A_Antibiotics, Antifungals, and Antiviral Compounds

A-1

Synthesis and Characterization of Carboxymethyl Cellulose/Silver Nanobiocomposites Using a Solution Plasma Process with Silver Electrodes

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Solution plasma process (SPP) was adapted to synthesize biocomposites of carboxymethyl cellulose (CMC)/silver nanoparticles (AgNPs). Unlike previous studies using $AgNO_3$ as the precursor and tungsten electrodes, silver electrodes were used in this study for the synthesis of pure CMC/AgNP biocomposites. CMC/AgNPs were synthesized by discharging plasma at 800 V, 30 kHz for 7 min in 0.3, 0.5, 0.75, or 1.0% CMC solutions. UV-Vis spectroscopy showed a peak at 406, 408, 407, and 406 nm with absorbance of 44.7, 37.3, 35.3, and 34.9, respectively. The absorbance was much higher than those obtained in the previous studies (3-3.5 at 440 nm). Blue shift transition of the peaks and high absorbances could be due to the formation of smaller particles and increased surface plasmon resonance (SPR), respectively. FTIR analysis showed no change in the molecular structure of CMC during SPP. Antimicrobial activity was investigated against 6 pathogens (*Escherichia coli*, *Vibrio vulnificus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans*). CMC (1.0%)/AgNP biocomposite showed minimal inhibition concentrations (MIC) in a range of 58.5-78 μg AgNPs/ml for the bacteria and 415.6 μg /ml for the yeast. *V. vulnificus* became more resistant to the biocomposites when culture in low salt medium, having MIC of 195 μg/ml. The results indicated that synthesis of antimicrobial biocomposites were possible using Ag electrodes instead of $AgNO_3$.

Keywords : Solution plasma process (SPP), CMC/AgNP biocomposites, antimicrobial activity

A-2

Antimicrobial Effect of MSF, a Novel Material Created by Fusion of Probiotics and *Filipendula glaberrima* Nakai Extract

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*Filipendula glaberrim*a Nakai has been used as traditional painkiller or to cure frostbite and burn. The conventional therapy suggests that this plant may have antimicrobial effects, but the efficacy of Filipendula glaberrima Nakai extracts on antimicrobial activity in HaCat cells was poor. hBD3 is known as a peptide with antimicrobial activity, and especially shows excellent efficacy against *Staphylococcus aureus*. To increase the antimicrobial efficacy, we prepared MSF, which is *L. plantarum* K8 lysates that manufactured after fermentation in a medium containing *F. glaberrima* Nakai extract. MSF significantly increased hBD3 transcripts and inhibited the internalization of *S. aureus* to HaCat cells as compared to Filipendula glaberrima Nakai extracts only or *L. plantarum* K8 lysates only. In the current study, we developed a new material with enhanced antimicrobial efficacy through the fusion of probiotics and the extracts of natural products. This novel material such as MSF could be used to develop cosmetic materials with antibacterial effects.

Keywords : *Filipendula glaberrima* Nakai, hBD3, MSF

Poster Session

A-3

Characterization of Alginate/AgNP Biocomposites Synthesizedby Solution Plasma Process Using Silver Electrodes as an Antimicrobial Agent

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Previously, alginate-AgNP biocomposites with antimicrobial activities were synthesized by a facile one-step solution plasma process (SPP) using $AgNO_3$ as a precursor, alginate from brown algae as the matrix, and tungsten electrodes. In this study, more efficient synthesis of cleaner biocomposites was attempted using Ag electrodes without the precursor. Plasma was discharged in 0.75% alginate solution using a pulsed field unipolar power supply at 800 voltages with 30 kHz of frequency for 2, 3, or 7 min with Ag electrodes with a gap of 1 mm. UV-Vis spectroscopy showed a peak at 407 ~ 413 nm, having blue-shift compared to the results obtained using $AgNO_3$ (415-440 nm). Also, the peaks had absorbance of 21.55~ 45.08, which was much higher than those of the previous results (1~2.5). It implied that the sizes of AgNPs produced using Ag electrodes might be smaller and had higher surface plasmon resonance. The results of FTIR showed that chemical bonds of alginate were not changed. The minimum inhibitory concentration of against *E. coli*, *V. vulnificus*, *P. aeruginosa*, *S. aureus*, *B. cereus*, were 9.12~ 18.24 μg/ml; *C. albicans*, 60.8 μg/ml of AgNPs that were synthesized using 0.75% alginate and discharging plasma for 2 min. Therefore, the SPP using Ag electrodes seemed to be a much more efficient way to produce antimicrobial agents than the previous SPP.

Keywords : Solution plasma, alginate-silver nanoparticle biocomposite, antimicrobial agent

A-4

Multi-Omics Based Characterization of Inhibition of Methicillin-Resistant *Staphylococcus aureus* by *lactobacillus* species

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As demands for new strategies to control methicillin-resistant *Staphylococcus aureus* (MRSA) increase, studies that gain insight from gut bacteria regarding growth inhibition and anti-virulence strategies have been reported. Although it was reported in several studies that *Lactobacillus* species, lactic acid-producing bacteria, have antibacterial activity against MRSA, the molecular mechanism under these phenomena is unclear. In here, we evaluated the inhibition effect against MRSA *Lactobacillus* species isolated from stools of infants and performed a multi-omics study about the highest growth inhibitory strains. Firstly, we observed that MRSA growth inhibition was not correlated with extracellular pH level, affected by *lactobacillus*. In the multi-omics study, it was observed that MRSA significantly upregulated arginine deiminase system, proteins related protein synthesis, and downregulated quorum-sensing system, capsular polysaccharide synthesis proteins in the *lactobacillus* co-culture model. In this study, the underlying mechanism involved in growth inhibition and anti-virulence of MRSA mediated by *lactobacillus* was discovered. It is expected that these results can provide insight into probiotics-pathogen interaction mechanisms and new opportunities for antibiotics development.

Keywords : MRSA, *Lactobacillus*, multi-omics

A-5

Manipulation of Bacterial Mechanisms for Engineering Applications

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Biofilms are composed of a single or multiple species of bacteria embedded in extracellular polymeric substance (EPS), which affects increasing antibiotic resistance through restriction of the transport of antibiotics to the bacteria cells. An alternative approach to treatment with antimicrobial agents is using biofilm inhibitors that regulate biofilm development without inhibiting bacterial growth. In the present study, we found mandarin peel extract (MPE)'s ability to inhibit *P. aeruginosa* PA14 biofilm formation. The result showed that 62-71% and 59-65% reduction in biofilm volume and thickness by 0.002-0.2% MPE treatment, respectively. Furthermore, MPE decreased production of EPS, whereas increased bacterial swarming motility. These results leads to the hypothesis that MPE reduced level of a second messenger c-di-GMP, which involved in bacterial biofilm development. To thes this hypothesis, a reporter-based measurement assay was used to evaluate the c-di-GMP inhibitory effect of MPE. The result showed that MPE slightly but significantrly reduced the c-di-GMP level through increased phosphodiesterase activity. Taken together, these finding suggest that MPE as a biofilm inhibitor has new potential for pharmacological and industrial applications.

Keywords : Mandarin peel extract, biofilm inhibition, c-di-GMP

A-6

Synthesis and Antimicrobial Activity of Cyclic mBjAMP1 from *Branchiostoma japonicum*

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Multidrug resistant (MDR) bacteria are rapidly increasing due to excessive use of antibiotics. Antimicrobial peptides (AMP) are in the spotlight as an important materials to overcome this problem. However, the disadvantage of AMP is that it has low bioavailability due to its unstable form. To overcome this, mBjAMP, one of the antimicrobial peptides, was newly designed and synthesized in a cyclic form (cyclotide form). Cyclotide has the advantage of being structurally stable against chemical, enzymatic and thermal conditions due to its multiple disulfide bonds and head to tail cyclization. mBjAMP1 consists of 21 amino acid residues and was isolated from *Branchiostoma japonicum*. And it contains two cysteines in the primary sequence. First, a wild-type sample of mBjAMP1 was synthesized through solid-phase peptide synthesis (SPPS). After purification by HPLC, air oxidation was used to oxidize the thiol group on the two cysteine residues to form a disulfide bond. Cyclic mBjAMP1 (C-mBjAMP1) was generated through head-to-tail cyclization. The CD spectrum of C-mBjAMP1 was used to confirm the conformational changes by cyclization of mBjAMP1. In addition, a minimum inhibitory concentration (MIC) was determined to assess the improvement of antimicrobial activity of the peptides.

Keywords : Antimicrobial peptides, cyclotide, bioavailability

A-7

Antifungal Metabolites Produced by *Bacillus siamensis* Isolated from Rhizosphere Soil of Weeds

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The establishment of strategy for dealing with plant disease is the one of the most important tasks in agricultural industry. The *Ralstonia solanacearum*, one of the severe plant fungal pathogens that occur in the wet tropics, subtropics and some temperature regions of the world, are well known for cause serious damage to tomato and other solanaceous plants. We isolated *Bacillus* strain from rhizosphere soil of weeds which showed antifungal activity against *R. solanacearum*. The strain was identified as *Bacillus siamensis* by the 16S rRNA sequence analysis. The LC-MS analysis of the culture supernatant of *B. siamensis* suggested that it produces various cyclic lipopeptides including iturins (m/z 1043.6 and 1057.8), fengycins (m/z 1464.5 and 1492.0) and surfactins (m/z 994.8, 1009.0, 1023.1 and 1037.3). To obtain the pure lipopeptides, crude metabolites were precipitated by adjusting culture media to pH 2 and the precipitate was dissolved in acetonitrile. Then the crude lipopeptides were further purified using preparative reverse phase-high performance liquid chromatography (RP-HPLC). The determination of minimal inhibitory concentration (MIC) and hemolysis assay were carried out to confirm the activities and toxicities of the purified lipopeptides. The molecular structure of the purified peptides was confirmed by 2D NMR spectroscopy.

Keywords : *Ralstonia solanacearum*, cyclic lipopeptides, antifungal compounds

A-8

Verification of Antibacterial Activity of Ag/Cu Nanoparticles against Several Pathogenic Bacteria

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The antibacterial activities of nanoparticles of silver and copper were studied by using food-borne pathogenic bacteria as test microorganisms including such as *Escherichia, Salmonella enteritidis, Listeria monocytogenes, Shigella boydii, Bacillus cereus* and *Staphylococcus aureus*. Agar well diffusion method showed Ag/Cu nanoparticles (Ag/CU NP) had antibacterial activity against all tested microbes. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of Ag/CU NP were determined and compared. The antibacterial activity of Ag/CU NP were not affected by temperature and pH changes. Cell wall and membrane were severely damaged by treatment of Ag/CU NP to bacterial cells, which was observed via SEM. The antibacterial activity was retained for up to three months at both 4℃ and -20℃. The Ag/CU NP inhibited growth of *L. monocytogenes* in beef, boiled rice and milk stored at 4℃ for 30 days. Taken these together, the Ag/CU NP might be applied to food storage containers.

Keywords : Ag/Cu nanoparticles, antibacterial activity, pathogenic bacteria

A-9

Antimicrobial use of Bacteriochlorophyll *a* to Inhibit the Growth of Cyanobacteria Which Cause Harmful Agal Boom

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Chlorophyll synthase (ChlG) esterifies chlorophyllide *a* with a C_{20}-moiety of either geranylgeranyl or phytyl group to form chlorophyll *a* (Chl *a*), a main pigment of the O_2-evolving photosynthetic organisms. Likewise, bacteriochlorophyll synthase (BchG) esterifies baceriochlorophyllide *a* with the same C_{20}-moiety to form bacteriochlorophyll *a* (Bchl *a*), a main pigment of anoxygenic photosynthetic bacteria. Interestingly, ChlG of *Synechocystis* sp. PCC6803 is competitively inhibited not only by bacteriochlorophyllide *a* but also by BChl *a* with an inhibition constant (K_{ic}) of 39.5 ± 2.2 μM, whereas the activity of ChlG was not affected by Chl *a*. Consistently, the exogenous addition of BChl *a* to the culture of *Synechocystis* sp. PCC6803 results in the retardation of its photosynthetic growth in a dose-dependent manner, while Chl *a* does not show any effect. The growth-inhibitory effect of BChl *a* was further illustrated with other cyanobacterial species; *Anabaena variabilis*, *Anabaena circinalis*, and *Microcystis aeruginosa*, which are the representative cyanobacterial species for algal blooms in river. Taken together, BChl *a* arrests the cyanobacterial growth by inhibiting ChlG and thus Bchl *a* may be practically used to control the cyanobacterial blooms.

Keywords : Cyanobacteria, chlorophyll synthase, bacteriochlorophyll *a*

A-10

Screening for Inhibitors of the Cyclic(Phe-Pro) Signal in Pathogenic *Vibrio* species

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To obtain chemicals interrupting a signal closely associated with the virulence expression in the human pathogen *Vibrio vulnificus*, we screened total 6,566 chemicals of the Chembank collection cordially provided by the Korean Chemical Bank for candidates that repress the expression of the gene *leuO*, which encode a master regulatory protein for the virulence pathway associated with the signal molecule cyclic-Phe-Pro (cFP). For this, we constructed a screening system in which *luxAB*-reporter genes were transcriptionally fused to the promoter region of *leuO*. The resulting strain was added with each of the collection chemicals dissolved in DMSO, and the Lux activity was quantitatively measured in 96-well plates employing DMSO and purified cFP as negative and positive controls, respectively. Through three rounds of screening, we could narrow down to two compounds which represses the reporter expression to a 30% level but do not affect growth of cells. These final candidates SG0010 and SGR0020 significantly inhibit the expression of *leuO* of *V. vulnificus* in concentration-dependent manner, and, furthermore, inhibit transcription of *ctxAB* of *V. cholerae* and *vvhBA* of *V. vulnificus*, which encode exotoxins known to be a target of the cFP-ToxR-LeuO signaling pathway. These compounds not only reduced resistance to ROS, but also significantly increased survivability of mice infected by *V. vulnificus*. These compounds exerted no significant harmful effects either on growth of *V. vulnificus* cells or viability of human epithelial cell line HaCaT as measured by the MTT assay. These results suggest that these two compounds can serve as agents controlling the virulence of pathogenic *Vibrio* species.

Keywords : *Vibrio vulnificus*, chemical compound, pathogenicity

A-11

Sargassum fusiforme and Its Components Inhibit Respiratory Syncytial Virus Infection In vitro and In vivo

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Sargassum fusiforme, a plant used as a medicine and food, is regarded as a marine vegetable and health supplement to improve life expectancy. Here, we demonstrate that *S. fusiforme* extract (SFE) has antiviral effects against respiratory syncytial virus (RSV) in vitro and in vivo mouse model. Treatment of HEp2 cells with a non-cytotoxic concentration of SFE significantly reducedRSV replication, RSV-induced cell death, RSV gene transcription, RSV protein synthesis, and syncytium formation. Moreover, oral inoculation of SFE significantly improved RSV clearance from the lungs of BALB/c mice. Interestingly, the phenolic compounds eicosane, docosane, and tetracosane were identified as active components of SFE. Treatment with a non-cytotoxicconcentration of these three components elicited similar antiviral effects against RSV infection as SFE in vitro. Together, these results suggest that SFE and its potential components are a promising natural antiviral agent candidate against RSV infection.

Keywords : *Sargassum fusiforme*, therapeutic effects, RSV

[The National Research Foundation of Korea (2018M3A9H4078703) and KRIBB Research Initiative Program (KGM9942011)]

A-12

Inhibition of Respiratory Syncytial Virus Replication *In Vitro* and *In vivo* by *Stichopus japonicus* (selenka) Extract

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The sea cucumber *Stichopus japonicus* (Selenka) is an invertebrate animal inhabiting the coastal sea around Korea, Japan, China, and Russia. It is one of the highest commercially valuable species as seafood and it has been commonly used for centuries in indigenous and folk medicine. Although it has many therapeutic effects, antiviral activity against RSV has not been reported in detail. In this study, we show that extracts from *S. japonicus* has antiviral effects against Respiratory Syncytial Virus (RSV) *in vitro* cell cultures and an *in vivo* mouse model. Treatment of human respiratory tract cell line (HEp2) with a non-cytotoxic concentration of *S. japonicus* extract significantly reduced RSV replication, RSV-induced cell death, RSV gene transcription and RSV protein synthesis. Moreover, the treatments significantly diminish syncytial formation after RSV infection in HEp2 cells. Time dependent treatment of *S. japonicus* after RSV infection in HEp2 cells showed that treatment with two-hour post infection of virus infection can provide better result by demolishing further replication of RSV virus in Hep2 cell line. Interestingly, oral inoculation with *S. japonicus* extract significantly improved viral clearance in the lungs of BALB/c mice. Collectively, these results suggest that extracts of *S. japonicus* could use as a potent natural anti-RSV candidate.

Keywords : *Stichopus japonicus* (selenka), RSV, therapeutic effects

[The National Research Foundation of Korea (2018M3A9H4078703) and KRIBB Research Initiative Program (KGM9942011)]

A-13

Studies on a Bacteriophage Specific for Antibiotic-Resistant *Escherichia coli* Isolated from Agricultural Environment

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Antibiotic-resistant (AR) bacteria are broadly present in the agricultural environment and their negative effects have significantly risen in the past few decades. The purpose of this study was to isolate and characterize AR *Escherichia coli* and its phage. Thirty-seven strains of *E. coli* were isolated from agricultural environment and IMViC test was performed prior to 16S rRNA sequencing. The presence of virulence genes and antibiotic resistance were investigated using PCR and E test, respectively. The *E. coli*-specific phage was isolated and purified from slaughter wastewater. Morphological characteristics, host range, and stability of *the* purified phage were performed using TEM, dot assay, and plaque assay. This phage had an icosahedral head (65.44 ± 8.90 nm in length, 10.08 ± 2.59 nm in width) and a tail (14.76 ± 3.58 nm in length, 2.03 ± 0.59 nm in width). In addition, it showed clear plaques against 11 strains of *E. coli* including *E. coli* CMD05061 (an AR E. coli) and was stable under various ranges of pHs (3 - 11) and temperatures (-20 - 60℃). This study demonstrated that *E. coli*-specific phage could be potentially useful as a biocontrol agent against AR *E. coli*.

Keywords : *Escherichia coli,* antibiotic resistance, bacteriophage

A-14

Heterologous Epression of the Nystatin-like *Pseudonocardia* Polyene B1 Biosynthetic Gene Cluster

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Many natural products produced by actinomycetes have been used as antibiotics for decades in various fields. Especially, Polyene antibiotics such as nystatin A1 and amphotericin B belong to a large family of very valuable antifungal polyketide compounds. Nystatin-like *Pseudonocardia* polyene (NPP) A1 which contains disaccharide moiety in tetraene macrolide backbone has been reported to be produced by a rare actinomycetes called *Pseudonocardia autotrophica*. Previously, a pharmacokinetically-improved NPP derivative named NPP B1 was developed by substituting two amino acids in the enoyl reductase domain in module 5 of the nppC. Although the NPP B1 productivity was increased by various strategies including elimination of the native plasmid in *P. autotrophica* and overexpression of pathway-specific regulatory genes, its titer is still far from satisfactory. To overcome this low-titer issue, the NPP B1 biosynthetic gene cluster (BGC) from *P. autotrophica* was isolated *via* bacterial artificial chromosome (BAC) system, followed by homologous and heterologous expressions in several *Streptomyces* hosts. More details related to NPP B1 BGC expression and its biological activities will be discussed.

Keywords : NPP B1, heterologous expression, Bacterial Artificial Chromosome (BAC)system

A-15

Isolation of Novel *Streptomyces via* Antifungal Bioassay-Based Screening

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Because of the side effects of chemical pesticides, research is proceed to develop microbial-based biological pesticides as the need for eco-friendly pesticide development has been raised. To develop an actinomycetes-based biological pesticide that produces a variety of useful secondary metabolites, we selected antifungal-active actinomycetes through antifungal activity tests on *F. oxysporum* and *C. albicans* using 2400 actinomycetes cultures medium. The selected strains conducted an additional antifungal activity test for 10 plant pathogenic fungi and selected one candidate strain for high antifungal activity. It was also pot-tested with selected strains, and the control efficacy was 61.6% for strawberries, 72.7% for tomatoes, and 60.0% for peppers. It showed high control efficacy Whole genome sequencing of this strain was performed to identify the putative biosynthetic gene clusters responsible for the antifungal compounds. the data was analyzed by antiSMASH. In silico analysis by antiSMASH, Candidate BGCs were identified at those showed high homology responsible for the biosynthesis of rifamycin with antifungal activity in the NRPS family. Additionally, we identified BGCs that showed high homology with elaiophyin and mediomycin in Type 1 PKS. These BGCs are predicted to show antifungal activity. As a result, the newly-screened actinomycetes species could be the promising candidates for development of novel antifungal agents or eco-friendly microbial pesticides.

Keywords : Antifungal, actinomyces, microbial pesticides

A-16

Antimicrobial Spectrum and Characterization of Purified Recombinant Micro Halocin HB384, Derived from Halophiles

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The problem of resistance due to excessive abuse of antibiotics is the cause of the birth of multidrug-resistant bacteria, and the problem has not been solved until now. Therefore, as a solution to the problem of existing antibiotics, attention was paid to the search for antimicrobial peptides (AMPs) whose resistance problem was not revealed. In this study, AMPs derived from halophiles were obtained based on the gene information of halocin peptides. It was named HB384, and the gene encoding HB384 was cloned into pGST vector and the recombinant HB384 was expressed in *E. coli* BL21. The recombinant protein was purified by GST affinity chromatography, and the molecular weight of HB384 was 3.14 kDa. Disk diffusion assays were performed to evaluate antimicrobial activity. HB384 showed antimicrobial activity against Gram-positive bacteria *S. aureus*, *B. subtilis* and Gram-negative bacteria *S. typhimurium*, *E. coli*. Moreover, HB384 was stable at 99℃ for 8 hours, as the concentration increased, antimicrobial activity against pathogen. In less than 6 μg, HB384 almost reached the maximum antimicrobial activity against pathogen. When comparing the number of moles of antimicrobial activity substances per area of the clear zone, the antimicrobial activity of HB384 against *B. subtilis* was 3.2 times better than that of ampicillin. As a result, purified HB384 is expected to be applicable not only as a functional peptide material, but also as a substitute for existing antibiotics.

Keywords : Micro halocin, antimicrobial kinetic activity, GST affinity chromatography

A-17

Screening of Antagonistic Bacteria Having Antifungal Activity against Phytopathogenic Fungi of Chilli pepper

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Chilli anthracnose, *Sclerotinia* stem rot (SSR), *Phytophthora* blight of chilli pepper are caused by fungal pathogens such as *Colletotrichum acutatum* and *Sclerotinia sclerotiorum*, and *Phytophthora* species threatening the production of chilli pepper. To search biological control agents (BCAs) of phytopathogenic fungi, five kinds of useful *Bacillus*-like isolates were selected from field soil of Sunchang in Korea. The selected isolates were characterized by production of enzyme, siderophore and indole-3-acetic acid (IAA). Also, antifungal activity against three of the phytopathogenic fungi that frequently occur in chilli pepper was tested. Among them, PBS-68 strain had the most excellent antifungal activity. Physiological characteristics of PBS-68 strain also confirmed by analysis of carbohydrate fermentation patterns and enzyme production ability. Based on the experimental results, PBS-68 strain was finally selected as a candidate for BCA. BLASTn search of the 16S rRNA gene sequence via National Center for Biotechnology Information (NCBI) database indicated that the isolate, PBS-68 strain matched *Bacillus subtilis* (GenBank accession no. NR027552) with similarity values of 99.59% (1442/1448 bp). Based on the above results, *B. subtilis* PBS-68 strain is expected to be used as a BCA for the chilli pepper pathogenic fungi.

Keywords : Antagonistic bacteria, *Bacillus subtilis,* biological control agents

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A-18

Strategic Approach for Reducing Aflatoxin Levels in Korean Traditional *Nuruk*

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Nuruk is a fermentation starter used for brewing alcoholic beverages. Since *Nuruk* is made from uncooked grains with naturally inoculated microorganisms, it can be infected with *Aspergillus flavus*, which produces carcinogenic aflatoxin (AF). To determine the safety of alcoholic beverages and reduce the AF levels in *Nuruk*, this study evaluated total AF levels in *Nuruk* and the transfer of AF from *Nuruk* to alcoholic beverages and isolated AF-producing fungi and antifungal lactic acid bacteria (LAB). Out of 61 *Nuruk*, 14 exceeded the Korean permissible total AF level of 15 ppb. Only AF G1 is transferred to alcoholic beverages at a rate of about 1.2% to 1.3%. The LC-MS-based non-targeted metabolomics approach allowed for the selection of two LAB species, *Weissella paramesenteroides* and *Pediococcus pentosaceus*, exhibiting high antifungal activity. They reduced AF via inhibition of mycelial growth, AF production, and AF surface binding. This present study was firstly considered the safety of Korean traditional alcoholic beverages in terms of the overall fermentation process starting from *Nuruk*.

Keywords : Aflatoxin, lactic acid bacteria, *Nuruk*

A-19

Antibiofilm and Antifungal Activities of Mmedium-Chain Fatty Acids against *Candida albicans*

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*Candida albica*ns is an opportunistic pathogen responsible for candidiasis and colonizes host tissues and implant devices via biofilm formation. These biofilms are tolerant to conventional antifungal therapeutics and the host immune system. The transition of yeast cells to hyphae is a crucial step in *C. albicans* biofilm development, and the quorum-sensing molecule farnesol inhibits this transition. We hypothesized that fatty acids mimicking farnesol might influence hyphal and biofilm formation by *C. albicans*. Amid 31 saturated and unsaturated fatty acids, six medium-chain saturated fatty acids, such as heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, and lauric acid, efficiently inhibited *C. albicans* biofilm formation by more than 75% at 2 μg/ml with MICs in the range 100–200 μg/ml. These six fatty acids at 2 μg/ml and farnesol at 100 μg/ml inhibited hyphal growth and cell aggregation. The addition of fatty acids to *C. albicans* cultures decreased the productions of farnesol and sterols. Furthermore, the downregulation of several hyphal and biofilm-related genes triggered by heptanoic or nonanoic acid closely resembled the changes instigated by farnesol. In addition, nonanoic acid, the most effective compound, diminished *C. albicans* virulence in a *Caenorhabditis elegans* model. Our results suggest that medium-chain fatty acids effectively inhibit hyphal growth and biofilm formation than farnesol.

Keywords : *Candida albicans,* fatty acids, quorum sensing

A-20

Iodoindoles as an Inhibitor of Biofilm Formation and Rapid Killing of Multidrug-Resistant *Acinetobacter baumannii* Strains and Other Microbes

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Multi-drug resistant *Acinetobacter baumannii* is well-known for its rapid acclimatization in hospital environments. The ability of the bacterium to endure desiccation and starvation on dry surfaces for up to a month results in outbreaks of health care-associated infections. Previously, indole and its derivatives were shown to inhibit other persistent bacteria. We found that among 16 halogenated indoles, 5-iodoindole swiftly inhibited *A. baumannii* growth, constrained biofilm formation and motility, and killed the bacterium as effectively as commercial antibiotics such as ciprofloxacin, colistin, and gentamicin. 5-Iodoindole treatment was found to induce reactive oxygen species, resulting in loss of plasma membrane integrity and cell shrinkage. In addition, 5-iodoindole rapidly killed three *Escherichia coli* strains, *Staphylococcus aureus*, and the fungus *Candida albicans*, but did not inhibit the growth of *Pseudomonas aeruginosa*. This study indicates the mechanism responsible for the activities of 5-iodoindole warrants additional study to further characterize its bactericidal effects on antibiotic-resistant *A. baumannii* and other microbes.

Keywords : *Acinetobacter baumannii,* antibiotics, biofilm

A-21

Antifungal Activity of *Bacillus subtilis* NK 2 against Phytopathogen

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This study was conducted to isolate bacteria having antifungal activity against various phytopahogens by producing multifunctional biocontrol agents. The use of biological fungicides can effectively reduce the consumption of chemical fungicides or toxic substances to control plant diseases. For the screen of biological fungicides, a total of 157 different bacterial isolates were obtained from undamaged soil by repeated cultivation in Sunchang, Korea, and screened for antibiotics agents, siderophores, and extracellular enzymes (protease, cellulase, and amylase) production. Among the isolates, NK2 strain with superior enzymatic and antifungal activity was selected for further experiments. The NK2 strain was identified as *Bacillus subtilis* by 16S rRNA sequence analysis. Finally, physiological and biochemical characteristics of *B. subtilis* NK2 were examined. The characteristic research results suggest that *B. subtilis NK2* has useful multifunctional biocontrol ability against various phytopathogens.

Keywords : Antifungal, *Bacillus subtilis*

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A-22

Screening and Isolation of a Novel Polyene-Producing *Streptomyces* Strain Inhibiting Phytopathogenic Fungi in the Soil Environment

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Microbial-based eco-friendly biological substances are needed to protect crops from phytopathogenic fungi and replace toxic chemical fungicides that cause serious environmental issues. To develop the substances, Bioassay-based antifungal screening of approximately 2,400 *Streptomyces* strains led to the isolation of 149 strains as tentative antifungal producers. Among them, one *Streptomyces* strain was selected as a potential novel fungicide producer to protect various crops in the soil environment through an *in vitro* antifungal assay against various phytopathogenic fungi. Whole-genome sequencing of the *Streptomyces* strain and an anti-SMASH genome mining approach revealed an approximately 150-kb polyene biosynthetic gene cluster in the chromosome. The target compound responsible for the anti-phytopathogenic activity was a giant linear polyene compound highly homologous to the previously reported neotrafibiricin A (NTF A). These results suggest that a bioassay-based screening of a novel antifungal *Streptomyces* strain followed by its genome mining for target compound BGC characterization would be an efficient approach to isolating a novel candidate phytopathogenic fungicide that can protect crops in the soil environment.

Keywords : *Streptomyces*, phytopathogenic fungi, antifungal activity

A-23

Black Phosphorus-Ddecorated Nanoparticle as a Revertant of Polymyxin B Usage against Mcr-1-Mediated Resistant *Escherichia coli*

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Treatment of multidrug-resistant Gram-negative bacterial infections by polymyxins has been impeded by the occurrence of resistance by Mcr-1, mobilized colistin resistance. Here, we report the synthesis of a nickel (Ni) doped Zinc oxide (NZO) combined with black phosphorus (BP) (NZB) nanocomposite and its effect on the action of polymyxin B (PolB) against polymyxin-resistant *Escherichia coli* carrying *mcr-1* gene. Checkerboard assays showed that the combination of NZB and PolB showed a positive synergy against Mcr-1-expressing *E. coli* cells, resulting in a more feasible integration of PolB into *E. coli*. Further mechanistic studies found that the synergy is attributed by the charge neutralization of the *E. coli* cell surface by NZB. Finally, we determined the lowest synergistic concentration of NZB with PolB and the concentration was biocompatible to mammalian cell *in vitro*. Therefore, NZB is the first biocompatible nano-adjuvant to polymyxins against polymyxin-resistant *E. coli* cells, recognizing the physical status of bacteria instead of known adjuvants targeting cellular gene products. Therefore, NZB has the potential to repurposing polymyxins as leading last-resort antibiotics to combat polymyxin-resistant Gram-negative bacterial infections.

Keywords : Nano-adjuvant, mobilized colistin resistance (mcr-1), charge neutralization

A-24

Toxicological Effects of Polyvinyl chloride and Low Density Polyethylene Microplastics on Earthworm

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Despite of benefits derived from plastic use, accumulation of plastic in ecosystems, is becoming an increasing environmental concern. Research on microplastic toxicity has mainly focused on aquatic environments, while studies of that on terrestrial ecosystems are limited. The aim of this study was to examine the potential toxic effects of widely used Polyvinyl chloride(PVC) and Low density polyethylene(LDPE) on earthworm in oxidative stress. To investigate the potential effects of microplastic, level of ROS/RNS, amount of GSH, activity of SOD, and ATP synthesis were measured with different concentrations of microplastic exposure. Although activity of SOD was not changed, the amounts of ROS and amount of GSH were increased in both PVC and LDPE groups. However, ATP synthesis was decreased in the PVC treated group and increased in the LDPE treated group. For PVC, an increase in antioxidant defense led to the elimination of ROS, but failed for LDPE treated group. These results indicate that each microplastic have different oxidative stress mechanisms in the earthworm. These findings will provide implications for risk of microplastic in terrestrial ecosystems.

Keywords : Polyvinyl chloride, low density polyethylene, oxidative stress

A-25

Changes of Antibiotic Resistance from Influent and Effluent in Wastewater Treatment Plants of Han River, Seoul, South Korea

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Antibiotic resistance has emerged as critical health problem by overuse and misuse of antibiotics. Wastewater treatment plants (WWTPs) are considered as a sink and a source of antibiotic resistance. In this study, we applied culture dependent and independent method for investigation of antibiotic resistance in two WWTPs. The number of multidrug resistant (MDR) bacteria carrying antibiotic resistance genes (ARGs) decreased after treatment process of WWTPs, even though they were resistant to corresponding antibiotics. Of the MDR bacteria, Gammaproteobacteria class seemed dominant in both influent and effluent, and mainly carried ARGs, possibly suggesting they are main carrier of ARGs in WWTPs. In addition, SmartChip analysis showed that the number and the abundance of ARGs were lower in the effluent than influent. However, some ARGs persisted the treatment processes. ARGs in WWTPs seemed to be correlated with mobile genetic elements (MGEs), especially integrons and insertional sequences. Both culture dependent and independent methods indicated that ARGs were reduced after treatment process but significant concentration of ARGs still remained in effluents of WWTPs. In addition, even in the mitigation of ARGs of MDR bacteria, they were already evolved and equipped with intrinsic resistance through selective pressure by contaminants in WWTPs. This study suggests that WWTPs work for mitigation of antibiotic resistance, at the same time, act as a hotspot of evolution of antibiotic resistance.

Keywords : Wastewater treatment plants, antibiotic resistance

A-26

Characterization of a New Antimicrobial Agent against Bovine Mastitis-Causing *Staphylococcus aureus* RF122

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Bovine mastitis is one of the most prevalent and costly diseases in the dairy industry, and *Staphylococcus aureus* RF122 is the most prevalent pathogens causing intra-mammary infections in dairy cows. 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. In this study, we present a novel unconventional antibacterial agent that inhibits FeoB in vitro enzyme activity, bacterial growth, and virulence factor expression related to milk quality. The small molecule synergistically enhanced bacterial antibiotic susceptibility and 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 may represent a promising biotechnological application for preventing *S. aureus*-induced bovine mastitis in the milk and dairy industry.

Keywords : Bovine mastitis, ferrous iron transporter, *Staphylococcus aureus*

A-27

Study on High Antibacterial Activity Condition of Sodium Hypochlorite Solution at Low Concentration

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Sodium hypochlorite (NaClO) is a disinfectant widely used in hospitals and food industries, and has antibacterial activity against not only bacteria but also fungi and viruses. The antibacterial activity of NaClO is thought to depend on the concentration of hypochlorous acid (HClO) in the solution rather than hypochlorite ion (ClO-). The pH of the solution determines how much HClO is formed. Therefore, when we used NaClO, acid is used to lower the pH and increase the antibacterial activity. To maintain HClO solution in a stable form, obtaining maximize its antimicrobial activities, and minimize undesirable side products, the pH must be maintained between 3.5 and 5. HClO easily penetrates bacterial cell due to its neutrality and attacks. The results of the time kill test were obtained by exposure to 4 ppm for 1 minute showed 99.9% antibacterial activity. In addition, the correlation between the change in chlorine concentration and antibacterial activity according to the factor of temperature, humidity and the mixing of surfactant or chlorhexidine (CHX) was confirmed. Our results provide conditions of high antimicrobial activity while minimizing toxicity with a low NaClO concentration.

Keywords : Hypochlorous acid, sodium hypochlorite, time kill test

A-28

Properties of Antimicrobial Peptide Lactoferricin and Lactoferrampin: Expression and Purification from *E. coli*

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Short peptide lactoferricin (LFcin) and lactoferrampin (LFampin) which are derived from human lactoferrin are known as antibacterial, antifungal, and even anti-viral peptide. LFcin is found at N-terminal domain and LFampin is at C-terminal, respectively. In this study, we modified the original amino acid sequences of both peptides to more strong one by comparison of several sequences. The strong LFcin peptide, HLSA and mLFampin were chosen and synthesized and the antibacterial and antifungal activity were analyzed. Both peptides showed strong and broad specificities. On the other hand, we tried to express both peptides in *E. coli*. For the expression, Mxe intein was used as fusion partner to obtain the short peptide more easily by cutting with DTT. For the purification by affinity column, CBD(chitin-binding domain) or (His)6-tag was used. After cutting the expressed fused protein with DTT, both peptides were purified with the chitin-affinity column or Ni-NTA column. The purified peptides were identified by SDS-PAGE. The isolated peptides showed strong antibacterial activity against *Staphylococcus aureus* as indicator. Here we report the results.

Keywords : Lactoferricin, antimicrobial peptide, lactoferrampin

A-29

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

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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-30

Comparison of 5 Types of Oral Cleansers

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An oral cleanser is a medicine that rinses the inside of the mouth and is used to remove bacteria left in the mouth after brushing teeth. As the Corona 19 situation worsens recently, the risk of tooth decay due to oral breathing while wearing a mask, and demands for oral cleanliness products are increasing to solve problems such as bad breath caused by wearing a mask. As demand for oral cleansers has increased, there has also been an increase in interest in whether or not they are effective in reducing oral bacteria. Therefore, we will conduct a study to select five types of oral cleansers sold throughout the city based on any criteria and see how effective they are and how different they are. Among the criteria for specifying oral cleansers, the highest priority was "products to be used by many people", and we selected oral cleansers that had different effects to emphasize in order to smooth out the difference in effectiveness between products. In addition, he established criteria for variables in smooth experiment progress. Before entering this experiment, the best visibility LB Agar was selected and used through an experiment comparing BAP, MacConkey, and LB Agar in the candidate group. In this experiment, we tried to reach a conclusion by selecting only the values of the results of the same experiment, such as wounds in the mouth and medication for oral treatment, to adjust the oral environment as much as possible.

Keywords : Oral cleanser, LB Agar, bacteria in the oral cavity

A-31

Cross-Resistance to Different Classes of Antibiotics in *Salmonella* Typhimurium

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The sequential antibiotic treatment has recently gained attention in clinical medicine to control multidrug-resistant (MDR) bacteria. However, relatively few studies have been conducted on the effect of sequential antibiotic therapy on the evolution of cross-resistance. Therefore, in this study, we aimed to investigate the antimicrobial activity of sequential antibiotic treatments against MDR *Salmonella* Typhimurium in association with the development of antibiotic cross-resistance. One-half of the MIC of ceftriaxone (CEF [1/2]), ciprofloxacin (CIP [1/2]), polymyxin B (POL [1/2]), or tetracycline (TET [1/2]), followed by 1×MIC of CEF [1], CIP [1], POL [1], and TET [1] were used to evaluate antimicrobial activities against *S.* Typhimurium (ST^{WT}), ciprofloxacin-induced *S.* Typhimurium (ST^{CIP}), and clinically isolated *S.* Typhimurium (ST^{CLI}). The development of cross-resistance were assessed based on the viability changes and relative fitness. The expression of efflux pump-related genes were evaluated by using a quantitative RT-PCR assay. The serial exposures of ST^{WT}, ST^{CIP}, and ST^{CLI} to CIP [1/2]+CIP [1] and ST^{WT} to TET [1/2]+TET [1] showed the highest viability. The CIP [1/2]+POL [1] treatment showed an increase in fitness cost and a decrease in viability for all strains. The efflux pump-related genes were overexpressed in all strains exposed to serial antibiotics. Consequently, the sequence of serial antibiotic treatments affected the development of cross-resistance to antibiotics. This study provides useful information for designing effective antibiotic treatments against antibiotic-resistance bacteria.

Keywords : *Salmonella*, serial antibiotic treatments, multidrug resistant

A-32

Characteristics of Collaterally Susceptible and Resistant *Acinetobacter baumannii* Exposed to Antibiotics

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The emergence and spread of antibiotic-resistant *Acinetobacter baumannii* is a major public health concern. Bacteria can evolve collateral susceptibility and resistance to additional antibiotics. In this study, we aimed to evaluate the relative fitness, collateral susceptibility, and collateral resistance of wild-type *A. baumannii* KACC 12454 (AB^{KACC}), wild-type *A. baumannii* CCARM 12088 (AB^{CCARM}), polymyxin B-(PMB-) adapted AB^{KACC}, PMB-adapted AB^{CCARM}, stabilized AB^{KACC}, and stabilized AB^{CCARM} to ciprofloxacin (CIP), meropenem (MER), PMB, tetracycline (TET), and tobramycin (TOB). Compares to wild-type AB^{KACC}, the susceptibility of PMB-adapted AB^{KACC} was decreased to PMB (from 2 to 128 μg/ml) but increased to CIP (from 2 to 1 μg/ml), MER (from 16 to 1 μg ml^{-1}), TET (from 16 to 2 μg/ml), and TOB (from 64 to 16 μg/ml). The resistance of PMB-adapted AB^{CCARM} was increased to CIP and PMB when compared to AB^{CCARM}, showing MIC 32 and 64 μg/ml respectively. However, the stabilized AB^{KACC}, and stabilized AB^{CCARM} were lost their resistance activity to all antibiotics except CIP and TET treatments. The presence of β-lactamase and efflux pump inhibitors increased the susceptibilities of CIP, MER, PMB, TET, and TOB in all strains. Stabilized AB^{KACC}, stabilized AB^{CCARM}, and PMB-adapted AB^{CCARM}, showed higher levels of relative fitness than PMB-adapted AB^{KACC}. Therefore, the collateral susceptibility and collateral resistance of *A. baumannii* were vary depending on the antibiotic exposure.

Keywords : *Acinetobacter baumannii*, collateral sensitivity, collateral resistance

A-33

Interaction between Atibiotic-Sensitive and Antibiotic-Resistant *Salmonella* Typhimurium in Associated with Antibiotic Resistance during Antibiotic Exposure

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Bacteria communities are heterogeneous, including antibiotic-sensitive and antibiotic-resistant bacteria. The heterogeneous populations can involve in bacteria competitive and cooperative interactions, resulting in the development of antibiotic resistance. In this study, we aimed to evaluate the mutual interaction between antibiotic-sensitive and antibiotic-resistant *Salmonella* Typhimurium. The single and mixed culture of *S.* Typhimurium ATCC 19585 (ST^S) and clinically isolated antibiotic-resistant *S.* Typhimurium CCARM 8009 (ST^R) with 1×MIC ceftriaxone (CEF) were used to determine the viability, β-lactamase activity, and gene expression. The susceptibility of ST^R to CEF was decreased with increasing inoculum densities from 10^2 to 10^7 CFU/ml, showing more than 5-fold increase of MIC_{50}. Compared to single culture, the number of ST^S in mixed culture was increased up to 10^8 CFU/ml in the presence of CEF after 20 h of incubation at 37℃. Furthermore, the mixed culture showed the highest β-lactamase activity as 18 μmol/min/ml, consistent to the highest relative expression of β-lactamase-related genes (bla_{TEM}). Therefore, the β-lactamase produced from ST^R can protected ST^S from CEF, thus the β-lactamase production play an important role in inducing cooperative interaction between ST^S and ST^R in mixed culture. This study provides valuable information for understanding the mutual interaction within bacteria heterogeneous populations.

Keywords : *Salmonella*, β-lactamase, mutual interaction, heterogeneous populations.

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A-34

Synergism of Enrofloxacin and Sulfamethoxazole/Trimethoprim and Its Clinical Application in Pig Infections

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Antibiotic resistance to certain antimicrobials has been constantly increasing worldwide due to the indiscriminate use of drugs, which has been recognized as a global livestock problem as well as pig production. Given this background, new approach to develop the antimicriobials is necessary for clinical use. Several previous studies have shown that combinations of antibiotics with different modes of action can reduce the amount of antimicrobial agents to minimize antimicrobial resistance and side effects. Combination therapy is proposed as a major breakthrough in suppressing the development of resistant bacteria and exploring new mixtures in veterinary medicine. Enrofloxacin (ENFX) is currently effective in the bactericidal activity of various gram-negative and gram-positive bacteria in pigs in some countries, including Korea. Sulfamethoxazole (SFX) / Trimethoprim (TRM) mixture is widely used as an antimicrobial agent that inhibits microorganisms. Unfortunately, no previous studies have implemented the combination (ENFX-SFX/TRM) to achieve the effect of antimicrobial activity even if the optimal ratio of SFX/TRM has been proven to be 5:1 in pigs. In this study the combinatory effects of the ENFX-SFX/TRM combination on six bacterial species (*E. coli, Salmonella* spp., *P.multocida, A.pleuropneumonia, M.hyopneumoniae, B.bronchiseptica*) was designed whether the combination exhibited a synergistic effect, or not. Antimicrobial pharmacodynamics were performed for minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), fraction inhibitory concentration (FIC) and time kill assays. To confirm the safety of the mixture, a single oral toxicity was conducted. Also, it is important to establish a residual washout period. Therefore, we set the withdrawal period after clinical application of this formulation to healthy pigs. From the results of the antimicrobial pharmacokinetics, we were able to obtain the optimal combinational ENFX 25 + SFX/TRM 75 ratio. On the other hand, it was determined as a safe formulation with an LD50 of 5000 mg/kg or more in a single oral toxicity test. Taking the above results together, it was confirmed in this study that the ENFX+SFX/TRM combination product is safe and has excellent antibacterial effect. Therefore, the optimal dosage regimen and efficacy using an integrated pharmacokinetic-pharmacodynamic model in diseased pigs should be additionally demonstrated.

Keywords : Combination therapy, antibiotic resistance, enrofloxacin-Sulfamethoxazole/Trimethoprim

A-35

Bacteriocinogenic and Safety Properties of *Bacillus tequilensis* ST816CD Isolated from *Kimchi*

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Traditional fermented food products are considered as a trend in the search for healthier lifestyle. In the last decade, different bacterial species involved in the fermentation processes of food products were evaluated as probiotic candidates for human and other animal application. However, safety evaluations on strain basis need to be considered as an essential milestone on the recommendation for application of any new strain. This project aims to identify and characterize bacteriocin-producing strains for biocontrol of *Staphylococcus* spp., clinical and food-associated pathogens, and to evaluate their safety. *Bacillus tequilensis* ST816CD, identified via 16S rRNA sequencing, was isolated from artisanal *kimchi* from the Pohang region. Treatment of the cell-free supernatant of *B. tequilensis* ST816CD with proteolytic enzymes resulted in deactivation of the produced inhibitory metabolite/s against Staphylococcus species. However, B. tequilensis ST816CD most probably expresses more than one type of antimicrobial metabolite/s since this hypothesis was confirmed by PCR analysis indicating the presence of surfactin (*srfa*), subtilosin (*sbo*), and iturin (*ituc*) genes in the DNA of B. tequilensis ST816CD. The stability of the expressed antimicrobials was found to be not affected after exposed to different temperatures (4-100℃), pH (2-10), and chemicals (NaCl, Tween 80, SDS, and skim milk). Bacteriocin activity of *B. tequilensis* ST816CD against *S. simulans* KACC 13241 and *S. auricularis* KACC 13252 was at most 1600 AU/mL, which was tested during 24 hours after incubation at 37℃. ST816CD was found to produce gelatinase, γ-hemolysin, and biogenic amines. PCR-based analysis showed that ST816CD does not harbor potentially beneficial adhesion genes (*map*, *mub*, *eftu*, *ef1249*, *ef2380*, *ef2662*, and *prg*) and genes related to the production of folate (*folPE*, *folKQ*, *pabB,* and *pabC*) and antimicrobials (*bli*, *thu*, *coa*, *nis*, and *ped*) but has *gad* genes encoding glutamate decarboxylase (GAD) for GABA production. Molecular-based screening for the presence of virulence genes in ST816CD showed an incomplete operon for hemolysin (*hbl*ABC) and no evidence for enterotoxin gene (*nhe*ABC) and vancomycin-resistant genes (*van*ABCDEG). The presence of the strains with virulence activity and biogenic amines production in the traditional fermented food products needs to be regarded as undesired and potential health hazard to the consumers. Moreover, due to lack of standardization, preparation of the traditional fermented food products can be hidden and unexpected problems related to their safety and quality. Good manufacturing practices are an assurance for the food safety.

Keywords : *Bacillus tequilensis,* bacteriocin, food safety

A-36

Antibacterial Properties of *Bacillus subtilis* ST829CD against Emerging Pathogens *Staphylococcus simulans* and *Staphylococcus auricularis*

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Bacteriocin production is considered an advantageous property for various beneficial cultures. In addition to their potential as biopreservatives. *Bacillus* spp., widely distributed with varied roles in nature, has been associated with different fermented food products. Their ability to produce a wide variety of antimicrobial by-products and enzymes has paved their way to the spotlight as a promising biotechnological tool for various industrial applications. This study aimed to screen and characterize a bacteriocin-producing strain from traditional fermented food products and evaluate their safety and functional properties. A potential producer of antimicrobial metabolites isolated from Korean fermented food products identified as B. subtilis ST829CD through 16S rRNA sequencing. The produced inhibitory substance(s) was determined to be partially inactivated upon treatment of its CFS with proteolytic enzymes, indicating an array of possible active inhibitory metabolite/s. To further confirm the production of other antimicrobial compounds, molecular-based screening for genes coding for lichenicidin (bli), surfactin (srfa), iturin (ituc), and subtilosin (sbo) was carried out. The bacteriocin activities were determined against *S. simulans* and *S. auricularis* at 800 AU/mL and 400 AU/mL, respectively. The mechanism of action was demonstrated to be cell lytic. Phenotypic safety evaluation showed B. subtilis ST829CD to be gelatinase positive and a producer of biogenic amines. PCR detection of potential virulence factors confirmed the absence of genes coding for enterotoxin (nheABC) and hemolysin (hblABC) enzyme production. Furthermore, beneficial properties were screened genotypically showing that adhesion genes (map, mub, eftu, ef1249, ef2380, ef2662, prg), GABA-associated genes (gad), folate-coding genes (folPE, folKQ, pabB, pabC), and additional bacteriocin-coding genes (bli, thu, coa, nis, ped) were all absent. Bio-molecular approaches provide information on the possible production of diverse antimicrobial metabolites by the same bacterial strain and their possible role(s) in the stability, safety, and beneficial properties of traditional fermented food products. Additionally, traditional skills and technologies employed for the preparation of fermented foods are considered as an essential factor in their preservation and microbial biodiversity. Thus, such traditional fermented food products can be valuable sources of new strains that are producers of various bioactive metabolites and with potential biotechnological applications.

Keywords : *Bacillus subtilis*, *Staphylococcus* spp., antimicrobials

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Structure Elucidation and Bioactivities of Bacteria-Derived Compounds Separated from Unique Habitats

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Investigating the secondary metabolites produced by bacteria associated with unique habitats, such as insect or extreme environments has become a promising strategy for discovering novel bioactive compounds. Formicins A-C (**1-3**) were discovered from *Streptomyces* sp. SFA33, associated with wood ant (*Formica yessensis*). The structures of formicin A and formicin B were elucidated to be structurally unique indenone thioesters bearing an *N*-acetylcysteamine moiety based on 1D/2D NMR and UV spectroscopy with MS/MS analysis. The absolute configuration of formicin C was determined by applying the phenylglycine methyl ester (PGME) method followed by ^{1}H chemical-shift analysis. Formicin A inhibited the growth of human triple negative breast cancer (TNBC) cells. Two new secondary metabolites, svalbamides A (**4**) and B (**5**), were isolated from a culture extract of *Paenibacillus* sp. SVB7 that was isolated from deep sea sediment from Svalbard archipelago in the Arctic Ocean. The combinational analysis of HR-MS and NMR spectroscopic data revealed the structures of **4** and **5** as being lipopeptides bearing 3-amino-2-pyrrolidinone, D-valine, and 3-hydroxy-8-methyldecanoic acid. The absolute configurations of the amino acid residues in **4** and **5** were determined using the advanced Marfey's method with deducing the stereochemistry of 3-hydroxy-8-methyldecanoic acid based on quantum mechanics-based calculations. Svalbamides A and B induced quinone reductase activity in Hepa1c1c7 murine hepatoma cells.

Keywords : Unique habitat, wood ants, arctic ocean

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Airborne Virus Reduction Effect of Violeds® in Indoor Space

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After the COVID-19 pandemic, social concerns are rising about the increase in transmission infection caused by airborne viruses in indoor spaces. Ultraviolet rays are emerging as a factor that reduces viruses. However, there are no studies on how to reduce airborne viruses using ultraviolet ray in indoor space. In this study, we provided the virus reduction effect of Violeds®. The Violeds®, developed by Seoul Viosys, is ultraviolet LED technology that guaranteed safety by optimizing the wavelength, irradiation dose, time, angle, and distance of ultraviolet. We checked virus reduction rate of air circulation device that is applied fan and Violeds® by space area of indoor space, air flow of fan and irradiation dose of Violeds®. As a result, Violeds® reduced airborne virus by more than 90% in a space of 120m^3. As a result of confirming the reduction effect according to the air flow condition, we confirmed the reduction effect of 90% at the air flow of 36m^3/min or more when driving for 30 minutes. Under the condition of 60m^3/min air flow in a 120m^3 space, we confirmed that it was possible to reduce airborne viruses even in a short time by showing a reduction effect of more than 90% in 20 minutes of applying Violeds®. These results were similar to the experimental results in KTL (Korea Testing Laboratory). In KTL, the air circulation device applied Violeds® reduced airborne virus by 99% in 30 minutes in a 60m^3 space. These results show that Violeds® effectively reduces airborne viruses in indoor spaces, and furthermore, it is considered to be a sufficient guide for the use of Violeds® for airborne virus reduction purposes.

Keywords : Violeds®, Indoor space, airborne virus reduction