누어 담는다. 염도를 기준으로 실험군을 나누는데, 평소 김치 담글 때의 50%, 75%, 100%의 염도로 김치를 각각 담근다. 발효를 진행시키기 전 종가집 김치의 김치 국물을 여섯 개의 컵 중 염도가 다른 세 컵에 김치 국물을 투입한다. 실험군들의 pH는 발효 후 증가되었고 특히 50%의 저염 김치는 급격하게 증가된다. 김치 국물의 흡광도는 김치 국물을 발효 전 첨가하였던 실험군이 무첨가군보다 높은 수치를 띠고 있었다. 김치 세균은 총 5가지 다른 종류로 구분 되어 있었다. 김치 세균은 A부터 E로 명명하였다. 김치 국물의 첨가는 김치 세균의 다양성을 증가시킬 수 있고 경쟁을 강화시킨다. 김치 세균 A는 5~6%의 염도에서 가장 높은 활성을 보였다. 김치 세균 B는 5~6%에서 높은 활성을 보였고 C는 4%에서 높은 활성을 보였다. 김치 국물에서 유산균을 추출하여 동정한 결과 김치 세균은 *Bacillus Subtilis, Bacillus tequilensis, Lactobacillus sakei* 임을 밝혀낼 수 있었다. *Bacillus Subtilis*는 다른 두 유산균과는 다르게 염도 감소에 매우 높은 효과를 나타냈다. 유산균에 의한 염도 감소 효과의 과정을 알기위해 고 흡수성 수지로 실험을 한 결과 나트륨 이온을 균 내로 흡수하여 염도를 감소시키는 것으로 결론을 내리게 되었다. 결론적으로 유산균을 선별하고 조절함을 통해 김치 발효의 속도를 조절할 수 있고 저염김치 개발의 가능성을 높일 수 있었다.

HS-9

정제당류 및 인공당류에 노출된 세균의 항생제 민감성 변화

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This study is conducted to better understand how each kind of sugar affects bacteria and their antibiotic sensitivity. *S. aureus* cultured in 1% aspartame solution or in 0.13% oligosaccharide solution, and *S. typhimurium* in 1% sucrose solution obtained sensitive trait to ampicillin 1x, kanamycin 10x and ampicillin 1x, respectively. Certain concentration of sugar strengthened *S. aureus* and *S. typhimurium* sensitivity to antibiotics; however, resistibility as well. Three out of five colon bacteria of guinea pig and two out of three colon bacteria found in mouse became sensitive after they were cultured in oligosaccharide solution. Moreover, the experiment was conducted to investigate how the yeast and bacteria break down different types of sugar. Yeast utilized sucrose and saccharine the most while the bacteria used fructose the most frequently. In conclusion, antibiotic sensitivity obtaining ability of bacteria by being exposed to sugar symbolizes that the reaction of human bodies can be more sensitive to environmental chemicals due to sugar ingestion.

HS-10

다양한 식품군의 섭취에 따른 햄스터 장내 세균의 분포 변화

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본 연구는 음식물의 종류에 따라 장내세균의 패턴이 어떻게 변하는지를 살펴보고, 특히 당을 섭취했을 때의 장내세균의 변화를 알아보기 위해 실시하였다. 먼저, 3대 영양소인 탄수화물, 단백질, 지방을 비롯하여 약품, 당류 등에 속하는 13종의 식품군을 나누어 각 식품군 마다 3마리씩의 햄스터를 배치한 후 3일간 음식물을 공급하였다. 이 후 장내 세균을 NA 배지와 MRS 배지에 발생하도록 하여 일반세균과 유산균의 발생을 관찰하였고, 이를 콜로니의 외형적 특성을 기준으로 분류한 후 분리 배양하여 16S rRNA 분석방법으로 유전자의 염기서열을 확인하여 세균의 종류를 동정하였다. 탄수화물만을 먹인 그룹에서는 주로 대장균이 자랐고, 지방을 먹인 그룹에서는 주로 대장균과 락토코코스 레티스균이 자랐다. 단백질을 먹인 그룹에서는 주로 바실루스 세레우스와 대장균이 자랐으며, 액상과당인 물엿을 먹인 그룹에서는 햄스터 한 마리에서 급성폐렴을 일으킬 수 있는 클랩시엘라 뉴모니에가 배양되었으며, 실험 종료 후 수일 안에 세 마리 모두 사망하였다. 이에 물엿과 설탕, 포도당 등 당류를 각 세 마리의 생쥐에게 급여하면서 장내세균을 비교한 결과 설탕을 섭취한 그룹은 대조군과 세균의 종류나 증식량의 큰 차이가 없었으나, 물엿과 올리고당을 섭취한 그룹 생쥐의 장내세균은 종류와 수가 모두 증가하고, 유산균은 감소하였다. 결론적으로 한 가지 식품군을 과도하게 섭취하면 장내세균의 균형이 깨지며 당류의 경우에는 물엿과 올리고당의 섭취가 장내세균의 패턴에 미치는 영향이 크다는 것을 알 수 있었다.

HS-11

비타민 C의 세포증식 억제 효과를 이용한 박테리아의 항생제 내성발생의 감소방안 및 원리의 규명

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대장균을 배양하는 과정에서 비타민 1%의 용액을 처리하면 대장균의 생장이 억제되고, 비타민 0.1%의 농도를 처리하면 대장균의 생장이 촉진되는 것처럼 보이지만, 항생제에 대한 내성을 가지게 되는 속도가 줄어든다는 결과를 확인 할 수 있었다. 이는 매우 중요한 결과로, 항생제 내성을 가지는 세균의 메커니즘에 대한 변화를 유도해 항생제 내성의 습득을 늦출 수 있는 중요한 단서가 된다. 이 현상의 증명을 위해 비타민 0.1%의 농도로 처리된 대장균은 대장균내의 과산화수소 농도가 증가하며, 이는 과산화수소를 분해하는 카탈라아제의 파괴로 인한 현상임을 철이온(Fe^{2+})의 농도변화를 통해 확인 할 수 있었다. 이러한 이유로 항생제에 내성을 가지게 되는 plasmid DNA가 손상을 입게 되어 항생제 내성을 가지게 되는 속도가 느려지는 것을 증명하였다. 이와 더불어 대장균의 카탈라아제를 생성하는 유전자인 katE유전자의 발현을 통해서도 항생제에 내성을 가지게 되는 plasmid DNA가 손상을 입게 되어 항생제 내성을 가지게 되는 속도가 느려지는 현상에 대해 상세한 입증이 가능했다.

HS-12

Molecular Analysis of Microbial Community in Anaerobic Digesters Treating Food Wastewater

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This study is aimed to investigate the methanogenic communities in anaerobic digesters treating food wastewater for promoting successful operation of long-term anaerobic process.

Hypothesis

Previously, analysis of anaerobic digestion has primarily relied upon qualitative or semi-quantitative methods such as clone libraries, molecular fingerprinting and nucleic acid hybridization. While these methods represent advancement over culture based characterization, they can only give a limited insight into the quantitative population dynamics that are important for the performance of anaerobic digesters. For the reason, culture-independent analysis for the microbial quantification like quantitative polymerase chain reaction(qPCR) and qualitative analysis using denaturing gel gradient electrophoresis(DGGE) based on 16S rRNA sequence will provide underpinnings for the improvement of anaerobic process performance.

Procedure

Samples taken at each steady state were analyzed to evaluate the process performance. Anaerobic sludge, which was collected from a local municipal wastewater treatment plant, was seeded, and raw food wastewater was also collected from the same facility.

Total DNA of the steady-state samples was extracted in duplicate. The 16S rRNA gene copy numbers of two hydrogenotrophic methanogen orders, *Metnanobacteriales*(MBT) and *Methanomicrobisles*(MMB) and one aceticlastic methanogen order, *Methanosarcinales*(MSL) were quantified using real-time PCR.

For DGGE analysis, the DNA samples from an acidogenesis bioreactor were used. The V3 to V5 region of 16S rRNA genes in the extracted DNA was amplified using PCR with a set of universal bacterial primers, BAC 228F with a 40-bp GC-clamp (5´-CGCCC GCCGC GCGCG GCGGG CGGGG CGGGG GCACG GGGGG-3´) and BAC 805R.

Observations/data/results

For the qualitative analysis of methanogens, 16 bands were observed. In the seed and the methanogenic digesters, seven and fourteen bands were detected, respectively. All detected bands were identified to be 9 known methanogenic species.

For the quantitative analysis of methanogens, MSL was dominant at 34days of seed. Therefore it was probably due to the food waste like kimchi including high salinity. After 32days, MSL was rapidly decreased over time as acetic acid depleted. In acid fermentation tank, MSL rapidly increased at 45 days. Meanwhile, MMB was observed continuously throughout the entire process. Therefore, to induce MMB proliferation would be one of the key to promote successful long-term operation.

HS-13

식물 영양제가 토양 속 미생물에 미치는 영향

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담당교사:정미선

본 연구에서는 식물 영양제가 식물뿐만 아니라 토양 미생물에게 미치는 영향을 알아보고자 하였다. 이를 위해 각기 다른 3종류의 영양제가 식물의 생장과 토양에 미치는 영향, 토양미생물의 분포에 미치는 영향, 미생물들이 식물의 생장에 미치는 영향에 대해 알아보았다. 이에 대한 결과는 다음과 같다.

먼저, 식초 계란 영양제는 뿌리에 좋은 효과를 보였다. 식초 영양제 투여 후 개체 수의 증가를 보였던 토양 미생물은 종 분석결과 Bacillus subtilis strain 16-5G으로 추정되었으며 이를 크놉액과 함께 수경재배에 처치했을 시, 식물의 생장에 약간의 도움을 주었다. 하지만 대체적으로 식물의 생장에는 큰 효과를 보이지 못하였다.

앰플 영양제는 토양 미생물의 수를 증가시켜 미생물이 식물의 생장에 긍정적인 영향을 끼쳤다. 앰플 영양제를 투여했을 때 분포에 가장 큰 변화를 보였던 종은 Bacillus subtilis strain ML102B로 추정되며, 이를 크놉액과 함께 수경재배 했을 때 식물의 잎과 뿌리의 생장에 긍정적인 영향을 끼쳤다. 이 미생물을 새로운 식물 영양제에 사용한다면 기존의 식물 영양제를 개선할 수 있을 것이다.

미생물 효소 영양제는 잎 생장에는 많은 영향을 주진 않았지만 뿌리 생장에는 좋은 효과를 보였으며 영양제에 포함된 미생물 몇 종을 수경재배 하였을 때 좋은 효과를 보였다. 이 미생물들은 영양제 투여 후 토양에서 우점 종으로 나타나지 않았지만 수경재배에서 긍정적인 영향을 준 것으로 보아 효소 영양제 미생물들이 Bacillus megaterium strain BJ51, Pseudomonas reinekei strain SN8의 다른 미생물과 상호작용 시 더 높은 효과를 내었다고 해석하였다.

HS-14

어병성 세균에 대한 노무라입깃해파리 추출물의 항균 활성 및 양식업에서의 응용 가능성 탐구

김대희, 서보인, 이형진, 임승진
전북과학고등학교
지도교사:박주현

국립수산과학원의 최근 3년간 표본조사 결과, 해파리로 인한 어업피해액은 연간 760억원～2,290억원으로 추산된다. 그 중 노무라 입깃 해파리는 정확한 원인파악이 불명한 상태에서 개체수가 급격히 증가하여 수산물 어획과정의 커다란 장애물로 등장하였다. 한 개체의 최대무게(습중량)가 200kg에 달하는 특징으로 인해 그물 등 어구파손, 어류-해파리 혼획으로 인한 상품성 저하 등 그 피해의 양상 및 범위 또한 다양하다.

한편, 해파리는 그 개체수가 많고 크기 또한 거대하기 때문에 피해저감과 더불어 이들의 수거 및 처리로 인한 경제적 손실이 발생한다. 이러한 손실을 최소화하기 위해 최근 해파리를 폐기하는 대신 유효자원화 하는 연구가 진행되고 있다. 이에 따라 본 연구는 국내 인근 해안에서 폐기되는 해파리에서 항균 활성을 갖는 물질을 색출하여 또 다른 자원으로써의 가능성을 재조명한다.

류결절증, 저수온기 비브리오 병, 선회병 등의 양식업에서 물고기 집단폐사를 야기하는 위와 같은 어병을 독성이 있어 버려지는 해파리에서 추출한 천연 항균활성물질을 통해 해결하는 것이 본 연구의 최종 목표이다.

HS-15

제주도에 자생하는 블루베리인 '삼동열매'의 항균 실험

공윤성, 강민철, 강병준, 김민우
남주고등학교
지도교사:강영철

제주도에 자생하는 삼동나무에 열리는 삼동열매에 대한 관심을 가지게 된 후 기존의 여러 연구 자료들을 검색해 보게 되었다. 연구 자료들을 통해 삼동열매는 눈의 피로회복 및 혈액 정화의 효능이 있다는 점을 알게 되었다. 하지만 이러한 자료에서 찾을 수 없었던 식중독 균에 대한 항균효과에 대하여 의문이 들었다. 최근 식중독의 위험성에 대한 신문 기사를 자주 접하게 된 결과 이번 탐구에 있어서 혹시 다양한 효능을 지닌 삼동열매에 이러한 효능이 있지 않을까 하는 생각에 삼동열매 술과 삼동열매 엑기스로 항균실험을 진행하였다. 우리는 삼동 술과 삼동열매 엑기스의 항균효과를 알아보고자 식중독균인 *Bacillus*균과 *Listeria* 균을 배지에 배양하여 음성대조군으로 15% 에탄올, 양성대조군으로 항생제를 지정하여 탐구를 진행하였다. 이번 탐구를 통해 삼동엑기스에 항균효과가 있음을 알게 되었고, 이 효능이 제주도의 삼동열매가 세계적으로 유명한 블루베리와 같이 세계적인 식품이 되는 밑바탕이 될 것을 기대한다.

HS-16

조릿대의 항균작용과 실제 활용에 관한 연구

원호빈, 허성호, 고기범, 이원형
남주고등학교
지도교사:강영철

조릿대는 제주도에서 흔하게 볼 수 있는 식물 중 하나다. 몇 년 전부터 제주조릿대 신산업창출사업으로 시작해 지금은 제주조릿대 RIS사업단으로 제주조릿대를 홍보, 가공 및 판매를 하고 있다. 이 사업단 홈페이지를 들어가 보면 제주조릿대의 효능에 관한 자료들이 있는데, 그 자료들을 보면서 항비만, 항산화, 항염활성 등의 효능이 조릿대의 있다는 것을 알 수 있었다. 그러나 그 홈페이지를 찾아봐도 제주조릿대의 항균효과에 관한 논문이나 자료는 찾아볼 수 없었다. 그래서 우리는 조릿대의 항균효과가 있는지 찾아보고자 한다.

우리는 우선 조릿대가 항균효과를 나타낼 경우 사람들이 쉽게 접할 수 있도록 입욕제라는 실용화방안을 정하고 실험에 임하였다. 조릿대의 항균효과를 알아보기 위해 온도와 시간을 달리해서 조릿대 추출물 4세트를 만들었고, 이것으로 항균실험을 진행했다. 그 결과 우리가 실험에 사용했던 칸디다 알비칸스(Candida albicans)에 대해 4세트 모두 항균효과가 나타나지 않거나 미미한 수준이었다. 비록, 우리가 실험했던 방법으로는 항균효과가 나타나지 않았지만 우리가 여러 논문을 찾아본 결과 항균효과가 나타났다는 결과들이 있던 것으로 봤을 때 다른 방법을 이용한다면 긍정적인 결과를 볼 수 있을 것이라고 생각된다.

HS-17

한라봉과 보리누룩 발효액의 아토피 및 여러 세균에 대한 항균 연구

정유준, 김태완, 강준우, 강용원
남주고등학교
담당교사:강영철

곰팡이 핀 한라봉과 누룩을 섞어서 발효액을 만들어 문제성 피부(아토피)에 적용시키는 것은 옛날부터 내려오던 민간요법이다. 하지만 민간 요법이라는 것이 효과가 검증된 것이 아니기 때문에 우리는 실험을 통해서 민간 요법의 효능을 검증해 보고, 어느 정도의 발효기간이 가장 큰 효과를 나타내는지 보고자 하였다.

우리는 한라봉 – 보리누룩 발효액의 최적 발효조건을 알아보기 위해서 발효기간을 조작변인으로 설정하고 3종류의 균주를 설정시켰다. 균주 배양의 과정에서 *Propionibacterium acnes* (여드름균), *Escherichia coli* (대장균)은 배양에 실패해서 실험에 적용할 수 없었고 *Staphylococcus aureus* (황색포도상구균)은 배양에 성공해서 항균효과를 실험했지만 황색포도상 구균에서는 한라봉 – 보리누룩 발효액의 항균효과가 검출되지는 않았다. 하지만 아토피는 포도상구균 외에도 여러가지 복합적인 요인이 작용하므로 한라봉 – 보리누룩 발효액이 다른 아토피 유발요인에 효과가 있을 수도 있으며 추가 연구가 필요하다.

HS-18

*Pseudomonas elodea*의 부산물(by-product; biopolymer)인 Gellan gum의 점성의 조절을 통한 강도의 변화 탐구

이준우, 윤예린, 김민정, 박종웅
하나고등학교
지도교사:김민정

*Pseudomas elodea*의 부산물인 Gellan gum은 점성을 갖는 High acyl과 강도를 가지고 있는 Low acyl로 나뉜다. High acyl은 pH, 온도 등에 영향을 받아 점성이 변화될 수 있으며, Low acyl은 수용액의 농도와 흙과 수용액의 혼합 비율에 의해 강도가 조절 될 것이라는 가설을 세우고 탐구하게 되었다. High acyl의 경우 40도에서 최고의 점성을 띄었으며, 상온으로부터 50도 까지는 점성을 유지하였다. pH에 의한 실험에서는 pH9의 경우 점성이 가장 높았으며, 산성일 경우와 비교해 염기성일 경우에서는 점성이 크게 감소했다. 농도에 의한 High acyl과 Low acyl의 실험에서는 각각 농도와 점도/강도가 비례하다는 것을 알 수 있었다. Low acyl과 High acyl 그리고 세 종류의 흙을 이용한 확장실험 에서는 점성과 강도의 차이점을 느끼게 되었다. 젤란검은 일상생활에서 건축자제, 접착제 등으로 활용이 가능한 것으로 보인다.

HS-19

햄스터 비만 억제에 영향을 미치는 유산균 효과 고찰

은영범
민족사관고등학교
담당교사:조진호

햄스터 중에서 눈에 띠는 비만 체형인 햄스터의 배설물로부터 세균을 배양했다. 비만 햄스터들의 장내 미생물의 패턴을 크게 3가지 그룹으로 나눌 수 있었다. 유산균도 많은 그룹A, 유산균이 적고 노란 콜로니가 눈에 띄게 많은 그룹B, 유산균이 적고 노란 콜로니가 별로 눈에 띄지 않는 그룹C로 나눌 수 있었다.

주변에서 흔히 섭취할 수 있는 유산균에 의한 비만 억제 효과를 관찰하기 위해, 유산균양이 적은 그룹B와 C를 중심으로 김치와 유산균 음료로부터 분리한 유산균 5가지 종류를 균주별로 1가지씩 선택적으로 섭취하게 하여 비만과 장내 미생물의 변화를 관찰하였다.

대부분의 햄스터들에게서 유산균의 양이 급증된 것이 관찰되었으며, 특히 김치로부터 분리한 Lactococcus lactis가 가장 뛰어난 비만 억제 효과를 보였다. 또한 많은 그룹 B에서 노란 콜로니들이 사라지는 등 LB배지에서 많은 세균총의 변화를 관찰할 수 있었다.

HS-20

적송(*Pinus resinosa*)과 리기다소나무(*Pinus rigida*)의 내생균에 대한 소나무재선충(*Bursaphelenchus xylophilus*)의 섭식 특성 비교

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담당교사:이재구

본 연구는 적송(*Pinus densiflora*)과 리기다소나무(*Pinus rigida*)에서 내생균을 분리 배양하여, 두 수종에 서식하는 내생균의 소나무재선충(*Bursaphelenchus xylophilus*)에 대한 피식성의 차이를 비교하였다. 소나무재선충은 소나무류에 기생하는 선형동물로, 리기다소나무는 소나무재선충에 일정 수준의 내성이 있으나, 적송 등 다른 소나무속의 식물은 소나무재선충에 감염되면 소나무재선충병을 보이며 2년 내에 고사한다. 소나무재 선충은 소나무의 내생균을 섭식하기 때문에 연구자들은 리기다소나무와 다른 소나무속 식물이 보이는 내성의 차이가 내생균 군집 분포의 차이에 기인한다고 가정하였다. 적송과 리기다소나무의 샘플을 표면살균하고 WA 배지에서 배양하는 과정을 거쳐 균을 분리하였다. 분리된 균은 PDA 배지에서 배양 후 소나무재선충을 접종하 였으며, 일정 기간이 지난 뒤 소나무재선충의 밀도 변화를 관찰하였다. 실험 결과 리기다소나무에서는 12종, 적송에서 6종의 내생균이 분리되었다. 균류의 종류에 따라 소나무재선충은 섭식 행동에서 큰 차이를 보였으며, 적송에 비해 리기다소나무로부터 소나무재선충이 섭식하지 못하는 내생균이 많이 분리되었다.

HS-21

해양 미생물(미세조류)의 방제효과 탐구

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담당교사:박선미

우리는 해양 미생물을 이용한 방제 효과에 대하여 탐구 해 보았다. 우리의 실험은 기름 유출사고를 보고 해양미생물이 방제능력을 가지고 있지 않을까 하는 생각에서 시작하게 되었다. 그래서 해양 미생물을 바닷물과 같은 염도의 소금물에서 휘발유, 벤젠, 톨루엔을 넣어준 뒤, 일정 시간마다 VOC측정기를 이용하여 값을 측정하며 오염도가 얼마나 줄어드는 지를 관찰하였다. 한 실험 마다 기간은 8일로 잡고 24시간 마다 동일한 시간에 정기적으로 측정하였으며 같은 조건에서의 대조군과 효모를 설정하여 측정값을 비교하였다. 우리는 미생물의 방제 효과를 탐구함과 동시에 우리 생활에서의 미생물이 얼마나 유용하게 사용될 수 있는지에 대하여 탐구해 보기로 하였다.

HS-22

미생물을 이용한 과다면역반응의 억제

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하나고등학교
담당교사:김민정

기생충은 알레르기를 억제한다는 연구 결과도 있지만, 부작용을 잘 일으키므로 세균에 의한 알레르기 치료가능성을 알아보려 했다. 개울, 공기, 김치, 햄스터 배설물 등에서 채집하거나 구입한 18가지 세균에 대해 비만세포(Mast cell) 탈과립화 억제효과를 관찰했다.

HMC-1 cell에 A23187 처리 후, 각각 18가지 세균으로 처리했다. 조사한 18종류의 세균추출물 10ul와 100ul에서 모두 비만세포의 탈과립을 억제한 세균은 3가지 였으며 *Pseudomonas* sp., *Bacillus subtilis, Pseudomonas mosselii*로 확인되었다.

선별된 3가지 세균을 처리한 상태에서 비만세포의 탈과립현상으로 방출된 히스타민을 정량분석한 결과 *Pseudomonas* sp., *Bacillus subtilis*의 2종에 대하여 tryptase 분비저해 효과가 관찰되었으며. 특히 *Pseudomonas* sp.의 경우 눈에 띄는 현저한 감소효과를 나타내었다. 시료준비과정에서 고온고압살균과정을 거쳤기 때문에 *Pseudomonas* sp. 체내에 있는, 열에 강한 화학물질이 아토피와 같은 자가면역질환 치료제로 쓰일 것으로 기대된다.

HS-23

Norovirus에 대한 Propolis의 억제 효과 측정

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담당교사:김세진

Norovirus는 *Caliciviridae* 과에 속하며 Single-Strand RNA 바이러스로 직경이 약 27~40 nm의 작은 구형 바이러스이다. 최근 전 세계적으로 발생하는 식중독 사례의 주요 원인체이며, 단체 급식 등 집단 피해 사례에서 Norovirus가 원인 병원체로 확인되면서 대규모 집단 발병에 대한 예방 대책이 시급하지만, 현재까지 Norovirus에 대한 백신이 개발되어 있지 않아 이에 대한 연구가 활발히 진행되고 있다.

프로폴리스는 벌이 나무, 꽃 등의 식물로부터 얻은 beewax와 resin을 그들의 타액과 혼합하여 만든 물질이며, raw 프로폴리스에는 resins (40-55%), beewaxs and fatty acids(20-35%), essential oils (about 10%), pollen (about 5%)의 주요 구성성분과 이 밖에 무기질, 비타민, 당 등을 포함 하고 있다. 여러 민간요법에서 사용되던 프로폴리스는 최근 influenza virus, HIV, adenovirus, herpes simplex virus등 antivirus 활성도 보고 되고 있으나, norovirus에 대한 프로폴리스의 활성은 보고되어 있지 않다. 따라서, 본 연구에서는 프로폴리스가 norovirus에 대한 새로운 항바이러스 활성 이 있는 천연소재로서의 가능성이 있는지 알아보고자 한다.

본 연구에서는 Norovirus의 surrogate로서 Murine Norovirus(MNV-1)와 Feline Calicivirus(FCV-F9)을 사용하였고, 이에 대한 host cell로 RAW264.7 cell 및 Crandell feline kidney(CRFK) cell을 각각 사용하였다. 두 cell은 Dulbecco's Modified Eagle's Medium (DMEM)을 기본 배지로 하여 37°C 5% CO_2 incubator에서 배양하였다. 실험에 사용된 프로폴리스는 (주) 서울 프로폴리스에서 sample 4가지를 받아 사용하였으며, 이중 3가지는 에탄올 추출물이며, 나머지 1개는 3개의 에탄올 추출물 중 1개 sample에서 에탄올을 제거하여 다시 물에 녹여 수용성으로 만들었다. 이들 sample은 아르헨티나, 국산, 중국으로 각각 다르며, 총 flavonoid 함량이 각각 에탄올 추출물은 2%, 수용성 추출물은 1%이 되도록 희석하여 사용하였다.

각 프로폴리스 sample의 항바이러스 활성 실험에 앞서, host cell 자체에 세포독성이 있는지 체크하기 위해 시간별, 농도별로 MTT assay를 실시하였다. 그 결과, RAW264.7 cell의 경우, 0.1%에서 24시간 처리 했을때 프로폴리스를 처리하지 않은 control과 비교하여 cell viability가 8.26%로 이 농도이상에서는 독성이 있는 것으로 나타났으며, 0.01% 이하 농도에서는 48시간까지 모두 90%의 cell viability를 보였다. CRFK cell의 경우, 역시 0.1%에서 48h처리 했을때 20.14%로 세포독성이 관찰됐으며, 0.01% 이하 농도에서는 모두 90%이상의 cell

viability를 보였다.

항바이러스 활성을 체크하기위한 Plaque assay를 진행하기에 앞서, 프로폴리스 sample을 국산 2% 에탄올 추출물, 중국산 1% 수용성 추출물로 축소하고, 에탄올 용매를 날리기 위한 동결건조과정을 진행하였다.

이후, 세포독성이 나타나지 않는 0.01% 이하의 프로폴리스농도에서 Norovirus와 Sample을 미리 반응하여 cell에 첨가하는 Pre-treatment와 Norovirus와 Sample을 동시에 cell에 첨가하는 Co-treatment, 두 가지 방식을 이용해 Plaque assay를 진행하였다. 기대와 달리 Norovirus를 프로폴리스가 경향성 있게 억제한다는 결과가 나오지는 않았지만, 이는 Sample 처리 방식에 따른 차이나 0.01%농도 이하에서의 농도 구획의 문제 등의 가능성이 있기 때문에 농도를 더 세분화 시키거나, Sample을 처리를 다르게 해 본다면 경향성 있게 활성을 띄는 농도를 찾을 수 있을 것으로 기대된다.

HS-24

탄소결핍 배지를 활용한 Pseudomonas 계열 세균의 폴리머 분해 탐구

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하천이나 호수 일대에서 채취한 토양과 물 시료로부터 PET (polyethylene terephthalic acid) 및 단위체인 terephthalic acid를 분해하는 세균을 찾고자 하였다. terephthalic acid를 탄소원으로 하는 한천배지에서 자라난 세균들을 동정하였는데, 이들은 *Pseudomonas fluorescens*, *Acinetobacter kyonggiensis*, *Acinetobacter* sp., Uncultured *Acinetobacter* sp., *Pseudomonas* sp., *Streptomyces gardneri*, *Acinetobacter* sp., *Microbacterium* sp. 였다. 이 중 *Pseudomonas* sp. KJF11-6는 terephthalic acid가 첨가된 액체배지에서도 잘 성장하였는데, 이를 오염물질 제거 등에 활용할 수 있을 것이다.

HS-25

일반렌즈에 비해 컬러렌즈에 세균이 더 쉽게 부착되는 이유 연구

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목적: 컬러렌즈 사용이 증가함에 따라 그로 인한 세균감염도 증가 추세이다. 그러나 왜 일반렌즈에 비해 컬러렌즈의 감염이 더 잘 발생되는지에 대한 연구는 아직 되어있지 않다. 본 연구는 렌즈표면의 특성을 중심으로 세균감염을 잘 일으키는 이유에 대해 연구하였다

방법: 하이드로겔 재질의 컬러렌즈와 일반렌즈를 대상으로 하였다. 각 렌즈의 표면 거칠기를 원자력현미경으로 분석하였고, Pseudomonas aeruginosa를 24시간까지 렌즈에 접촉시킨 후 표면에 부착된 세균을 분리·배양하였다. 또한 두 종류의 렌즈를 사람이 실제 착용한 뒤, 렌즈에 붙은 세균정도를 세균배양을 통해 확인하였다.

결과: 원자력현미경으로 렌즈 표면을 분석한 결과, 일반렌즈에 비하여 미용컬러렌즈의 거칠기가 유의하게 증가되어 있었고, 세균용액에 담갔던 렌즈의 세균을 분리, 배양, 관찰한 결과 컬러렌즈에 세균이 더 많이 붙는 것을 확인하였다. 흥미로운 것은, 1시간만 배양해도 컬러렌즈에서의 세균부착이 의미 있게 증가하였다. 실제 사람에게 착용된 렌즈를 배양한 결과 일반렌즈와 달리 컬러렌즈는 착용시간에 따라 세균부착이 증가하는 것을 확인하였다.

결론: 본 연구는 컬러렌즈의 표면 거칠기가 증가되어 세균부착이 일반렌즈에 비해 쉽게 일어나며 이로 인한 세균감염이 증가될 가능성을 객관적으로 밝혔다.

HS-26

빗방울 속 미생물 종류 및 연관성 탐구

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인천진산과학고등학교
담당교사: 김효남

최근 세계적으로 미세먼지의 영향이 매우 커지고 사람들의 대부분이 대기오염 상태 변화에 관심이 집중되고 있다. 이처럼 대기오염의 심각성이 떠오르면서 우리는 주변의 대기의 오염도와 대기 속 미생물 종류의 상관관계에 대해 궁금증이 생겼다. 대기의 오염성분의 변화에 따라 대기에 분포하는 미생물의 종류도 영향을 받을 것이라는 가정 하에 강우 시에 대기 중의 미생물이 빗방울 또는 눈 안에 갇힐 것이라고 예상하였다. 그래서 비 또는 눈이 내리기 전과 비 또는 눈이 내린 후의 대기 미생물을 공기 중에 고체 배지에 노출시키는 방법으로 포집을 하고, 강수 시에는 멸균시킨 집기병으로 빗방울 또는 눈을 포집한다. 우리는 본 실험에서 크게 강우 전과 강수 시, 강수 후의 미생물을 분류한 뒤 그 날의 대기오염의 상태와 미생물의 종류를 비교해 보고자한다.

HS-27

미생물과 세포를 활용한 항균 물티슈의 독성평가

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담당교사:최현주

There are a variety of products such as wet wipes used in everyday life. However, they could contain toxic chemicals. Therefore, question has been raised about if wet wipe has adverse effects on our aquatic ecosystem and human health. To answer this question, ecotoxicity tests were conducted on water extracts of wet wipes using microtox bioassay. Results were compared to *Daphnia magna*, phytoplankton and Japanese medaka(*Orizias latipes*) which are widely used for evaluating aquatic ecotoxicity. Microtox bioassay may be a good candidate for pre-screening the environmental toxicities of water pollutants, since the testing method with microtox bacteria was relatively easier and more economic than other bioassays. To determine the toxicity effect of human health by wet wipes, human lung cells was treated with effluent of wet wipes (EWWs) for 24 hours. EWWs led to reduction of cell viability with morphological change. However, comet analysis showed that treatment of cells with EWWs did not increase DNA damage.

HS-28

광합성미생물을 이용한 음식물 쓰레기의 효율적 처리 방안 탐구

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본 연구는 *Rhodobacter sp.*을 음식물쓰레기에 사용했을 때 *Rhodobacter sp.*이 가진 효과들이 성공적으로 발현되어 음식물쓰레기의 효율적, 친환경적 처리 및 퇴비화를 도울 수 있는지 여부에 대한 탐구이다. 실험에서 광합성미생물로는 *Rhodobacter sp.*를 사용하였다. 광합성미생물의 악취제거효과를 알아보기 위해 후각이 건강한 사람들에게 직접 냄새를 맡도록 하는 직접 관능법과 플라스틱 시험관에 암모니아수를 넣고 *Rhodobacter sp.*을 증류수와의 비율을 달리하여 넣은 암모늄 이온의 감소량을 측정하는 방법을 사용하였다. 또한 *Lactobacillus casei*와 *Escherichia coli*를 이용하여 *Rhodobacter sp.*의 발효균 활성화 및 부패균 비활성화 능력을 확인하였다. 스티로폼 상자 두 개에 흙과 음식물쓰레기를 넣은 후 실험군에만 *Rhodobacter sp.*을 넣은 뒤 온도를 측정하여 *Rhodobacter sp.*이 음식물쓰레기의 퇴비화 속도에 미치는 영향을 탐구하였다. 연구 결과 *Rhodobacter sp.*이 증류수에서의 자연적인 암모늄이온의 증발량보다 더 많은 양의 암모늄이온을 제거한다는 것을 알 수 있었다. *Rhodobacter sp.*은 발효균의 증식을 활성화시키며, 부패균의 증식을 미약하게나마 억제한다. *Rhodobacter sp.*은 퇴비화속도를 향상시킨다는 것을 본 연구를 통해 확인할 수 있다. 이러한 *Rhodobacter sp.*의 특징으로 인해 *Rhodobacter sp.*은 음식물쓰레기의 효율적 처리를 위하여 효과적으로 사용할 수 있으며, 실제 처리과정에서 본 연구를 통해 확인한 효과들뿐만 아니라 다른 여러 미생물과의 긍정적 상호작용을 기대할 수 있어 음식물쓰레기로 인한 경제적 환경적 피해를 줄이는 일에 크게 기여할 수 있을 것이다.

HS-29

Platycodon grandiflorum (길경)을 이용한 치아 바이오필름 형성 세균 억제 방안 연구

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야생초가 치아 바이오필름 형성억제에 어떻게 이용될 수 있는 지를 알아보기 위해 연구를 진행하였다. 영양소(10% 펩톤, 10% 포도당, 오일)를 달리해서 처치했을 때에 비해 쑥, 길경, 구절초 추출액을 처리한 경우는 세균 생장이 현저하게 감소한 것을 알 수 있었다. XTT Assay에서 10% 포도당과 추출액을 1:1로 섞어 배양액으로 사용한 경우에 비해 야생초 추출액만을 배양액으로 넣어준 경우가 흡광도 수치가 낮게 측정되어 항생효과를 보이는 물질이 있음이 확인되었다. 야생초 추출액에 대한 Paper Disc 실험을 통해 모두 항생 효과를 보인다는 것을 알 수 있었다. 또한 탄닌으로 추정되는 성분이 확인되었으며 길경에서 탄닌 추정 물질에 대한 농도가 특별히 높게 나타났다. 길경은 모두 높은 항생효과를 보여 치약이나 가글 제재 합성을 위한 재료로 사용될 수 있는 가능성을 보였다.

HS-30

PGPR 의 생육촉진현상과 ISR 기작 활성화 탐구

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Nitrogenous manure, which has been used to boost a yield since early 20 century, is pointed as a crucial reason of the environmental pollution such as eutrophication. Not only does it occur the rapid difference of species but also vigorous side-effect to human lives. To solve this matter, many of researchers and institutes have studied to invent new type of manure that is not harmful to nature.

PGPR (Plant Growth Promoting Rhizobactaria) is root-colonizing bacteria which form symbiotic relationships with plants. PGPR can be generated through direct or indirect way so that the plant immunized from the diseases which are caused by bacteria. (PGPR can promote plant growth through direct and indirect means, and prevent plant diseases that are caused by other bacteria fungi.) It is hard to investigate, however, because the concrete mechanisms of immunizing process haven't been well-characterized and researched.

Therefore, the present writers conducted an experiment with the aim of analyzing roll of PGPR in plant growth (especially for ornamental bulbs), and process of inducing ISR based on hypothesises : PGPR can promote plant growth, which can boost a productivity of onion and increase the average number and length of garlic's root hair. Experiment was conducted, based on advanced scientific research on PGPR and variables of experiment are accurately controlled. The test is conducted, and data are statistically analyzed and digitized.

HS-31

고초균의 영양소 분해능력을 이용한 음식물쓰레기의 분해 및 퇴비화에 관한 탐구

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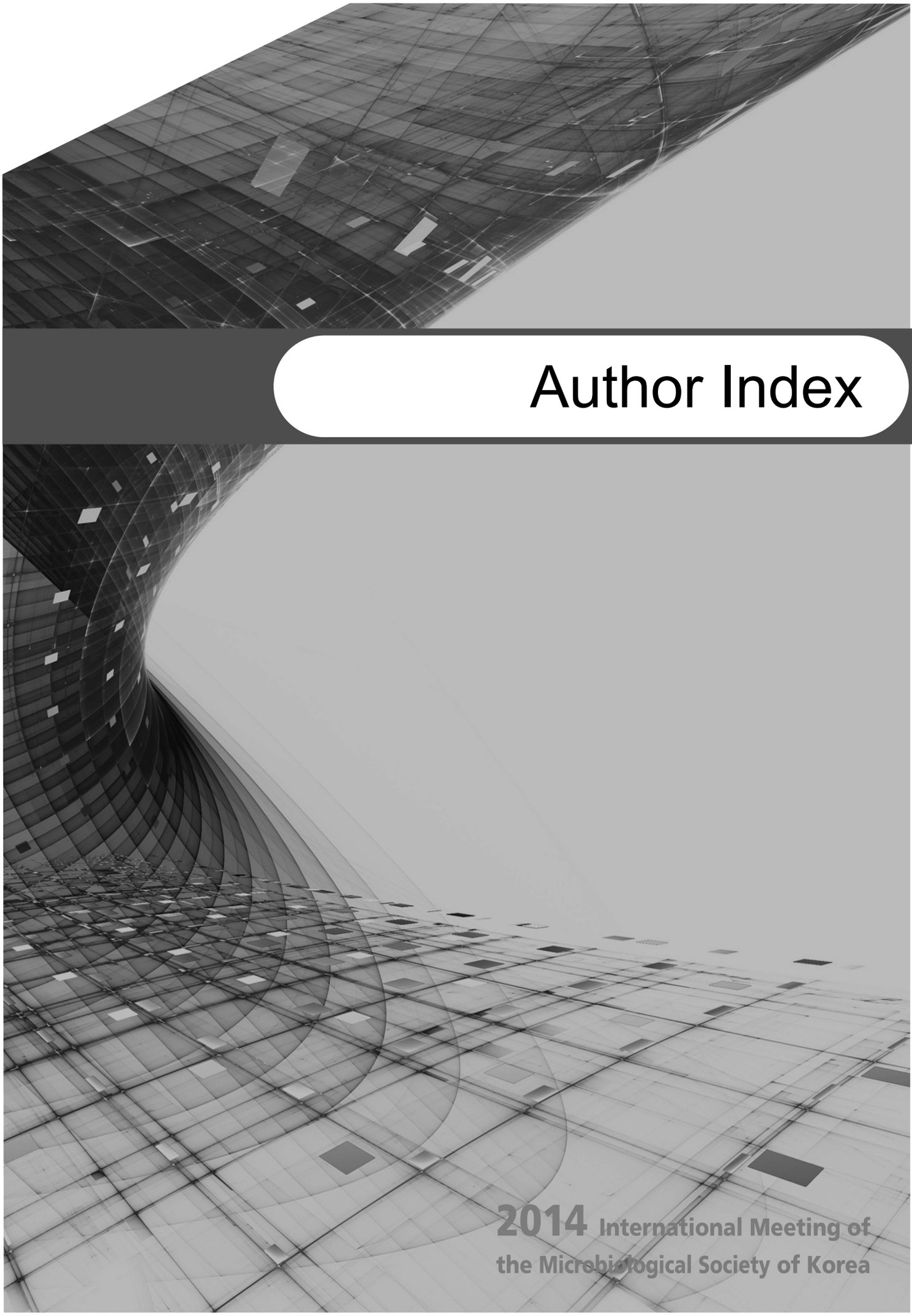
현대사회에서 음식물쓰레기의 문제는 심각하다. 1년에 음식물쓰레기를 처리하는데 드는 비용은 약 6000억원이나 된다. 그래서 우리는 고초균(*Bacilus subtilis*)을 음식물쓰레기 처리에 사용해 보기로 했다.

고초균은 볏짚에 많이 존재한다. 우리 조상들은 예로부터 볏짚을 많이 사용했고, 특히 볏짚을 썩혀서 퇴비로 주곤 했다. 그래서 우리는 음식물 쓰레기를 고초균으로 분해할 수 있는지 그리고 생성된 생성물이 퇴비로서의 효과가 있는지 실험해 보았다.



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