A_Antibiotics, Antifungals, and Antiviral Compounds

A-1

Utility of Antimicrobial Peptide in Diagnosis and Therapeutics

Seong-Cheol Park, Mi-Kyeong Jang*
Department of Polymer Science and Engineering, Sunchon National University, Chonnam 57922, Republic of Korea

The increasing emergence of resistant pathogens to conventional drugs and the increasing frequency of microbial infections in immunosuppressed hosts have needed to find new-typed antibiotics or therapeutic methods. Despite great efforts on the development of antibiotics, there is still a great need to develop a strategy to early detect bacterial infections and eradicate bacteria effectively and simultaneously. Among new antimicrobial agents, antimicrobial peptides have recently attracted increasing attention as an alternative to conventional antibiotics in antibacterial medications. Here, we report a new family of antibacterial agents, which are formulated from self-assembly of chimeric antimicrobial lipopeptide and amphiphilic biodegradable polymers. Micelles could effectively bind the bacterial membrane to kill a wide spectrum of bacteria and bacterial biofilms. In the studies of mouse models of drug-resistant bacterial infections, Micelles could target bacterial infections with high specificity and also kill drug-resistant bacteria effectively, demonstrating the great potential of micelles as imaging and targeted antibacterial agents. These findings also provide new insight in the design of antimicrobial peptide-based nanomedicine for detection and treatment of bacterial infections (NRF-2019R1I1 A3A01062547).

Keywords : Antimicrobial peptide, diagnosis, micelle

A-2

Antifungal Efects of *Oryza sativa* Thioredoxin Proteins

Seong-Cheol Park, Mi-Kyeong Jang*
Department of Polymer Science and Engineering, Sunchon National University, Chonnam 57922, Republic of Korea

An antifungal protein was isolated from rice (*Oryza sativa*) leaves and N-terminal sequence was analyzed by *Ederman* degradation, resulted in matching to the *O. sativa* Thioredoxin m-type isoform (OsTrxm). Ubiquitous disulfide reductases, thioredoxins (Trxs), function in the redox balance of all living organisms. Although roles of OsTrxm in chloroplast development have been already published, biochemical and molecular functions of OsTrxm remain to be elucidated for decades. To determine antimicrobial activity of OsTrxm, we purified recombinant OsTrxm and its two active cysteine mutant protein (OsTrxm C/S) in *Escherichia coli*. The recombinant OsTrxm proteins inhibited the cell growth of various pathogenic fungal strains. Interestingly, OsTrxm C/S mutant showed higher antifungal activity than OsTrxm in rice. The importance of active cysteines on the activity was also confirmed by a growth inhibitory assay against various fungal pathogens and yeasts. The proteins showed significant intracellular accumulation for fungal and yeast cells. The OsTrxm protein variants could penetrate the fungal cell wall and membrane and act as inhibitors of fungal growth via generation of cellular reactive oxygen species (ROS). These finding suggest that in addition to its role in redox regulation, it appears to be a natural antimicrobial reagent.

Keywords : Antifungal protein , thioredoxin, reactive oxygen species

A-3

Identification of Both New Resistance Pathway and Synergistic Combination for Zidovudine against Gram-Negative Bacteria

Jaehun Yu, Kwang-sun Kim*
Department of Chemistry, Chemistry Institute for Functional Materials, Pusan National University, Busan 46241, Republic of Korea

Zidovudine (ZDV), an originally known as antiretroviral, have recently received a high attention as repurposing antibacterial agent against Gram-negative bacteria. However, ZDV is prone to resistance although it has been widely used in clinics. One known resistance mechanism of ZDV is derived from *tdk*-mutation in the chromosome. However, it is expected that additional resistance mechanisms might be presented. In this study, we performed MIC assays of ZDV using *tdk* mutated *E. coli* and found that the value was increased to 10,000-fold compared to wild-type cells. To see whether additional mechanisms for the ZDV resistance are presented, we further exposed ZDV to *tdk* mutated *E. coli* cells and found that MIC of ZDV was increased >20-fold. Therefore, we conclude that the high-level of resistance to ZDV is *tdk*-independent. To circumvent the resistance, we searched for antibiotics that could be used as combination therapy with ZDV. From the screenings, the combination of novobiocin (NOV) with ZDV increased bacterial susceptibility by 4-fold compared to ZDV alone and the activity was considered as synergy based on the FICI value (0.325-0.5) against *E. coli*. All our results showed that combination therapy could be one way to reduce the emergence of drug resistance by ZDV.

Keywords : Zidovudine, synergistic combination, tdk-independent pathway

A-4

Adjuvant Effect of Silene Armeria Extract with Polymyxin B against *Acinetobacter baumannii* ATCC17978

Mingyeong Kang, Jaebok Lee, Bora Shin, Jaeeun Park, Woojun Park*
Department of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea

Non-toxic supplements are desirable to reduce amounts and side effects of polymyxins for the treatment of *Acinetobacter baumannii*. Among the 120 kinds of plant extracts, only *Silene armeria* extract (SAE) showed the synergistic effect with polymyxin B (PMB) in our fractional inhibitory concentration and time-kill analyses. Pretreatment of cells with SAE made *A. baumannii* more susceptible to PMB in exposure time- and concentration- dependent manner, indicating that SAE induced alteration of cells. To confirm the synergistic effects of PMB and SAE *in vivo*, the killing assay of *Galleria mellonella* was performed and showed the highest survival rate of *G. mellonella* infected with *A. baumannii* cells under synergistic condition. Fluorescence and scanning electron microscopic observation confirmed alteration on surface of SAE-treated *A. baumannii* cells. SAE triggered an increase in cell width and total negative charge of surface area of cells. The *pmrA* gene linked to PMB stress was overexpressed in *A. baumannii* cells under synergistic condition. Addition of osmolytes canceled synergistic effect of SAE with PMB. Morphological alteration of cells might increase the effectiveness of PMB by enhancing PMB binding to surface of cells. This study provides a promising approach for utilization of plant extracts to reduce the toxicity of PMB in clinical trials.

Keywords : Antibiotics, natural extract, synergy effect

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Poster Session

A-5

Bacteriostatic Effects of Edible Herbal Mixtures on *Streptococcus mutans*

Hyejin Seo, Hyun Ju Lee, Han Soo Kim, Byung Hee Chung*
BAREUN Co.,LTD, No. 418, Bio-1, Chuncheon Biotown, 32, Soyanggang-ro, Chuncheon-si, Gangwon-do 24232, Republic of Korea

Although many attempts have been made to create a vaccine for the organism, such have not been successful in humans so far. Commercial mouthwashes that are toxic to bacteria are mostly alcohol-based formula. With its antiseptic effect Ethanol has been commonly used as dissolvent in the mouthwash, but is recently known to cause mouth dryness. Cleaning Time is a consumable oral care powder product made from nine natural food extracts (green tea, mint, lemon, quince, mogroside, balloon flower root, and enzyme salt, xylitol, propolis), which has antibacterial and anti-inflammatory effects without containing any chemical ingredients. In this study, we investigated bacteriostatic effects of nine consumable natural ingredients known for their anti-bacterial effects. The experiment showed that the ingredients have 63.48%~99.64% of bacteriostatic action depending on the raw materials. Furthermore, compounding the raw materials into the products of powder (CTP) and tablet (CTT), we tested their antibacterial activity against *S. mutans*. As a result, it was proved that our products are as much bacteriostatic as the available mouthwashes.

Keywords : Bacteriostatic effects, herbal mixtures, *Streptococcus mutans*

A-6

Exploring Beneficial Properties of the Bacteriocinogenic *Enterococcus faecium* ST10Bz Strain Isolated from Boza, a Bulgarian Cereal-Based Beverage

Svetoslav Dimitrov Todorov, Samantha Joy D. Valledor, Wilhelm H. Holzapfel
Advanced Green Energy and Environment Institute (AGEE), Handong Global University, Pohang, Gyeongbuk 37554, Republic of Korea

Samantha Joy D. Valledor, Wilhelm H. Holzapfel, Svetoslav D. Todorov
Bacteriocins produced by lactic acid bacteria (LAB) are proteinaceous antibacterial metabolites that normally exhibit bactericidal or bacteriostatic activity against genetically closely related bacteria. Bacteriocins are generally low molecular weight proteins that gain entry into target cells by binding to cell surface receptors. Their bactericidal mechanism may vary, and may include pore formation, degradation of cellular DNA, disruption through specific cleavage of 16S rRNA, and inhibition of peptidoglycan synthesis. The bacteriocin-producing strain *Enterococcus faecium* ST10Bz, isolated from *boza*, a Bulgarian cereal-based beverage, exhibited strong activity against *Listeria* strains, vancomycin-resistant *Enterococcus* spp. and other *Enterococcus* strains, but not against most of the other lactic acid bacteria (LAB) included in the test panel. Bacteriocin ST10Bz was proved as a stable antimicrobial, when exposure to various environmental conditions, including varying pH and temperature. The proteinacuses nature of the inhibitory substance was confirmed through the treatment of pepsin and α-chymotrypsin, whereas the inhibitory properties were observed to have no significant changes when subjected to Tween 80, glycerol, NaCl, SDS and milk. Bacteriocin activity against *L. monocytogenes* ATCC15313 was recorded as 25600 AU/mL when producer was cultured in MRS broth at 25℃ and 30℃, and 19200 AU/mL, when cultured at 37℃. Additionally, bacteriocin ST10Bz exhibited bactericidal mode of action when added to initially growing cultures of *L. monocytogenes* ATCC15313 and *Enterococcus faecalis* 200A. *E. faecium* ST10Bz was susceptible to the antibiotics: vancomycin, kanamycin, gentamycin, ampicillin, streptomycin, tylosin, chloramphenicol, clindamycin and tetracycline. PCR analysis of DNA from the strain was generated positive results for presence of some bacterial adhesion genes, including, *map*, *mub* and *ef-tu* genes. Under simulated gastrointestinal conditions in single and co-culture with *L. monocytogenes* ATCC15313 and *E. faecalis* 200A, *E. faecium* ST10Bz, showed a high survival rate and the ability to reduce the viable numbers of the two test strains.

Keywords : Bacteriocins, *Listeria monocyogenes*, lactic acid bacteria

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A-7

Bacteriocin Production of *Leuconostoc citreum* Isolated from Organic Farm Soil

Chaerin Woo, Sungmin Jung*, Samantha Joy D. Valledor*, Joanna Ivy I. Fugaban*, Vazquez Bucheli Jorge Enrique*, Wilhelm H. Holzapfel*, Svetoslav D. todorov*
Advanced Green Energy and Environment Institute (AGEE), Handong Global University, Pohang, Gyeongbuk 37554, Republic of Korea

The application of LAB bacteriocins in biopreservation has been continually explored worldwide. In recent decades, bacteriocins have especially been exploited in their application against multi-drug resistant strains and viruses. This study aimed to isolate a bacteriocinogenic strain and characterize its expressed bacteriocins in application against *Listeria monocytogenes*. Potential bacteriocinogenic LAB were isolated from organic soil samples via a three-level approach analysis using *L. monocytogenes* ATCC15313 as a sensitive test. Selected bacteriogenic isolates were differentiated based on morphology, sugar fermentation profile via API50CHB/CHL, and RAPD-PCR using primers OPL01, OPL09 and OPL11. Isolate ST110LD, a strong producer of anti-*Listeria* bacteriocins (25,600 AU/ml), was identified as *Leuconostoc citreum*. The protein nature of *L. citreum* ST110LD bacteriocins was confirmed with pepsin and α-chemostripsin. Bacteriocin activity was not affected by the presence of milk, NaCl, SDS, Tween 80, and glycerol. Bacteriocin ST110LD effectively inhibited *L. monocytogenes* ATCC15313 growth during a 10h incubation in BHI at 37°C while showing minimal inhibition on a few *Leuconostoc* spp. and no inhibition on beneficial cultures as tested in the spectrum of activity microbial test panel.

Keywords : *Leuconostoc citreum*, *Listeria monocytogenes*, bacteriocin

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A-8

Evaluation of Antimicrobial Activity of Symbiotic Bacteria Associated with a Korea Native Sea Roach, *Ligia exotica*

Ho Seok Sim[1], Eunha Kim[1], Yongjoon Yoon[1], Bukyeong Yoon[1], Sam Woong Kim[2], Woo Young Bang[3*], Ki Hwan Moon[1*]
[1]Division of Marine Bioscience, Korea Maritime and Ocean University, Busan 49112, Republic of Korea, [2]Swine science and Technology Center, Gyeongnam National University of Science and Technology, Jinju 52725, Republic of Korea, [3]National Institute of Biological Resources, Environmental Research Complex, Incheon 22689, Republic of Korea

Symbiotic marine bacteria are renowned for the synthesis of bioactive compounds. With the emergence of various antibiotic resistant bacteria and the delay in the development of new antibiotics, it is urgent to find novel antibacterial agent. The objectives of this study were to identify a new bioactive compound with antibacterial activity of symbiotic bacteria isolated from Sea roach, *Ligia exotica*. Sea roach play important roles in the intertidal ecosystem as a decomposer. Lacking the well-developed immune system, invertebrates largely depend upon their innate defense system to protect themselves against pathogens. We hypothesized that the symbiotic bacterium of *L. exotica* may produce a novel secondary metabolites, and those bioactive molecules are non-toxic and biodegradable with antimicrobial and/or immune boosting activities. In this study, we isolated twelve distinguishable symbiotic bacterial colonies from *L. exotica*. To tested its antimicrobial activity, we performed with killing assay against two bacterial pathogens *Streprococcus iniae* and *Salmonella* Typhimurium. Only one isolates displayed antimicrobial activity against *S. iniae*. To identify the isolates, we conducted 16S rDNA sequence analysis and whole genome sequence analysis, resulting *Pseudomonas* sp. was detected. Although this research is in very early stage, we will be explored the possibility of symbiotic bacterium utilization as a new type of drug producer or a probiotics for fish in aquaculture industry.

Keywords : Antimicrobial activity, *Ligia exotica*, *Pseudomonas* sp.

A-9

Evaluation of the Therapeutic Potential of Antibiotic Combination for Controlling Antibiotic-Resistant *Salmonella* Typhimurium

Jirapat Dawan, Juhee Ahn*
Department of Medical Biomaterials Engineering, Kangwon National University, Chuncheon, Gangwon 24341, Republic of Korea

Recently, the combination antibiotic therapy has been used to treat the late stage of severe bacterial infections. However, the combination antibiotic therapy can impose selection pressure and evolve antibiotic resistance in bacteria. Therefore, the aim of this study was to evaluate the mutual antimicrobial activity of combination antibiotic treatments against multidrug-resistant (MDR) *Salmonella* Typhimurium. Cephalothin (CEP), ciprofloxacin (CIP), ceftriaxone (CEF), tobramycin (TOB) alone and combination treatments (CEP-CIP and CEF-TOB) were used to evaluate antimicrobial activities against *S.* Typhimurium (ST^{WT}), ciprofloxacin-induced *S.* Typhimurium (ST^{CIP}), and clinically isolated multidrug-resistant *S.* Typhimurium (ST^{MDR}). The physical states of the cells after antibiotic treatments were assessed based on the rates of antibiotic-injured *S.* Typhimurium cells. The antibiotic-injured cells were estimated by the difference between the counts obtained on trypticase soy agar (TSA) and those obtained from xylose lysine desoxycholate (XLD) agar. The susceptibilities to CEP between CEP and CEP-CIP treatment were not significant differences for all tested strains, whereas those of ST^{WT}, ST^{CIP}, and ST^{MDR} to TOB were significant differences between TOB and CEF-TOB treatments. The CEF-TOB treatment increased bactericidal activity against ST^{WT}, ST^{CIP}, and ST^{MDR} without causing injured cells. The results suggest that cephalosporin could be used as potentiator to combine with other classes of antibiotics for the treatment of *S.* Typhimurium infections. This study provides useful information for optimizing antibiotic combination to effectively treat antibiotic-resistant bacteria.

Keywords : *Salmonella*, antibiotic combination, multidrug resistance

A-10

Rapid and Sensitive Carbapenem Susceptibility Test Using Parylene-Matrix Chip for MALDI-ToF MS

Tae Gyeong Yun[1], Jong-Min Park[2], Joo-Yoon Noh[1], Moon-Ju Kim[1], Jae-Chul Pyun[1*]
[1]Department of Materials Science and Engineering, Yonsei University, Seoul 03722, Republic of Korea, [2]Department of Laboratory Medicine and Research Institute of Antimicrobial Resistance, Yonsei University College of Medicine, Seoul 03722, Republic of Korea

Carbapenem is the strongest β-lactam antibiotics and acts as inhibitors of the enzymes that catalyze formation of peptidoglycan in the cell wall of bacteria. Recently, the emergence of carbapenem-resistant bacteria seriously threatens this class of lifesaving drugs. Therefore, rapid detection of carbapenemase-producing enterobacteriaceae (CPE) is very important to prevent spread of these strains. Carbapenemase is an important enzyme that are produced by CPE and catalyze the hydrolysis of carbapenem. Typically, MALDI-TOF MS is not appropriate for small molecule analysis because organic matrices make a lot of noise at low m/z range. Parylene-matrix chip was developed for matrix noise reduction, and analyzing small molecules. Recently, the Parylene-matrix chip has been used for analysis of penicillin-resistant bacteria. The applicability of the Parylene-matrix chip was demonstrated in a quantitative β-lactamase assay that required the quantification of penicillin (*m/z*: $[PEN+H]^+$ = 335.1 and $[PEN+Na]^+$ = 357.8), as well as its hydrolyzed product, penicilloic acid (*m/z :* $[PA+H]^+$ = 353.1). In this study, the Parylene-matrix chip was used for the carbapenemase assay. The assay measured the hydrolysis of 4 carbapenems such as doripenem, ertapenem, imipenem, and meropenem into their hydrolyzed form. Finally, MALDI-TOF MS based carbapenem susceptibility test was carried out with different 60 isolates using Parylene-matrix chip.

Keywords : Antibiodic-susceptibility test, MALDI-TOF MS, Parylene-matrix chip

A-11

Identification of Antimicrobial Protein with Membrane-Disruption Activity Produced by Gut Microbiota against *Clostridium difficile*

Byeong Seob Oh[1], Ji-Sun Kim[1], Seoung Woo Ryu[1], Seung Yeob Yu[1], Seung-Hwan Park[1], Se Won Kang[1], Kook-Il Han[1], Keun Chul Lee[1], Mi Kyung Eom[1], Min Kuk Suh[1], Jam-Eon Park[1], Seung-Hyeon Choi[1], Jung-Sook Lee[1,2], Jae-Ho Jung[3], Ju Huck Lee[1*]

[1]Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsin-gil, Jeongeup-si, Jeollabuk-do 56212, Republic of Korea, [2]University of Science and Technology (UST), 217 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea, [3]Department of Microbiology, Chonnam National University Medical School, Jeollanam-do 61186, Republic of Korea

Clostridium difficile is the causal agent of antibiotic-associated diarrhea and intestinal infection, called CDI, in individuals who have dysbiosis of the gut microbiota following antibiotic treatment. Antibiotic use for therapeutic strategy often leads to a high recurrence rate, so that new approaches are required to reduce the risk of CDI. It was reported that the metabolites of gut microbiota (GM) inhibit the growth and colonization of *C. difficile* in the gut. Thus, we exploited the cell free culture supernatants (CFSs) of GM for screening antibacterial activity against *C. difficile*. To do this study, we prepared CFSs of 100 GM isolated from healthy human faeces. As a result of the antibacterial assays, one CFS of GM called GM86 had more than 50% antibacterial activity against *C. difficile*, and the activity was the highest from the CFS prepared 30h cultured GM86. Additionally, we determined the activity based on the incubation temperature, proteinase treatment and strain specificity. Since the activity was reduced in over 60℃ and the enzyme treatment, we assumed that the antibacterial agents against *C. difficile* was protein. Therefore, the CFS of GM 86 was precipitated to obtain the crude protein by ammonium sulfate for further study, and we confirmed that the crude proteins (GM86PPT) still had anti-Cd activity. To study the action of the anti-Cd activity, we observed the *C. difficile* treated using GM86PPT by electron microscope, showing that the anti-Cd agents induced the disruption of bacterial membrane. Currently, we are establishing the CDI mouse model and examining this anti-Cd activity *in vivo*.

Keywords : Gut microbiota, antibacterial activity, *Clostridium difficile*

A-12

Inhibitory Effects of Metergoline on Nav1.2 Voltage-Dependent Sodium Channel

Junho Lee

Department of Biotechnology, Chonnam National University, Gwangju 61186, Republic of Korea

Metergoline is an ergot-derived psychoactive drug that acts as a ligand for serotonin and dopamine receptors. In the present study, we investigated the effects of metergoline on neuronal Nav1.2 voltage-dependent sodium channel activity. The two-microelectrode voltage clamp technique was used to study the regulation of metergoline on Na^+ current in *Xenopus oocytes* expressing cRNA-encoding Nav1.2 α and β1 subunits in the rat brain. In oocytes that expressed neuronal Na^+ channels, metergoline induced inhibitory effects on the peak of Na^+ currents. The metergoline-induced tonic inhibitions of peak Na^+ currents were voltage- and concentration-dependent and reversible. The half maximal inhibitory concentration (IC_{50}) in peak currents of rat brain Na1.2 channels was 4.6 ± 1.3 uM. Metergoline treatment produced a 3.6 ± 0.4 mV depolarizing shift in the activation voltage but did not alter the steady-state inactivation voltage. In addition, metergoline produced a use-dependent blockade of the Na^+ channel after high-frequency stimulation, indicating that metergoline could exert an inhibitory effect on the open state of the Na^+ channel. Taken together, these results indicate that metergoline might regulate neuronal Nav1.2 voltage-dependent channels that are expressed in *X. oocytes*. Our study further suggests that metergoline can be an important pharmacological target as a potent inhibitor of neuronal Nav1.2 channels.

Keywords : Metergoline

A-13

Identification and Characterization of *Salmonella* spp. Isolated from Fresh Produces and Environment

Ji Min Han, Gi Yeon Song, Heejeong Lee, Mi-Kyung Park*

School of Food Science and Biotechnology, Kyungpook National University, Daegu 41566, Republic of Korea

The prevalence of antibiotic-resistant *Salmonella* in fresh produces has increased worldwide due to the abuse of antibiotics. The purpose of this study was to isolate antibiotic-resistant *Salmonella* from fresh produce and the environment. One hundred twenty samples were enriched in TSB and then RV broth, followed by streaking on MacConkey and XLD agar for single colony isolation. The isolated single colony was identified using 16s rRNA sequencing. PCR was performed to confirm the virulence gene of *Salmonella* targeting to *inv*A, *stn*, *spv*C, *spv*R, and *fim*A. The antibiotic-resistance of *Salmonella* strains were investigated by disc diffusion method against 22 antibiotics. Five presumable *Salmonella* strains were confirmed from thirty isolated colonies. The *inv*A, *stn*, and *fim*A gene were detected in five identified *Salmonella* strains. In addition, one *Salmonella* strains contained *spv*R gene. Furthermore, four strains of *Salmonella* were resistant to at least one antibiotic. Overall, four pathogenic and antibiotic-resistant *Salmonella* strains were identified and confirmed in this study. They will be used as target pathogens for investigating a new bacteriophage for developing an on-site, applicable, and all-in-one rapid detection method.

Keywords : *Salmonella*, antibiotic-resistance, pathogenicity

A-14

Antibacterial Activity of ZnO and ZnO-Ag Nanocomposite: ROS-Mediated Antibacterial Mechanism Caused by UV-A Light Exposure

Wonbin Bae, Hyeonbin Son, YeongJun Jeon, Kwang-Hwan Jhee*
Department of Applied Chemistry, Kumoh National Institute of Technology, 61 Daehak-ro, Gumi, Gyeongbuk 39177, Republic of Korea

We previously studied the antibacterial activity of ZnO nanoparticles in dark conditions by Zn^{2+} ions released from nanoparticles. In this study, we investigated the antibacterial activity caused by reactive oxygen species (ROS) induced by UV-A light. Prior to the experiment on microorganisms, it was confirmed whether the synthesized ZnO nanoparticle were well formed by the analysis of XRD pattern, TEM image, and XPS spectra of the nanoparticles. Two microorganism, *E. coli* and *S. aureus* were exposed to several different concentration of ZnO nanoparticles under dark condition or the irradiating UV-A light. The adopted concentrations of the ZnO nanoparticles were quite low, so the antibacterial effect of the dissolved Zn^{2+} ions were negligible. We found that the oxygen defects of the ZnO crystals enhanced the photogeneration of ROS and consequently, the ZnO nanoplates (NPs) with the polar facets exhibited the most pronounced antibacterial activity under UV-A stimulation. To enhance antibacterial activity of nanoparticles, we synthesized ZnO-Ag nanocomposites and report their antibacterial activity compared to ZnO nanoparticles.

Keywords : ZnO nanoparticles, antibacterial activity, oxygen defects

A-15

Phenotypic and Genotypic Properties of Persister Cells Formed by Antibiotic-Resistant *Staphylococcus aureus*

Jirapat Dawan[1], Gibeom Jeon[1], Geun-Pyo Choi[2], Juhee Ahn[1*]
[1]Department of Medical Biomaterials Engineering, Kangwon National University, Chuncheon, Gangwon 24341, Republic of Korea, [2]Department of Barista and Bakery, Gangwon State University, Gangneung, Gangwon 25425, Republic of Korea

The emergence and spread of antibiotic-resistant *Staphylococcus aureus* is a major cause of chemotherapeutic failure and leading to hospital and community-acquired infections. The antibiotics stress can induce phenotypic switching of bacterial from normal to persister, tolerant, and resistant. Among them, the persistence has been overlooked in the evolution of antibiotic resistance in bacteria. Therefore, this study was aimed to evaluate phenotypic and genotypic properties of persister cells formed by *S. aureus* ATCC 15564 (SA^{WT}), oxacillin-induced *S. aureus* (SA^{OXA}), ciprofloxacin-induced *S. aureus* (SA^{CIP}), and clinically isolated multidrug-resistant *S. aureus* CCARM 3080 (SA^{MDR}). The inactivation characteristics of SA^{WT}, SA^{OXA}, SA^{CIP}, and SA^{MDR} were examined by using dose-dependent biphasic killing patterns ranging from 0- to 2-fold minimum inhibitory concentrations (MICs) of ciprofloxacin. The metabolic-based assay was used to measure the surviving persister cells after ciprofloxacin treatment. SA^{CIP} showed the lowest of persister formation, showing 58% of persistence. Moreover, SA^{CIP} also showed the lowest fitness cost of resistance for the recovered persister cells (relative fitness = 0.95), followed by SA^{MDR} (relative fitness = 0.70), while SA^{WT} showed the highest fitness cost (relative fitness = 0.26). The expression of virulence-related genes were analyzed by RT-PCR assay. The highest expression of stress- and efflux- related genes were observed in the recovered persister cells of SA^{OXA} and SA^{MDR}. Therefore, this study provides valuable information for understanding the phenotypic and genotypic properties of persister cells in different antibiotic-resistant *S. aureus* strains.

Keywords : *Staphylococcus*, persister, ciprofloxacin

A-16

Identification and Characterization of Lipopeptides Isolated from *Bacillus velezensis*, the Dominant Strain of *Malva verticillata* Leaves

Hyeonju Lee, Keumok Moon, Seola Lee, Seonmin Lee, Jaeho Cha*
Department of Microbiology, Pusan National University, Busan 46241, Republic of Korea

A bacterial strain isolated from *Malva verticillata* was identified as *Bacillus velezensis*, which is known as Gram-positive and a soil rhizobacterium with potent biocontrol activity. In this study, we figured out that the supernatant of *B. velezensis* culture has antimicrobial activity against not only Gram-positive bacteria but also phytopathogenic fungi and this activity reached maximum at the early stationary phase. Generally, *Bacillus* sp. are known to produce many secondary metabolites. Among them, lipopeptides are classified as non-ribosomal peptide and consist of short linear chains or cyclic structures of amino acid. They are known to show various biological functions such as antibacterial, antiviral and antifungal. The antimicrobial substance from the supernatant of *B. velezensis* was partially purified by hydrophobic and ion-exchange chromatography. We detected two clusters of peaks by LC-MS; the clusters were identified as bacillomycin families and surfactin families, respectively. As the result of mode of action analysis, these substances were shown to control the bacterial growth by batericidal effect. The lipopeptides were stable over a wide pH range of 2-9 and also stable at high temperature up to 121℃. Furthermore, various proteases cannot inhibit the antimicrobial activity of these substances. These molecules also inhibit growth of fungi, suggesting that these compounds have potential for application such as industries of alternative antibiotic research, biopesticide and food processing.

Keywords : Lipopeptide, *Bacillus velezensis*, antimicrobial activity

A-17

Characterization of Bacteriophage-Resistant Mutant Strains of *Salmonella* Typhimurium

Gibeom Jeon, Juhee Ahn*
Department of Medical Biomaterials Engineering, Kangwon National University, Chuncheon, Gangwon 24341, Republic of Korea

Recently, bacteriophage has received growing attention as a possible alternative treatment over chemical antibiotics. However, the development of bacteriophage resistance still remains a challenging question to answer. Therefore, this study was designed to characterize the bacteriophage-insensitive *Salmonella* Typhimurium mutants in association with bacteriophage adsorption, antibiotic susceptibility, and receptor-related gene expression. Bacteriophage-sensitive (BS) *Salmonella* Typhimurium ATCC 19585 ($BSST^{WT}$), ciprofloxacin-induced *S.* Typhimurium ATCC 19585 ($BSST^{CIP}$), *S.* Typhimurium KCCM 40253 ($BSST^{LAB}$), and clinically isolated multidrug-resistant *S.* Typhimurium CCARM 8009 ($BSST^{MDR}$) were used to isolate bacteriophage-resistant *S.* Typhimurium (BRST) mutants ($BRST^{WT}$, $BRST^{CIP}$, $BRST^{LAB}$, and $BRST^{MDR}$) against P22. $BSST^{WT}$, $BSST^{CIP}$, $BSST^{LAB}$ treated with P22 showed more than 3 log reduction, while most bacteriophages had the least lytic activity against $BSST^{MDR}$. $BSST^{WT}$ had varied in cell state (CV>40%) and highest mutant frequency (62%) in the exposure of P22. $BRST^{WT}$, $BRST^{LAB}$, and $BRST^{MDR}$ were sensitive to PBST-10, PBST-13, PBST-32, and PBST-35. $BRST^{CIP}$, $BRST^{LAB}$, and $BRST^{MDR}$ showed the increased susceptibility to ciprofloxacin, ampicillin, and tetracycline. The expression levels of bacteriophage-binding receptor-related genes (*rafL*, *btuB*, *fliC*, *fhuA*, *ompC*, and *tolC*) were decreased in $BRST^{CIP}$ and $BRST^{MDR}$ in connection with the low adsorption rates. The virulence factor was less produced by $BRST^{CIP}$ and $BRST^{MDR}$, showing the decrease in *stn* expression. The results suggest that the bacteriophage-resistant (BR) mutants could alter the bacteriophage -binding receptors, leading to the cross-resistance to different antibiotics. These findings could pave the way for designing an effective bacteriophage therapy.

Keywords: Bacteriophage; *Salmonella*, bacteriophage-binding receptors

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A-18

Discovery and Characterization of a Novel Antibacterial Agent against *Staphylococcus aureus* and Gram-Positive Bacteria

Minhye Shin[1], Shelley M. Payne[2], Kyoung Heon Kim[3*], Younghoon Kim[1*]
[1]*Department of Agricultural Biotechnology, Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea,* [2]*Department of Molecular Biosciences, College of Natural Science, The University of Texas at Austin, Austin, TX, 78712, USA,* [3]*Department of Biotechnology, Graduate School, Korea University, Seoul 02841, Republic of Korea*

The emergence of multidrug-resistant *Staphylococcus aureus* strains has become a serious clinical problem. Iron is absolutely required for the bacterial growth, virulence associated with colonization and survival from the host immune system. The bacterial ferrous iron transporter protein FeoB functions as a major iron transporter in prokaryotes that has been shown to play a crucial role in virulence of some pathogenic bacteria. However, FeoB is still uncharacterized in Gram-positive pathogens, and its effects on *S. aureus* pathogenesis are unknown. In this study, we present a novel unconventional antibacterial agent that inhibits FeoB in vitro enzyme activity, bacterial growth and virulence factor expression. Genome-editing and metabolomic analyses revealed that the molecule inhibited FeoB function and affected additional, unidentified mechanisms in *S. aureus*. Significantly, the small molecule prevented the evolution of gentamicin resistance and enhanced nematode survival against *S. aureus* infection. It was also effective against a broad range of Gram-positive pathogens, suggesting therapeutic potential to overcome the emergence of antibiotic-resistant bacteria against conventional antibiotics. This novel inhibitor will provide new insight for developing a next-generation antibacterial therapy.

Keywords : Ferrous iron transporter, staphyloxanthin, antibiotics

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A-19

Isolation and Characterization of Strain *Rouxiella* sp. S1S-2 with Antimicrobial Activity

Young Ho Nam, Buyng Su Hwang, Ahyoung Choi, Eu Jin Chung*
Nakdonggang National Institute of Biological Resources, Sangju, Gyeongsangbuk-do 37242, Republic of Korea

A gram-negative bacterial strain S1S-2 with antibacterial activity was isolated from sediment collected from Osipcheon in Yeongdeok-Gun. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that strain S1S-2 belonged to the genus *Rouxiella* and shared 98.7%-99.5% sequence similarity with *Rouxiella* species. Based on the results of API kit testing and some physiological characteristics, the strain was identified and named *Rouxiella* sp. S1S-2. The strain revealed antibiosis against pathogenic bacteria, including *Bacillus cereus* KCTC 3624, *Escherichia coli* KCTC 2443, and *Staphylococcus aureus* KCCM 40510 (methicillin-resistant strain). The effects of commercial culture media, temperature, and initial pH on the cell growth and antibacterial activity were confirmed for culture optimization of strain S1S-2. When the strain was cultured in LB, NB, TSB, R2A media, the antibacterial activity did not show. The optimal conditions for growth and antibacterial activity of strain S1S-2 were found to be YPD medium and 25℃. We also isolated the antibacterial compound from ethyl acetate fraction of the bacterial culture and its chemical structure was identified as maculosin(1) by ESI-MS, ^{1}H-NMR, and ^{13}C-NMR analyses. This study is the first report that newly isolated strain *Rouxiella* sp. S1S-2 showed antibacterial activity against pathogenic bacteria.

Keywords : Antibacterial, optimization, maculosin

A-20

Antimicrobial Activities of Strain *Acetobacter* sp. Hyunduk-27 and Optimization of Culture Conditions

Young Ho Nam[1], Kiwoon Baek[2], Eu Jin Chung[1*]
[1]*Microbial Research Department, NNIBR, Sangju-si, Gyeongsangbuk-do 37242, Republic of Korea,* [2]*Bioresources Collection and Bioinformation department, NNIBR, Sangju-si, Gyeongsangbuk-do 37242, Republic of Korea*

In this study, we isolated and identified bacteria from freshwater and soil collected from Dodaecheon in Pyeongtaek, to screen antimicrobial bacteria against various pathogenic bacteria. 92 strains were isolated and based on 16S rRNA gene sequence analysis. Among them, strain Hyunduk-27 showed a good growth inhibition against methicillin-resistant *Staphylococcus aureus* subsp. *aureus* (MRSA) strains and *Escherichia coli*. As a result of the 16S rRNA gene sequence analysis, strain Hyunduk-27 show the high similarity with *Acetobacter orientalis* 21F-2^T, *Acetobacter cibinongensis* 4H-1^T, *Acetobacter cerevisiae* LMG 1625^T 99.93, 99.01, 98.73% respectively. We investigated cell growth and antimicrobial activity according to commercial culture medium, temperature, NaCl for culture optimization of strain Hyunduk-27. Culture filtrate of strain Hyunduk-27 showed antimicrobial activity against MRSA strains and *E. coli* with inhibition zone. Culture optimization of strain Hyunduk-27 can be improved on antimicrobial activity. Therefore, the antimicrobial activity of *Acetobacter* sp. Hyunduk-27 had potential as a novel antibiotics for pathogens including MRSA.

Keywords : Antimicrobial activity, antibiotics , optimization

A-21

Bacteriocinogenic Properties of *Enterococcus faecalis* ST651EA Isolated from Korean Traditional Fermented Soy Paste

Svetoslav Dimitrov Todorov, Joanna Ivy I. Fugaban, Wilhelm H. Holzapfel
Advanced Green Energy and Environment Institute (AGEE), Handong Global University, Pohang, Gyeongbuk 37554, Republic of Korea

Joanna Ivy I. Fugaban, Wilhelm H. Holzapfel, Svetoslav D. Todorov The search for better alternatives in the control of spoilage contaminants in food industry with the consideration of the continuous increase in the occurrence and development of antibiotic-resistant organisms has been studied for the past decade. One of the most promising alternatives in the field of biopreservation is the ability of microorganisms to produce small peptides that can acts as an inhibitory molecule to other organisms. Inhibitory properties of antimicrobial peptide produced by *Enterococcus faecalis* ST651EA isolated from Korean traditional fermented soy paste (doenjang) against *Listeria monocytogenes* ATCC 15313 was evaluated in this study. Different samples of locally prepared doenjang were collected and subjected to microbiological analysis for isolation of potential bacteriocin producing lactic acid bacteria (LAB) through the three-level approach. A total of 18 putative bacteriocin producers isolated from doenjang, a three unique profiles were differentiated through rep-PCR (5'-$(GTG)_5$ -3') and RAPD-PCR (with primers OPL-01: 5' - GGC ATG ACC T - 3'and OPL-11: 5' - ACG ATG AGC C - 3', respectively). One of the isolates, ST651EA, identified as *E. faecalis* based on 16s rRNA sequencing and recommended biochemical and physiological tests, was observed to be a stronger producer of bacteriocin against *L. monocytogenes* ATCC 15313 (12 800 AU/mL). The proteinacuses nature of the inhibitory substance was confirmed through the treatment of pepsin and α-chymotrypsin, whereas the inhibitory properties were observed to have no significant changes when subjected to Tween 80, glycerol, NaCl, SDS and milk. The actively growing *L. monocytogenes* ATCC 15313 in BHI for 10 h at 37℃ was effectively suppressed by presence of bacteriocin produced by ST651EA. In addition to different *Listeria* spp. used in the determination of spectrum of activity, bacteriocin produced by *E. faecalis* ST651EA has inhibited several vancomycin-resistant *Enterococcus* strains, but did not affect the growth of industrially significant strains, including several probiotics.

Keywords : Bacteriocins, vancomycin-resistant *Enterococcus* , lactic acid bacteria

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A-22

Screening of Potential β-Lactamase Inhibitors from Plant Extracts

Jirapat Dawan[1], Nana Nguefang Laure[1], Geun-Pyo Choi[2], Juhee Ahn[1*]
[1]*Department of Medical Biomaterials Engineering, Kangwon National University, Chuncheon, Gangwon 24341, Republic of Korea,* [2]*Department of Barista and Bakery, Gangwon State University, Gangneung, Gangwon 25425, Republic of Korea*

This study was aimed to evaluate the potential of using medicinal plant extracts as β-lactamase inhibitors (BLIs) to control antibiotic-resistant *Staphylococcus aureus*. The β-lactamase-producing abilities of *S. aureus* ATCC 15564 (SA^{WT}), ciprofloxacin-induced *S. aureus* ATCC 15564 (SA^{CIP}), oxacillin-induced *S. aureus* ATCC 15564 (SA^{OXA}), and clinically-isolated *S. aureus* CCARM 3008 (SA^{CLI}) were determined by a modified Hodge test. The ampicillin susceptibilities of SA^{WT}, SA^{CIP}, SA^{OXA}, and SA^{CLI} were evaluated in the absence and presence of medicinal plant extracts, including *Cleyera japonica* (CJ), *Carpinus laxiflora* (CL), *Euphorbia helioscopia* (EH), *Euscaphis japonica* (EJ), *Oenothera erythrosepala* (OE), and *Rosa multiflora* (RM). Nitrocefin-hydrolyzing assay was used to assess the inhibitory effects of medicinal plant extracts on the production of β-lactamases. SA^{WT}, SA^{CIP}, and SA^{OXA} showed the clear zone of inhibition around ampicillin disc, showing the production of ampicillinase. The MICs of ampicillin against SA^{WT}, SA^{CIP}, and SA^{OXA} were decreased from 4 to 0.5 μg/mL in the presence of CL, 16 to 4 μg/mL in the presence of RM, and 32 to 2 μg/mL in the presence of CL, EH, and RM, respectively. Compared to the control, SA^{WT}, SA^{CIP}, and SA^{OXA} in the absence of medicinal plant extracts showed the highest β-lactamase activities, while the reduction of β -lactamase activities was observed in the presence of OE, EJ, and CL extract for SA^{WT} (78%), SA^{CIP} (57%), and SA^{OXA} (76%). This results demonstrated that the medicinal plant extracts used in this study can be used as BLIs to enhance antibiotic activities and control antibiotic-resistant *S. aureus*.

Keywords : *Staphylococcus*, β-lactamase inhibitors, plant extracts

A-23

Antimicrobial Activity Re-Evaluation of Korean *Dendropanax morbifera*

Wonbin Bae[1], Hyeonbin Son[1], YeongJun Jeon[1], Dae Suk Bang[2], Kwang-Hwan Jhee[1*]
[1]*Department of Applied Chemistry, Kumoh National Institute of Technology, 61 Daehak-ro, Gumi, Gyeongbuk 39177, Republic of Korea,* [2]*Department of Chemical Engineering, Kumoh National Institute of Technology, 61 Daehak-ro, Gumi, Gyeongbuk 39177, Republic of Korea*

In this study, we compared the antibacterial activity of *Dendropanax morbifera* (*D. morbifera*) hot water extracts and fermented broth using *Lactobacillus plantarum* (*L. plantarum*). The minimum inhibitory concentration (MIC) method and a disk diffusion assay were used to evaluate the antimicrobial activity of the fermented extracts of the leave/branches and sap against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Our result suggest that antibacterial activity was enhanced against three tested strains by fermenting *D. morbifera*. In the case of *D. morbifera* sap fermentation, the antibacterial activity was relatively weaker than that of *D. morbifera* leave/branches extracts. In addition, the antibacterial activity increased in proportion to the content of the fermented extract of *D. morbifera*. As a result of this study, it is expected that *D. morbifera* leave/branches and sap extracts fermentation can be used as a natural preservative.

Keywords : Antimicrobial activity, fermentation, *Dendropanax morbifera*

A-24

Synergistic Growth Inhibition by Mixing *Artemisiae Capillaris* Herba and Sanguisorbae Radix against *Candida albicans* Causing Vaginal Candidiasis

Hyunjeong Na, Tae-Jong Kim*
Department of Forest Products and Biotechnology, Kookmin University, Seoul 02707, Republic of Korea

Candida albicans is the main cause of vaginal candidiasis. As a result of treatment with the constant use of antifungal agents, it causes resistant *C. albicans*. Therefore, a new alternative method is required. Natural extracts have been used as medicinal ingredients for a long time and have the advantage of being evaluated for safety to the human body. This study proposes a combination of *Artemisiae Capillaris* Herba and *Sanguisorbae Radix* as a new method for preventing vaginal candidiasis by identifying the synergistic effects of two extracts with antifungal effects against *C. albicans*. *Artemisiae Capillaris* Herba and *Sanguisorbae Radix* were extracted twice with water at 100℃ for 4 hours, concentrated to 20 - 25 brix at 57℃, and then spray dried. The CLSI M27-A2 method was used to evaluate the growth inhibitory effect of *C. albicans*, and a checkerboard assay was used to measure synergistic growth inhibitory effects. The concentration ranges with synergical growth inhibition were 0.125 - 0.232 g/L for *Artemisiae Capillaris* Herba and 0.018 - 0.020 g/L for *Sanguisorbae Radix*. In the identified concentration ranges, the antifungal effect was more than twice that of the treatment of a single extract.

Keywords : *Candida albicans*, synergistic antifungal effect, natural plant extract

A-25

Emergence of Extensively-Drug Resistant *Escherichia coli* Positive for blaNDM-5 from Influent of JN Wastewater Treatmentplant of Han River, South Korea

Hanseob Shin, Yeonghyeon Kim, Hor-Gil Hur*
Department of Earth Science and Environmental Engineering, Gwangju Institute of Science and Technology, Gwangju 61005, Republic of Korea

Carbapenemase producing *Enterobacteriaceae* have been considered as clinically critical due to difficulty of treatment of infections. In this study, NDM-5 producing *Escherichia coli* was isolated from influent of JN WWTP of Han River, South Korea. This strains is resistant to 15 antibiotics and high level of MICs of 14 antibiotics was evaluated. This strain also exhibited resistance to colistin but did not harbor a series of mcr genes. Colistin resistance is mediated by chromosomal mutations, resulting in alteration of outermembrane. A total of 21 antibiotic resistance genes (ARGs) were found in whole genome of *E. coli* ST746, named after N7. Of them, 17 ARGs were equipped within pKJNI-2, incorporated by IS257 and class 1 integron. On the other plasmid, pKJNI-5, blaNDM-5 was located and bracketed by several insertion sequences (ISs). On the opposite site of blaNDM-5 region, Type IV secretion system was also found. *E. coli* N7 is equipped with several mechanisms for genetic transfer as followings: ISs, Integron, Plasmid and Type IV secretion systems. On pKJNI-5, genetic environment of blaNDM-5 was linearized and compared with other previously reported regions, suggesting that structure of IS3000-ISAba125-IS5-blaNDM-bleMBL-trpF-dsbD-IS26 was conserved. Particulary, structure of IS3000-blaNDM-bleMBL-trpF-dsbD-IS26 was commonly shown in all of 170 structures analyzed in this study. This conserved region is thought to have been evolved, but not clear yet. *E. coli* N7 is not classified as sero- and patho-type but carries eight virulence factors. This strain has XDR pattern with several mobile genetic elements and virulences, suggesting XDR *E. coli* can be evolved into PDR pathogenic *E. coli*, and transfer ARGs and virulence genes between bacterial strains. It can increase selective pressure in the aquatic environment and threaten public health risk. Thus, continuous surveillance is needed in clinical as well as environmental settings.

Keywords : Carbapenem, antibiotic resistance, pathogen

A-26

Coralmycin Derivatives with Potent Anti-Gram Negative Activity Produced by the Myxobacteria *Corallococcus coralloides* M23

Ha-young Choi[1,2], Nguyen Van Minh[1], Bo-Min Kim[1,2], Won-Gon Kim[1,2*]
[1]Superbacteria Research Center, Korea Research Institute of Bioscience and Biotechnology, Yusong, Daejeon 34141, Republic of Korea, [2]Department of Bio-Molecular Science, KRIBB School of Bioscience, Korea University of Science and Technology (UST), Yusong, Daejeon 34113, Republic of Korea

Seven new coralmycin derivatives, coralmycins C - I, along with three known compounds, cystobactamids 891-2, 905-2, and 507, were isolated from a large-scale culture of the myxobacteria *Corallococcus coralloides* M23. The structures of these compounds, including their relative stereochemistries, were elucidated by interpretation of their spectroscopic and CD data. The structure-activity relationships of their antibacterial and DNA gyrase inhibitory activities indicated that the *para*-nitrobenzoic acid unit is critical for the inhibition of DNA gyrase and bacterial growth, while the nitro moiety of the *para*-nitrobenzoic acid unit and the isopropyl chain at C-4 could be important for permeability into certain Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and the b-methoxyasparagine moiety could affect cellular uptake into all tested bacteria. These results could facilitate the chemical optimization of coralmycins for the treatment of multidrug-resistant Gram-negative bacteria.

Keywords : Coralmycin, Gram negative, structure-activity relationships

A-27

Prebiotic Effect of the Carthami Fructus Oil and Linoleic Acid on Skin Microbiome

Jisoo Shin, Tae-Jong Kim*
Department of Forest Products and Biotechnology, Kookmin University, Seoul 02707, Republic of Korea

Prebiotics promote the growth of beneficial bacteria selectively. Our previous study showed that the Carthami Fructus oil and linoleic acid, its major component, inhibited the growth of *Staphylococcus aureus* and enhanced the growth of *Staphylococcus epidermidis* as a skin prebiotic. In this study, we isolated many bacteria of two strains from human skin and evaluated the skin prebiotic effect of Carthami Fructus oil and linoleic acid on isolated bacteria. After swab on the human skin, bacteria of two strains were isolated and cultured in the mannitol salt media. Carthami Fructus oil and linoleic acid were treated to each isolated skin bacterium and their final cell concentration was measured by colony forming unit. The change in the final cell concentration was determined by comparison with the control culture without any treatment. The number of isolated bacteria were 28 for *S. aureus* and 61 for *S. epidermidis* by 5 times of swab. As a results of Carthami Fructus oil treatment, the final cell number of *S. epidermidis* increased on average by 56%, while the final cell number of *S. aureus* increased by 7% on average. As a result of treatment with linoleic acid, the final cell number of *S. aureus* showed an average reduction rate of 83%, while the final cell number of *S. epidermidis* showed an average decrease of 15%. All the results showed that Carthami Fructus oil and linoleic acid were skin prebiotics that improve the skin microbiome by differentially regulating growth not only for laboratory strains but also for *S. aureus* and *S. epidermidis* on the skin.

Keywords : Skin microbiome, skin prebiotics, Carthami Fructus

A-28

Identification of the 2,2-Bipyridyl Family Compounds from *Streptomyces* Species Using Tandem Mass Screening

Byeongsan Lee[1,2], Kyung Taek Heo[1], Sangkeun Son[1], Jae-Hyuk Jang[1], Bang Yeon Hwang[2], Young-Soo Hong[1*]
[1]Anticancer Agent Research Center, Korea Research Institute of Bioscience and Biotechnology(KRIBB), Cheongju 28116, Republic of Korea, [2]College of Pharmacy, Chungbuk National University, Cheongju 28160, Republic of Korea

The advances of genomic sequence analysis and LC-MS technology have enabled the exploration of untapped microbial natural products. The hybrid polyketides (PKs)-nonribosomal peptides (NRPs), among the most structurally diverse and pharmaceutically important secondary metabolites, are synthesized by modular multifunctional nonribosomal peptide synthetases and polyketide synthases, and the core structures are often subsequently modified via the action of tailoring enzymes. The 2,2'-bipyridyl family compounds, which is a member of small hybrid PK-NRP, were found to have strong antifungal, antiamoebic, antitumor, and mild antibacterial activities. In particular, caerulomycin A was demonstrated to have novel bioactivity with remarkable promise in immunosuppression. These significant bioactivities have motivated continual attention to the discovery of novel 2,2'-bipyridyl natural products. Herein we report the discovery of several 2,2'-bipyridyl family compounds using tandem mass screening from *Streptomyces* sp. culture broths and identification of the biosynthetic gene cluster using bioinformatics-based predictions from genome data.

Keywords : *Streptomyces*, 2,2′-bipyridyl compounds, tandem mass

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A-29

The Nitrite Transporter Facilitates Biofilm Formation via Suppression of Nitrite Reductase and is a New Antibiofilm Target in *Pseudomonas aeruginosa*

Ha-young Choi[1,2], Ji-su Park[1,2], Won-Gon Kim[1,2*]
[1]Superbacteria Research Center, Korea Research Institute of Bioscience and Biotechnology, Yusong, Daejeon 34141, Republic of Korea, [2]Department of Bio-Molecular Science, KRIBB School of Bioscience, Korea University of Science and Technology (UST), Yusong, Daejeon 34113, Republic of Korea

Biofilm-forming bacteria, including the gram-negative *Pseudomonas aeruginosa*, cause multiple chronic infections and are responsible for serious health burdens in humans, animals and plants. Nitric oxide (NO) has been shown to induce biofilm dispersal via triggering reduction in cyclic-di-GMP levels in a variety of bacteria. However, how NO, at homeostatic levels, also facilitates biofilm formation is unknown. Here, we found that complestatin, a structural analog of vancomycin isolated from *Streptomyces*, inhibits *P. aeruginosa* biofilm formation by upregulating NO production via nitrite reductase (NIR) induction and c-di-GMP degradation via phosphodiesterase (PDE) stimulation. The complestatin protein target was identified as a nitrite transporter from a genome-wide screen using the Keio *Escherichia coli* knockout library and confirmed using nitrite transporter knockout and overexpression strains. We demonstrated that the nitrite transporter stimulated biofilm formation by controlled NO production via appropriate NIR suppression and subsequent diguanylate cyclase (DGC) activation, not PDE activity, and c-di-GMP production in *E. coli* and *P. aeruginosa*. Thus, this study provides a mechanism for NO-mediated biofilm formation, which was previously not understood.

Keywords : Biofilms, *P. aeruginosa*, nitric oxide

A-30

Characterization of Bacteriophage PhiAB01 and Its Potential as Alternative Antibiotics for MDR- and Foodborne *Acinetobacter* spp.

Soyul Im, Minsik Kim*
Department of Food and Nutrition, College of Human Ecology, Yonsei University, Seoul 03722, Republic of Korea

Nosocomial infection of *Acinetobacter baumannii* with multi-drug resistance (MDR) is a worldwide threat, and recent studies highlighting the concern of *Acinetobacter* spp. isolation from foods. Here, we isolated *Acinetobacter*-infecting bacteriophage phiAB01 from sewage to use as alternative antimicrobials. Morphological and genomic analysis suggested that phiAB01 is classified as a member of family *Myoviridae*. PhiAB01 could infect 6 *A. baumannii* strains including 5 MDRs among 23 strains tested, and inhibited 10 foodborne *Acinetobacter* spp. isolates among 96 food isolates tested. The lytic activity of phiAB01 was maintained in broad environmental conditions, ranged from 4 to 60℃, and from pH 3 to 11. Approximately 80% of the virions were attached to the host *A. baumannii* CCARM12090 cells within 3 min, and more than 98% were bound within 9 min. Eclipse and latent periods of the phiAB01 were determined to be 10 and 20 min, respectively, and the burst size was calculated as 133 PFU/infected cell. Host bacterial growth was effectively inhibited for 6.5 (at MOI =10) to 8 (at MOI = 0.1) hours by phiAB01 treatment. Based on the results, we suggest the virulent phage phiAB01 as one of the potent candidate for alternative antimicrobial to control MDR and foodborne *Acinetobacter* spp.

Keywords : Bacteriophage, *Acinetobacter* spp., alternative antimicrobials

A-31

Antifungal Mechanism of Hn-Mc, A Chimeric Peptide, against Pathogenic Fungal Strains

Jin-Young Kim, Mi-Kyeong Jang*
Department of Polymer Science and Engineering, Sunchon National University 255 Jungang-ro, Suncheon, Jeollanam-do 57922, Republic of Korea

The finding of new antifungal agents was very difficult because mammalian and fungal cells belong to the same eukaryotes, unlike bacteria. However, antimicrobial peptides have an excellent ability to distinguish cell types, due to their variable amino acids. Here, the antifungal activity and mechanism of Hn-Mc, a chimeric peptide that designed by combining the N-terminus of HPA3NT3 and the C-terminus of melittin were evaluated and investigated. In this study, we evaluated its potent antifungal activity with low minimal inhibitory concentrations (MICs), ranging from 1 to 16 μM against pathogenic yeast and mold cells. Cell selectivity of Hn-Mc was conducted by formation from random to α-helical structure in mimic fungal membrane environment. Furthermore, we found that the death of *Candida tropicalis* and *Fusarium oxysporum* was caused by the induction of apoptosis in cells through the generation of reactive oxygen species (ROS), which is produced in the mitochondrial damage in the cell.

Keywords : Antifungal peptide, ROS generation, apoptosis

This work was supported by the National Research Foundation of Korea(NRF) and the Center for Women In Science, Engineering and Technology(WISET) Grant funded by the Ministry of Science, ICT & Future Planning of Korea(MSIP) under the Program for Returners into R&D.

A-32

Differential Effects of Alkyl Gallates on Quorum Sensing in *Pseudomonas aeruginosa*

Ha-young Choi[1,2], Bomin Kim[1,2], Ji-Su Park[1,2], Jin-Hwan Kwak[3], Won-Gon Kim[1,2*]

[1]*Superbacteria Research Center, Korea Research Institute of Bioscience and Biotechnology, Yusong, Daejeon 34141, Republic of Korea,* [2]*Department of Bio-Molecular Science, KRIBB School of Bioscience, Korea University of Science and Technology (UST), Yusong, Daejeon 34113, Republic of Korea,* [3]*School of Life Science, Handong Global University, Pohang, Kyungbuk 37554, Republic of Korea*

Virulence factors and biofilms constitute attractive targets for the prevention of infections caused by multidrug-resistant bacteria. Among alkyl gallates, propyl gallate (PG) and octyl gallate (OG) are used as food preservatives. Here we found that alkyl gallates differentially affect virulence, biofilm formation, and quorum sensing (QS) in *Pseudomonas aeruginosa*. Ethyl gallate (EG), PG, and butyl gallate (BG) inhibited biofilm formation and virulence factors including elastase, pyocyanin, and rhamnolipid, in *P. aeruginosa* without affecting cell viability by antagonizing the QS receptors LasR and RhlR. PG exhibited the most potent activity. Interestingly, hexyl gallate (HG) inhibited the production of rhamnolipid and pyocyanin but did not affect elastase production or biofilm formation. Notably, OG inhibited the production of rhamnolipid and pyocyanin but stimulated elastase production and biofilm formation. Analysis of QS signaling molecule production and QS gene expression suggested that HG inhibited RhlR, while OG activated LasR but inhibited PqsR. This mechanism was confirmed using QS mutants. Additionally, PG prevented the virulence of *P. aeruginosa* in *Caenorhabditis elegans* and a mouse model. This is the first report of the differential effects of alkyl gallates on QS systems and PG has great potential as an inhibitor of the virulence and biofilm formation of *P. aeruginosa*.

Keywords : Alkyl gallates, *Pseudomonas aeruginosa*, quorum sensing

A-33

Synthesis, Characterization, and Anti-Algal Activity of Molybdenum Doped Zinc Oxide

Sondavid Nandanwar[1], Myung Won Lee[2], Shweta B. Borkar[2], Jeong Hyung Cho[1], Hak Jun Kim[2*]

[1]*Department of Marine Convergence Design, Pukyong National University, Busan 48513, Republic of Korea,* [2]*Department of Chemistry, Pukyong National University, Busan 48513, Republic of Korea*

In this study, we attempted to synthesize visible light active nano-sized photocatalysts using metal oxides such as zinc oxide, zirconium oxide, tungsten oxide, and strontium titanium oxide with $(MoCl_5)_2$ as a dopant by the simple ball-milling method. FT-IR data confirmed the presence of M-O-Mo linkage (M= Zn, Zr, W, and SrTi) in all the molybdenum-doped metal oxides (MoMOs), but only MoZnO inhibited the growth of the bloom-forming *Microcystis aeruginosa* under visible light in a concentration-dependent manner up to 10 mg/L. Further, structural characterization of MoZnO using FESEM and XRD exhibited the formation of typical hexagonal wurtzite nanocrystals of approximately 4 nm. Hydroxyl radical (·OH), reactive oxygen species (ROS), and lipid peroxidation assays revealed ·OH generated by MoZnO under the visible light seemed to cause peroxidation of the lipid membrane of *M. aeruginosa*, which lead to an upsurge of intracellular ROS and consequently introduced agglomeration of cyanobacteria. These results demonstrated that nano-sized MoZnO photocatalyst can be easily synthesized in a cost-effective ball-mill method and be utilized for biological applications such as the reduction of harmful algal blooms. Further, our study implies that a simple ball-milling method can provide an easy, green, and scalable route for the synthesis of visible light active doped metal oxides.

Keywords : Harmful cyanobacteria, molybdenum-doped zinc oxides, visible light active

A-34

Application of Single-Stranded DNA Aptamers for Quorum Quenching

In-Hwan Oh[1], Woo-Ri Shin[1], Simranjeet Singh Sekhon[1], Sang Yong Kim[2], Ji-Hyang Wee[2], Ji-Young Ahn[1], Jiho Min[3], Yang-Hoon Kim[1*]

[1]*Major in Microbiology School of Biological Sciences College of Natural Sciences Chungbuk National University, 1 Chungdae-Ro, Seowon-Gu, Cheongju, Chungbuk 28644, Republic of Korea,* [2]*Department of Food Science and Biotechnology, Shin Ansan University, 135 Sinansandaehak-Ro, Danwon-Gu, Ansan 15435, Republic of Korea,* [3]*Graduate School of Semiconductor and Chemical Engineering, Chonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju, Jeonbuk 54896, Republic of Korea*

Bacteria communicate with other bacteria in the community through a mechanism called quorum sensing, which uses a chemical signaling agent called autoinducer (AI). In Gram-negative bacteria, AHL (acyl-homoserine lactones) is the most common type of AI. AHL consists of an acyl chain of 4-18 carbons containing some changes in structure and N-acylated homoserine-lactone in the middle of the structure. AI accumulate in the environment as bacteria grow, and when the concentration of AI reaches a certain level, the bacteria regulate gene expression at the population level. Typically, gram-negative pathogens exhibit pathogenicity such as virulence, biofilm formation, conjugation, and sporulation through quorum sensing. In this study, ssDNA aptamer specific for homoserine lactone, a common structure of AI, was selected through 10 rounds of SELEX process. After in vitro selection, the optimal aptamer candidates were selected through SPR analysis, and it was confirmed that the formation of biofilm was suppressed in the sample treated with the obtained aptamer.

Keywords : Autoinducer, aptamer, quorum quenching

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A-35

Biocontrol of Carbapenem-Resistant *Klebsiella pneumonia* (a CRE) Using Bacteriocin-Mediated Antagonism in the Gut Microbiota

Seohee Jeong, Seungmin Park, Heenam Stanley Kim*

Division of Biosystems and Biomedical Sciences, College of Health Sciences, Korea University, Seoul 02841, Republic of Korea

Klebsiella pneumonia is a commensal bacterium residing in the human gastrointestinal (GI) tract and a prevalent nosocomial pathogen, causing pulmonary diseases, pyogenic liver abscess, and bacteremia. Carbapenem-resistant *K. pneumonia* (CRKP), particularly, has been a serious problem to human health due to its multidrug resistance. In this study, we characterized 68 *K. pneumoniae* isolates that produce various bacteriocins with specific killing activity against diverse *K. pneumoniae* strains. Among these, three strains (Kpn101, Kpn102, Kpn103) were selected as potential biocontrol agents for CRKP infections based on their potent killing effect against CRKP strain ATCC-BAA 1705. We found that at least in strain Kpn102, a plasmid confers the bacteriocin-mediated killing activity. Our data suggest that bacteriocin-producing commensal *K. pneumoniae* can be developed as an effective and safe tool to eliminate CRKP in the gut, without causing collateral damage on the gut microbiota as in current antimicrobial therapies.

Keywords : Gut microbiota, *Klebsiella pneumoniae*, multidrug-resistance

A-36

A Novel Myxobacterial Strain Producing Bacteriolytic and Antimicrobial Activities

Tra Thi Huong Nguyen[1,2], Song-Gun Kim[1,2*]
[1]*Biological Resource Center/Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, Jeonbuk 56212, Republic of Korea,* [2]*University of Science and Technology (UST), Daejeon 34113, Republic of Korea*

Myxobacteria are Gram-negative bacteria with a complex developmental life cycle of swarming, forming fruiting body, and producing abundant secondary metabolites. In the previous study, 27 myxobacterial strains were isolated from samples collected in Naejangsan Mountain. A strain RL12ST isolated from decayed leaf was determined to belong to the genus *Myxococcus* based on 16S rRNA sequence and morphology of vegetative cells and fruiting body. Notably, the strain RL12ST exhibited bacteriolytic and antifungal activities. Indeed, strain RL12ST was able to lysis bacterial cells of *Escherichia coli* and yeast cells of *Saccharomyces cerevisiae*. Moreover, strain RL12ST also displayed antifungal activities against pathogenic fungi of *Trichosporon pullulans*, *Alternaria alternate* and *Trichophyton rubrum.* The antimicrobial compounds produced by strain RL12ST were investigated. From the crude extract, an antifungal compound was separated by silica gel column chromatography by a solvent of ethyl acetate in hexane. Based on this result, the antifungal compound will be further purified by high-performance liquid chromatography and its chemical and antifungal activity will be determined in detail.

Keywords : Myxobacteria, antifungal activity, cell lysis

A-37

Class D Beta-Lactamase Gene (OXA) Abundance and Activity from Marine Microbial Metagenome

Sung Jae Ahn[1], Taeyune Kim[1], Mina Rho[2,3], Woo Jun Sul[1*]
[1]*Department of Systems Biotechnology, Chunag-Ang University, Anseong 17546, Republic of Korea,* [2]*Department of Computer Science and Engineering, Hanyang University, Seoul 04763, Republic of Korea,* [3]*Department of Biomedical Informatics, Hanyang University, Seoul 04763, Republic of Korea*

It is an important concern in clinical practice as the number of antibiotic-resistant species has recently increased. The class D beta lactamase gene, one of the resistances of antibiotic-resistant species, is hydrolyzes the antibiotic structure and allows to the microbes resist to the antibiotics which have β-lactam ring like penicillin, cephalosporin, and carbapenem. In this study, we performed a functional analysis using marine metagenome sequence data. The marine is a reservoir of many antibiotic-resistant genes, among which was tested to identify class D β-lactamase and to provide suitable criteria about annotated gene. We sampled sediment and seawater from Jindo, Shipborne Pole-to-Pole Observations (SIPPO), Sokcho, Wando, Tara Ocean (downloaded), Tongyeong, and Uljin. We searched against CARD database with the criteria: E-value < 1e-10, similarity > 70 %, and coverage > 70 % and found the ORFs having class D -lactamase. The class D -lactamase sequences obtained from this analysis were cloned into *E. coli* BL21 (DE3) and their activities were confirmed by paper disc assay with 10 antibiotics (ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, imipenem, meropenem, and ticarcillin).

Keywords : Antibiotics, beta lactamase, marine metagenome

A-38

Endolysin PA90 from Phage PBPA90 Infecting *Pseudomonas aeruginosa*

Yongwon Jung[1], Jae Y. Jang[2*], Heejoon Myung[1,2,3*]
[1]*Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Yong-In, Gyung-gi Do 17035, Republic of Korea,* [2]*LyseNTech Co. Ltd., Yong-In, Gyung-gi Do 17035, Republic of Korea,* [3]*Bacteriophage Bank of Korea, Yong-In, Gyung-gi Do 17035, Republic of Korea*

Endolysin is an enzyme that degrades peptidoglycan cell wall when a bacteriophage bursts out in lytic replication. In this study, we characterized endolysin PA90 from phage PBPA90 infecting *Pseudomonas aeruginosa* and confirmed its antibacterial activity. It was shown that the enzyme was active against various Gram-negative pathogens including *P. aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii,* and *Escherichia coli*. Antibacterial efficacy was increased in the presence of EDTA, a substance inducing disruption of the outer membrane of Gram-negative bacteria. Antimicrobial efficacy was also improved when used in combination with antibiotic colistin. In vivo efficacy was confirmed using *Galleria mellonella* as an animal model.

Keywords : Endolysin, antibacterial activity, *Pseudomonas aeruginosa*

A-39

Comparison of *Enterococcus faecalis* Biofilm Removal Efficiency Among Bacteriophage PBEF129, Its Endolysin, and Cefotaxime

Yoonjung Hwang[1], Hyun Keun Oh[1*], Hee Joon Myung[1,2*]
[1]*Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Yong-In, Gyung-gi Do 17035, Republic of Korea,* [2]*LyseNTech Co.Ltd, Yong-In, Gyung-gi Do 17035, Republic of Korea*

Enterococcus faecalis is a Gram-positive pathogen which colonizes human intestinal surfaces, forming biofilms, and demonstrates a high level of resistance to many antibiotics. In this study we have isolated and characterized a novel bacteriophage, PBEF129, infecting *E. faecalis.* PBEF129 infected a variety of strains of *E. faecalis*, including those exhibiting antibiotic-resistance. Its genome is a linear double stranded DNA 144,230 base pairs in length. Its GC content is 35.9%. The closest genomic DNA sequence was found in *Enterococcus* phage vB_EfaM_Ef2.3, with a sequence identity of 99.06% over 95% query coverage. 49 open reading frames (ORFs) were functionally annotated. ORF 2 was annotated as a phage endolysin having an L-acetylmuramoyl-L-alanine amidase activity. We purified the enzyme as a recombinant protein and confirmed its enzymatic activity. The endolysin's host range was observed to be wider than its parent phage PBEF129. When applied to bacterial biofilm on the surface of in vitro cultured human intestinal cells, it demonstrated a removal efficacy of the same degree as cefotaxime, but much lower than its parent bacteriophage. Additive effects were observed with both cefotaxime and bacteriophage PBEF129.

Keywords : Endolysin, bacteriophage, *Enterococcus faecalis*

A-40

A Novel Hybrid Oligosaccharide/Copper Composites for Antibacterial Applications

Sung -Bin Park[1], Ye-Jin Jeong[1], Young-Jung Kim[1], Gyeong Ho Han[1], Moon-Hee Sung[1,2*]
[1]*KookminBio Corporation, Seoul 02826, Republic of Korea,* [2]*Department of Bio and Fermentation Convergence Technology, BK21 PLUS Project, Kookmin University, Seoul 02707, Republic of Korea*

Copper particles have been used as a potential antimicrobial agent in many biomedical applications due to their extensive bioactivity. In this study, copper particles were synthesized by chemical reduction and precipitation methods. The Ascorbic acid (Vitamin C) was used as a reducing as well as a capping agent to produce highly stable and dispersed copper particles. The antibacterial property of the synthesized copper particles was tested by inhibition zone assay. To expand the application of the prepared antibacterial copper particles, an oligosaccharide/copper composite film was prepared through solvent casting after mixing the edible oligosaccharide solution and the copper particle powder. Prepared copper particles and hybrid oligosaccharide/copper composite films were characterized. In addition, the antibacterial activity of the oligosaccharide/copper composite film was evaluated against both Gram-positive and Gram-negative bacteria. The results indicate the applicability of the developed composite as a safe antibacterial material.

Keywords : Hybrid composites, copper particles, antibacterial materials

A-41

Elimination of Bacterial Biofilm via Synthetic Antimicrobial Peptides

Hyeonseok Kim, Mi-Kyeong Jang, Seong-Cheol Park*
Department of Polymer Science and Engineering, Sunchon National University, Chonnam 57922, Republic of Korea

Antibiotic resistance of bacterial cells growing in a biofilm can increase up to 1000-fold and biofilm-associated infections are difficult to manage or treat as biofilms or biofilm-embedded bacteria are difficult to eradicate. Therefore, we suggest synthetic antimicrobial peptides with repeated sequences of "XWZX" (X: lysine or arginine, Z: leucine, tyrosine, valine, or glycine) as anti-biofilm agent in this study. $(KWYK)_3$ peptide with the highest therapeutic index was selected as an anti-biofilm target peptide in this study. To determine which of the lysine and arginine residues as a cationic amino acid is effective for anti-biofilm activity, $(RWYR)_3$ peptide was compared. In addition, although $(KWLK)_3$ peptide was found to be highly cytotoxic, it was chosen to compare the effects of aliphatic and aromatic side chains on anti-biofilm prevention. All peptides have an inhibiting effect on biofilm formation and disrupting activity for preformed biofilms in drug-susceptible and drug-resistant *P. aeruginosa* and *S. aureus* strains. Moreover, they reduced the biofilm by detaching the carbohydrates, nucleic acids, and lipids of the EPS but did not detach the proteins. *In vivo*, $(KWYK)_3$ peptide showed the greatest efficacy against preformed biofilms with no cytotoxicity (NRF-2019R1I1A3A01062547).

Keywords : Antimicrobial peptide, anti-biofilm

A-42

Identification of Antibacterial Compound from *Streptomyces* sp. SJ1-7

Dasom Kim, Won-Jae Chi*
Microorganism Resources Division, Biological Resources Research Department, National Institute of Biological Resource, Incheon 17058, Republic of Korea

A bacterial strain was isolated and identified from soil sample collected from Sanju, Korea. The isolate was showed strong antimicrobial activity against *Staphylococcus aureus, Photobacterium damselae, Edwardsiella tarda, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus, Bacillus subtilis, Streptococcus iniae, Sclerotinia sclerotiorum, Rhizoctonia solani, Fusarium oxysporum, Pythium aphanideratum,* and *Clavibacter michiganensis.* The 16S rRNA gene sequence of the isolate showed high similarity (>99%) to *Streptomyces* spp.; hence, it was classified as a species of genus *Streptomyces*. The isolated was named as *Streptomyces* sp. SJ1-7 in this study. Antibacterial compound from *Streptomyces* sp. SJ1-7 was purified from cell-free culture broth by 5 steps of chromatography. Each step of the process was guided by bioassay for antibacterial activity using *S. aureus* as the indicator strain. The antibacterial compound was identified as Chromomycin A3 by using Electrospray ionization mass spectrometry and Nuclear magnetic resonance analysis.

Keywords : *Streptomyces*, antimicrobial activity, Chromomycin A3

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A-43

Influence of Chronic Antibiotics Exposure on Microbiome and Immune/ Stress Response in the Korean Native Ricefish, *Oryzias latipes*

Jun Hyeok Yang[1], Sungmin Hwang[2], Min Yeong Lee[1], Jeong Woo Park[1], So Young Jeong[1], Seungki Lee[3], Woo Young Bang[3], Ki Hwan Moon[1*]
[1]*Division of Marine Bioscience, Korea Maritime and Ocean University, Busan 49112, Republic of Korea,* [2]*Department of Biology, Duke University, Durham, NC 27708, USA,* [3]*National Institute of Biological Resources, Environmental Research Complex, Incheon 22689, Republic of Korea*

Antibiotic pollutants in the milieu escalate the selective pressure on microbial flora and promote the increase of antibiotic resistance in the aquatic environment. The indiscriminate use of antibiotics can affect not only microorganisms but also their hosts in both aquatic and terrestrial environments. In this study, we hypothesized that chronic antibiotic exposure can alter the composition of gut microbiome in the host, and modulate expression levels of immune and stress-related genes. In order to confirm this, we procured the Korean native ricefish, *Oryzias latipes* from the National Institute of Biological Resources. To set up the antibiotic concentration for chronic exposure assays, we selected three antibiotics, and six microbes from intestine of ricefish for minimal inhibitory concentration (MIC) testing. Based on MIC data, we established the antibiotic concentration for chronic exposure assay. Our ricefish were exposed for a month to each antibiotic. Then, total DNA from fish intestine was purified for future gut microbiome analyses. Total ricefish RNA was also purified to check immune and stress-related gene expression with future differentially expressed genes (DEG) analyses. Through this work, we have already made progress in elucidating the relationship of antibiotics as an environmental factor for modulating the intestinal microbiome of host and its influences on the immune and stress responses. In addition, we provide a solid reason to halt the indiscriminate use of antibiotics.

Keywords : Antibiotics, microbiome, ricefish

A-44

A Novel Decoy Strategy of *Acinetobacter baumannii* for Polymyxin Resistance

Jaeeun Park, Misung Kim, Bora Shin, Woojun Park*
Laboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea

Modification of lipopolysaccharides by a polymyxin B (PMB)-induced PmrAB two-component system appears to be a dominant phenomenon in PMB-*resistant Acinetobacter baumannii*. Here, PMB-resistant variants (PMR^{Low} and PMR^{High}), which showed common mutations of the sensor kinase PmrB (or the response regulator PmrA), were found to produce more outer membrane vesicles (OMVs) than wild type. Furthermore, adding purified from the PMB-resistant variants into the cultures of PMB-susceptible *A. baumannii* and the clinical isolates protected those susceptible bacteria from PMB. Terminal restriction fragment length polymorphism analysis of the feline fecal microbiota revealed that this protective effect of OMVs was not specific to the microbiota. Additionally, a *Galleria mellonella* infection model showed that OMVs increased the mortality rate of larvae by protecting *A. baumannii* from PMB. OMVs can protect the human pathogen *A. baumannii* from PMB under in vivo and in vitro conditions possibly by functioning as decoys for PMB binding. Our data showed that mutation of the pmrB gene and the resulting OMVs increase bacterial survival by reducing the binding of PMB to the bacterial membrane.

Keywords : *Acinetobacter baumannii*, outer membrane vesicles, polymyxin-resistance

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A-45

Killing Effect of Deinoxanthin on Cyanobacterial Bloom-Forming *Microcystis aeruginosa*

Wonjae Kim, Woojun Park*
Laboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea

Cyanobacterial blooms caused mainly by *Microcystis aeruginosa* could be controlled using application of antagonistic bacteria. Killing effect of *Deinococcus*-derived carotenoids have not been tested on *M. aeruginosa* cells. *Deinococcus metallilatus* MA1002 cultured under the light increased the production of several carotenoid-like compounds. When *D. metallilatus* MA1002 cells were cocultured with axenic *M. aeruginosa* PCC7806, growth defect of *M. aeruginosa* was observed. Ethyl acetate-concentrated compounds from ethyl alcohol extracts of *Deinococcus* cells were tested to find an active compound in *Deinococcus* cell-death products. Compounds were identified using thin-layer chromatography, high-performance liquid chromatography, and liquid chromatography-mass spectrometry. Deinoxanthin-like compounds could inhibit growth of axenic *M. aeruginosa* cells probably due to its interference with *Microcystis*-membrane synthesis during cell elongation. Combinatory treatment of our deinoxanthin-derivative with H_2O_2 is more effective against *M. aeruginosa* cells. Deinoxanthin-derivative can be used as a novel candidate for preventing cyanobacterial blooms. [Supported by a Korea University grant (K2006821).] Cyanobacterial blooms caused mainly by *Microcystis aeruginosa* could be controlled using application of antagonistic bacteria. Killing effect of *Deinococcus*-derived carotenoids have not been tested on *M. aeruginosa* cells. *D. metallilatus* MA1002 cultured under the light increased the production of several carotenoid-like compounds. When *D. metallilatus* MA1002 cells were cocultured with axenic *M. aeruginosa* PCC7806, growth defect of *M. aeruginosa* was observed. Ethyl acetate-concentrated compounds from ethyl alcohol extracts of *Deinococcus* cells were tested to find an active compound in *Deinococcus* cell-death products. Compounds were identified using thin-layer chromatography, high-performance liquid chromatography, and liquid chromatography-mass spectrometry. Deinoxanthin-like compounds could inhibit growth of axenic *M. aeruginosa* cells probably due to its interference with *Microcystis*-membrane synthesis during cell elongation. Combinatory treatment of our deinoxanthin-derivative with H_2O_2 is more effective against *M. aeruginosa* cells. Deinoxanthin-derivative can be used as a novel candidate for preventing cyanobacterial blooms.

Keywords : Pigment, carotenoid, cyanobacterial bloom

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