

2023 한국미생물학회

생리생화학 심포지움

Advances in Microbial Physiology and Biochemical Research

일자 **2023.8.17(목)~18(금)**

장소 부산대학교 첨단과학관 205호

주최



한국미생물학회
The Microbiological Society of Korea

공동
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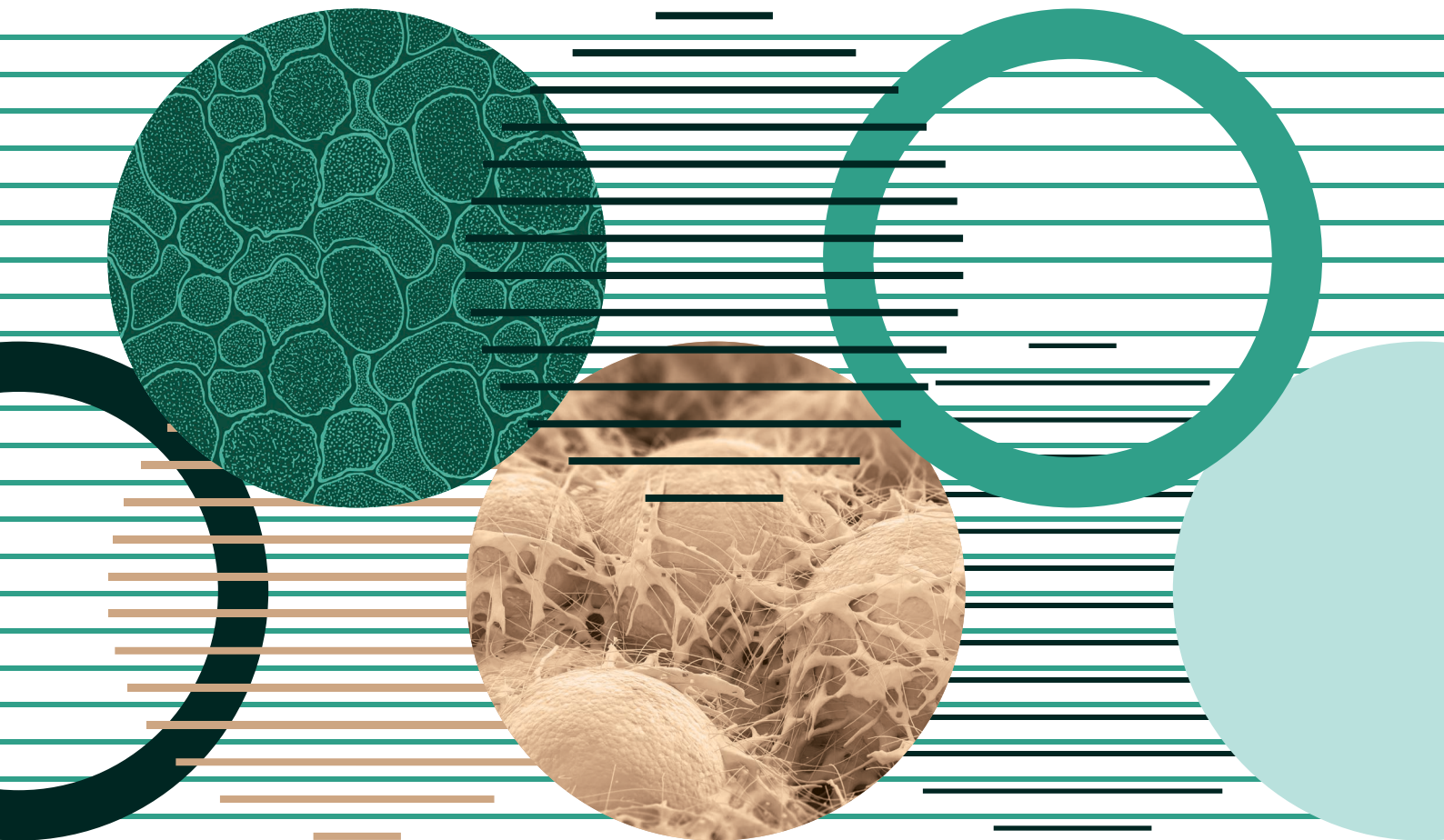
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장수·해양바이오
혁신인력 양성 교육연구단

후원



부산대학교
미생물자원연구소



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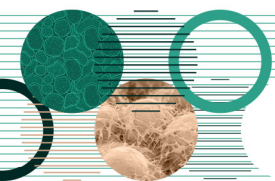
(사)한국미생물학회

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Timetable

| 8.17 (Thu) | | |
|-------------|---|----------------------|
| 14:30~ | Registration | |
| 15:00~15:10 | Opening Address | |
| | 축사: 정용석 교수 (경희대학교, 한국미생물학회 회장) | 사회: 차선신 교수 (이화여자대학교) |
| 15:10~16:10 | S1. Scientific Session 1 좌장: 김효정 교수 (우석대학교) | |
| | S1-1. 이준희 교수 (부산대학교) | |
| | S1-2. 이창한 교수 (아주대학교) | |
| | S1-3. 염진기 교수 (서울대학교) | |
| 16:10~16:30 | Coffee Break | |
| 16:30~17:30 | S1-4. 김민규 박사 (한국원자력연구원) | |
| | S1-5. 김지선 박사 (한국생명공학연구원) | |
| | S1-6. 오준택 교수 (경희대학교) | |

| 8.18 (Fri) | | |
|-------------|---|--|
| 09:20~09:40 | S2. Scientific Session 2 좌장: 이창한 교수 (아주대학교) | |
| | S2-1. 김옥빈 교수 (이화여자대학교) | |
| | S2-2. 천병희 교수 (부경대학교) | |
| 10:00~10:20 | Coffee Break | |
| 10:20~11:00 | S2-3. 이현숙 박사 (한국해양과학기술원) | |
| | S2-4. 차선신 교수 (이화여자대학교) | |

Scientific Program

S1

Scientific Session 1

Chair: Hyo Jung Kim (Woosuk University)



S1-1 15:10-15:30

Bacterial Biofilm: Environmental Cues and Signaling

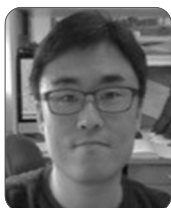
Joon-Hee Lee (Pusan National University)



S1-2 15:30-15:50

Various Chaperoning Elements in Bacteria

Changhan Lee (Ajou University)



S1-3 15:50-16:10

A Signal Transduction System Responds Oxidative Stress to Confer Pathogenesis in Bacteria

Jinki Yeom (Seoul National University)



S1-4 16:30-16:50

HPr Prevents FruR-mediated Facilitation of RNA Polymerase Binding to the *fru* Promoter in *Vibrio cholerae*

Min-Kyu Kim (Korea Atomic Energy Research Institute)



S1-5 16:50-17:10

Function of Novel Korean Gut Bacteria in Human Disease

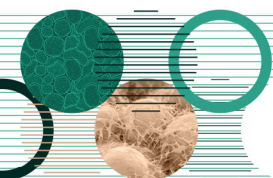
Ji-Sun Kim (Korea Research Institute of Bioscience and Biotechnology)



S1-6 17:10-17:30

Transcriptional Processing of Elongation Barriers by RNA Polymerases

Juntaek Oh (Kyung Hee University)



S2

Scientific Session 2

Chair: Changhan Lee (Ajou University)



S2-1 09:20-09:40

The Orphan Enzymes in Anaerobic Allantoin Degradation: Oxamate Carbamoyltransferase and Catabolic Carbamate Kinase

Ok Bin Kim (Ewha Womans University)



S2-2 09:40-10:00

A Study on Functional and Metabolic Characteristics of Fermented Microorganisms in Korean Traditional Fermented Foods Based on Genome Analysis

Byung Hee Chun (Pukyong University)



S2-3 10:20-10:40

Anaerobiosis of the Hyperthermophilic Archaeon *Thermococcus onnurineus* NA1

Hyun Sook Lee (Korea Institute of Ocean Science & Technology [KIOST])

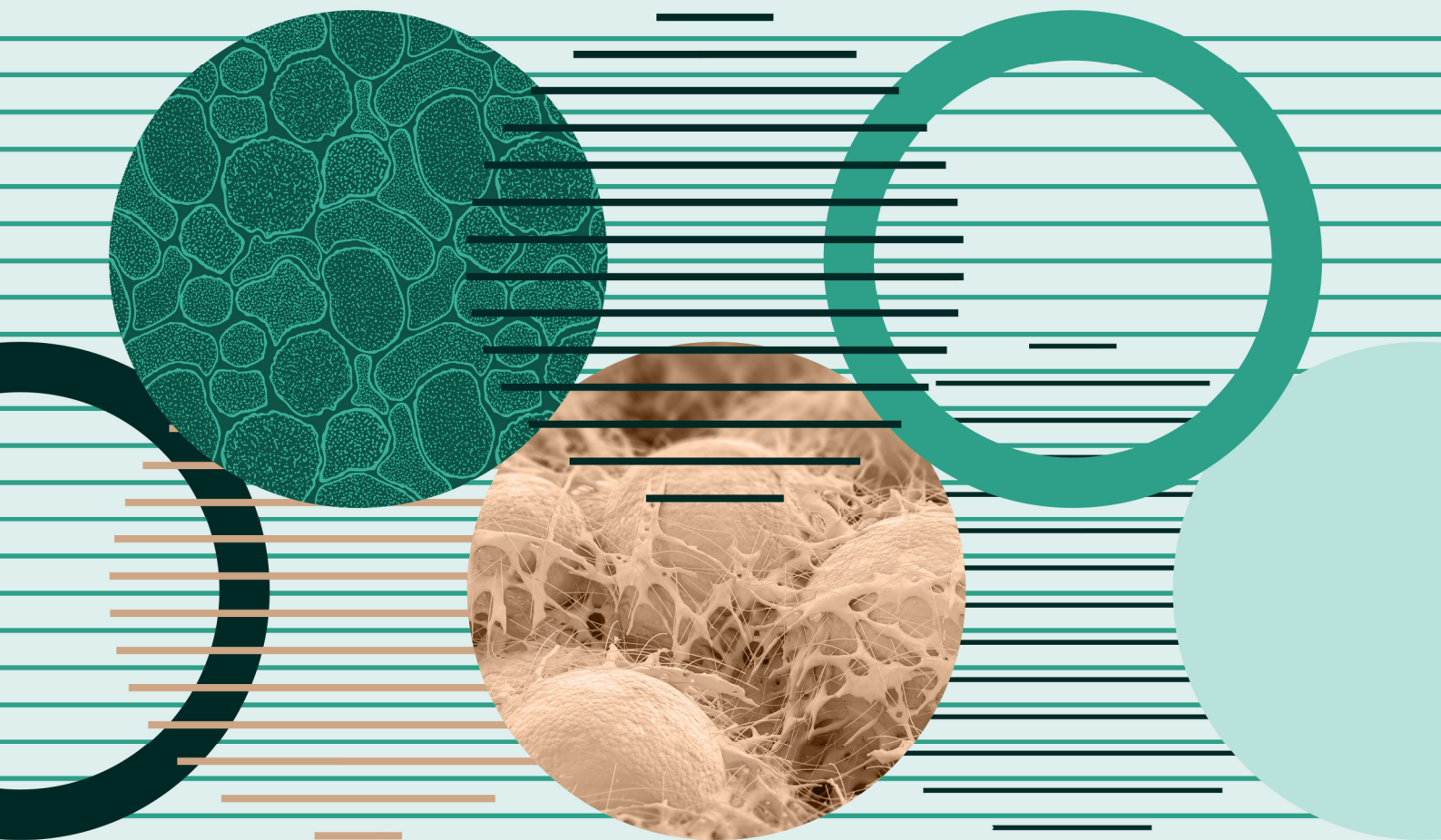


S2-4 10:40-11:00

Crystal-guided Discovery of the Broad Inhibition Activities of Halisulfates against β -Lactamases

Sun-Shin Cha (Ewha Womans University)

Scientific Session 1



Bacterial Biofilm: Environmental Cues and Signaling

Hyeon-Ji Hwang, Alexandra Winkler, Huiyan Li, and Joon-Hee Lee*

Department of Pharmacy, College of Pharmacy, Pusan National University

Biofilms are complex microbial architectures that encase microorganisms in a matrix composed of self-producing extracellular polymeric substances (EPSs), such as polysaccharides, extracellular DNAs (eDNA), and proteins. Bacterial biofilm is a social community of bacterial cells in which complicated bacterial group behaviors occur. Since bacterial cells in biofilms become highly tolerant to anti-microbial treatments, various environmental challenges, and host immunity, and thus cause serious problems in human bodies and civil/industrial facilities, it is of great interest for scientists to understand bacterial biofilm formation and to resolve the biofilm-mediated infections and biofouling. Biofilm development is not a simple passive process, but an active process occurring as a result of complex spatial differentiation and molecular events in biofilm cells, and finely controlled in population by bacterial cell-to-cell microbial signaling and communications in response to various environmental cues. Here, we show how *Pseudomonas aeruginosa*, an important opportunistic human pathogen regulates its biofilm formation according to environmental cues and cell-to-cell signaling.

Various Chaperoning Elements in Bacteria

Changhan Lee

Department of Biological Sciences, Ajou University

Proteins are indispensable for the survival of all living organisms. They possess unique structures that are directly linked to their functions. While the folding of proteins occurs spontaneously, larger and more complex proteins face challenges in attaining their correct structures. Moreover, various environmental changes such as heat and acidity can induce denaturation of proteins, leading to functional loss. Additionally, the denatured proteins often exhibit toxicity within cells. Therefore, cells employ a molecular chaperone system to facilitate the proper folding of proteins and protect them against various stresses. Molecular chaperones were initially discovered as proteins induced by heat and have been found to be conserved in all organisms from bacteria to humans. Apart from these proteins, various biomolecules exist within the cell, which also exhibit chaperone-like effects. In this talk, I will present the chaperone-like function of nucleic acids and the function of chaperones in opportunistic human pathogen *Pseudomonas aeruginosa*.

A Signal Transduction System Responds Oxidative Stress to Confer Pathogenesis in Bacteria

Hoan Van Ngo^{1,2}, Donghyuk Shin³, Kiwook Kim⁴, and Jinki Yeom^{1,2*}

¹Department of Biomedical Science, College of Medicine, Seoul National University

²Department of Microbiology and Immunology, College of Medicine, Seoul National University

³Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University

⁴Department of Pharmacology and Regenerative Medicine, University of Illinois College of Medicine, Chicago, IL, USA

All living organisms respond to environmental stresses in order to survive. In response to environmental cues, signal transduction systems govern global gene expression via post-translational modification. Under stressful conditions, the two-component signal transduction system in bacteria regulates gene expression for survival and proliferation. Here, we report that a metal cofactor of sensor protein in two-component signal transduction system activates a gene cluster for iron-sulfur assembly in response to oxidative stress that enable bacteria survive in the host. The transcriptional regulator PmrA and the sensor PmrB form a two-component system in pathogenic bacterium *Acinetobacter baumannii* are required for survival within the innate immune cells of the host. PmrB detects hydrogen peroxide in the periplasmic space of bacteria, and then activates the PmrA regulator protein by phosphorylation. Activated PmrA stimulates gene expression for iron-sulfur cluster assembly to alleviate bacterial oxidative stress. Histidine residues in PmrB are involved in the detection of hydrogen peroxide. This signal sensing system comprised of PmrB residues is sufficient for macrophage survival. Inactivation of histidine residues in PmrB renders is hypersensitive to cellular killing by hydrogen peroxide and innate immune cells. Our findings indicate that a two-component signal transduction system employs a histidine core for sensing oxidative stress to survive inside the immune cells of an infected host.

HPr Prevents FruR-mediated Facilitation of RNA Polymerase Binding to the *fru* Promoter in *Vibrio cholerae*

Chang-Kyu Yoon^{1,2†}, Seung-Hwan Lee^{1†}, Jing Zhang^{3†}, Hye-Young Lee^{1,2},
Min-Kyu Kim^{3*}, and Yeong-Jae Seok^{1*}

¹*School of Biological Sciences and Institute of Microbiology, Seoul National University*

²*Research Institute of Basic Science, Seoul National University*

³*Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute*

Phosphorylation state-dependent interactions of the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) components with transcription factors play a key role in carbon catabolite repression (CCR) by glucose in bacteria. Glucose inhibits the PTS-dependent transport of fructose and is preferred over fructose in *Vibrio cholerae*, but the mechanism is unknown. We have recently shown that, contrary to *Escherichia coli*, the fructose-dependent transcriptional regulator FruR acts as an activator of the *fru* operon in *V. cholerae* and binding of the FruR-fructose 1-phosphate (F1P) complex to an operator facilitates RNA polymerase (RNAP) binding to the *fru* promoter. Here we show that, in the presence of glucose, dephosphorylated HPr, a general PTS component, binds to FruR. Whereas HPr does not affect DNA-binding affinity of FruR, regardless of the presence of F1P, it prevents the FruR-F1P complex from facilitating the binding of RNAP to the *fru* promoter. Structural and biochemical analyses of the FruR-HPr complex identify key residues responsible for the *V. cholerae*-specific FruR-HPr interaction not observed in *E. coli*. Finally, we reveal how the dephosphorylated HPr interacts with FruR in *V. cholerae*, whereas the phosphorylated HPr binds to CcpA, which is a global regulator of CCR in *Bacillus subtilis* and shows structural similarity to FruR.

Function of Novel Korean Gut Bacteria in Human Disease

**Ji-Sun Kim¹, Ju Huck Lee¹, Seung-Hwan Park¹, Se Won Kang¹, Dong-Ho Lee²,
Hyuk Yoon², Je Hee Lee³, and Jung-Sook Lee^{1*}**

¹*Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology*

²*Seoul National University Bundang Hospital*

³*CJ Bioscience, Inc.*

The human gut is colonized with complex and diverse communities known as billions of gut microbiota. Metagenome-based diversity analyses to date have shown the potential that the gut microbiome may mediate or modulate human health and disease risk. Therefore, in order to study the direct relationship between human disease and the gut microbiome, it is very important to conduct physiological and multi-omics studies using gut microbiota isolated from human. However, many researchers have encountered difficulties in follow-up studies using disease-related gut microbiota due to the difficulty of their isolation/cultivation and the different characteristics of strains. To solve these problems, we isolated 13,066 strains belong to 457 species from 835 healthy Koreans through Korean Gut Microbiome Bank (KGMB) project. Based on multi-omics analyses of these isolates, the therapeutic effect of these gut bacteria on various human diseases such as cancer, obesity, and inflammatory bowel disease was screened. Furthermore, we are investigating therapeutic and preventive strategies of these diseases of novel gut microbes.

Transcriptional Processing of Elongation Barriers by RNA Polymerases

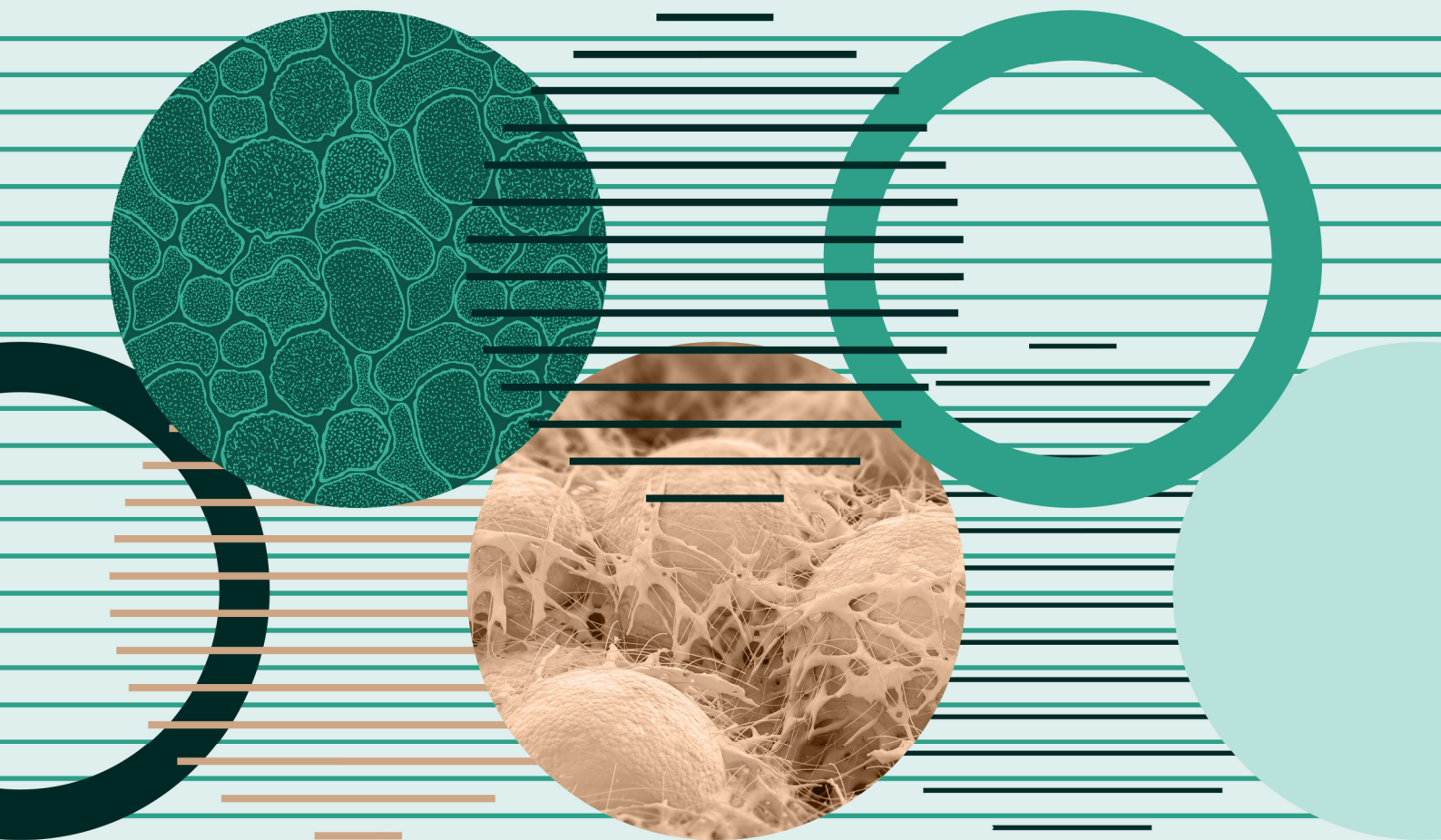
Juntaek Oh^{1,2}, Jun Xu¹, Jenny Chong¹, and Dong Wang^{1*}

¹*Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, USA*

²*Department of Pharmacy, College of Pharmacy, Kyung Hee University*

Oxidation of guanine generates 8-oxoguanine (8OG) and purine ring-broken DNA lesions, such as 5-guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp). These guanine-derived oxidative DNA lesions interfere with both replication and transcription. However, the molecular mechanism of transcription stalling of Gh and Sp remains unknown. In this study, by combining biochemical and structural analysis, we revealed distinct transcriptional processing of these chemically related oxidized lesions: 8OG allows both error-free and error-prone bypass, whereas Gh or Sp cause strong stalling and only allows slow error-prone incorporation of purines. Our structural studies provide structural snapshots of how Pol II gets stalled by a non-bulky Gh lesion in a stepwise manner, including the initial lesion encounter, ATP binding, ATP incorporation, jammed translocation, and arrested states. We show that while Gh can form hydrogen bonds with AMP during incorporation, this base pair hydrogen bonding is not sufficient to hold an ATP substrate in the addition site and is not stable during Pol II translocation after the chemistry step. Intriguingly, we reveal a unique structural reconfiguration of Gh lesion that its hydantoin ring rotates $\sim 90^\circ$ and is perpendicular to upstream base pair planes. The perpendicular hydantoin ring of Gh is stabilized by non-canonical lone pair- π interactions and C-H- π interaction as well as hydrogen bonds. As a result, the Gh lesion, as a functional mimic of 1,2-intrastrand crosslink, occupies canonical -1 and +1 template positions and compromises the loading of the downstream template base. Furthermore, we suggest Gh and Sp lesions are potential targets of transcription-coupled repair.

Scientific Session 2



The Orphan Enzymes in Anaerobic Allantoin Degradation: Oxamate Carbamoyltransferase and Catabolic Carbamate Kinase

Ok Bin Kim

Department of Life Science, Division of EcoScience, Ewha Womans University

Escherichia coli can use allantoin as its sole nitrogen source under anaerobic condition. Allantoin is an intermediate in anaerobic purine degradation pathway. When the allantoin is deaminated twice, it becomes ureidoglycolate, which can flow into either i) the glyoxylate shunt or ii) further catabolic transcarbamoylation. Although the former pathway is well studied, the latter pathway are not yet completely known. In the catabolic pathway, ureidoglycolate is oxidized into oxalurate, which is then converted into carbamoyl phosphate (CP) and oxamate. Oxamate is a dead-end product, whereas CP is dephosphorylated and completely degraded into CO₂ and NH₃. In this study we find an unidentified gene cluster *fdrA-ylbE-ylbF-ybcF*. We were able to identify that former three genes (*fdrA*, *ylbE*, and *ylbF*) encode a global orphan enzyme oxamate carbamoyltransferase and that the last gene *ybcF* encodes a local orphan enzyme catabolic carbamate kinase, and the activity of these enzymes was measured to characterize their function.

A Study on Functional and Metabolic Characteristics of Fermented Microorganisms in Korean Traditional Fermented Foods Based on Genome Analysis

Byung Hee Chun¹ and Che Ok Jeon^{2*}

¹*Department of Microbiology, Pukyong National University*

²*Department of Life Science, Chung-Ang University*

Functional and metabolic characteristics of various fermenting microorganisms isolated from Korean traditional fermented foods were analyzed through pan-genome, metatranscriptome, and comparative genome analyses. Through pan-genome analysis of *Bacillus velezensis*, which is known as a fermentation microorganism for meju, doenjang, and ganjang, it was confirmed that *B. velezensis* possesses various genes related to the production of antibacterial substances, as well as genes that metabolize pectin, compared to other strains. Pan-genome analysis of *Tetragenococcus halophilus*, when combined with metatranscriptome analysis at different salt concentrations, revealed metabolic characteristics and key metabolites that contribute to the salt tolerance adaptation of *T. halophilus*. Additionally, pan-genome and metatranscriptome analyses of *Leuconostoc mesenteroides* during kimchi fermentation revealed the genomic diversity and features of *Leu. mesenteroides* strains that undergo heterolactic fermentation, as well as the fermentative metabolic characteristics for various carbohydrates during kimchi fermentation. Furthermore, by conducting comparative genomic analysis between strains that evolved resistance to high concentrations of acetic acid through laboratory evolution and the wild-type strains, genes involved in enhancing acetic acid tolerance in acetic acid bacteria could be identified.

Anaerobiosis of the Hyperthermophilic Archaeon *Thermococcus onnurineus* NA1

Hyun Sook Lee^{1,2}

¹Marine Biotechnology Research Center, Korea Institute of Ocean Science and Technology

²Department of Marine Biotechnology, Korea University of Science and Technology

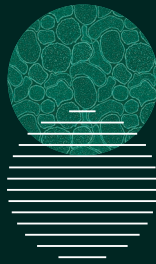
A hyperthermophilic anaerobic archaeon was isolated from deep-sea hydrothermal vent sediment and identified as belonging to the genus *Thermococcus*. The hyperthermophilic properties of strain *Thermococcus onnurineus* NA1 have been exploited to develop thermostable enzymes useful for biotechnological applications. Oxygen-insensitive proteins were readily purified from *Escherichia coli* as recombinant proteins, biochemically characterized, and some could be licensed out for commercial use. Although the anaerobic nature of the strain has posed challenges in cultivation, genetic engineering, and characterization of oxygen-sensitive proteins, anaerobic metabolism studies have led to several new discoveries. i) Dimethyl sulfoxide served as an electron sink coupled with the thioredoxin system to regenerate the reducing cofactor NAD(P)⁺. The NA1 strain is obligate anaerobic, but oxygen (O₂) can act as an electron sink. ii) Formate oxidation leading to H₂ gas production enabled the growth of hyperthermophilic NA1 at 80°C through ATP generation, whereas it is thermodynamically impossible in mesophilic microbes. The adaptation approach led to remarkable enhancement in cell growth, H₂ production, and formate consumption. A point mutation (G154A) in a formate transporter greatly impacted the formate metabolism. iii) NA1 acquired enhanced O₂ tolerance through overexpression of the *frhAGB* genes and was able to grow under the oxic condition omitting O₂ removal steps such as the addition of the reducing agent Na₂S, autoclaving, and inert gas purging. Interaction and electron transfer were observed between the *frhAGB*-encoded hydrogenase and the thioredoxin reductase by direct contact. TrxR partner proteins may play a role in regulating transcription factors involved in the oxidative stress response. Various physiological features of NA1 related to anaerobic metabolisms will be discussed in the presentation.

Crystal-guided Discovery of the Broad Inhibition Activities of Halisulfates against β -Lactamases

Sun-Shin Cha

Department of Chemistry and Nanoscience, Ewha Womans University

AmpC BER is an extended-spectrum (ES) class C β -lactamase with a two-amino-acid insertion in the H10 helix region located at the boundary of the active site compared with its narrow spectrum progenitor. The crystal structure of the wild-type AmpC BER revealed that the insertion widens the active site by restructuring the flexible H10 helix region, which is the structural basis for its ES activity. Besides, two sulfates originated from the crystallization solution were observed in the active site. The presence of sulfate-binding subsites, together with the recognition of ring-structured chemical scaffolds by AmpC BER, led us to perform *in silico* molecular docking experiments with halisulfates isolated from marine sponge. Inspired by the snug fit of halisulfates within the active site, we demonstrated that halisulfate 3 and 5 significantly inhibit ES class C β -lactamases. Especially, halisulfate 5 is comparable to avibactam in terms of inhibition efficiency; it inhibits the nitrocefin-hydrolyzing activity of AmpC BER with a K_i value of 5.87 μ M in a competitive manner. Furthermore, halisulfate 5 displayed moderate and weak inhibition activities against class A and class B/D enzymes, respectively.



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