N-2 Genomic and Transcriptomic Landscapes of Multidrug Resistant
Pseudomonas aeruginosa
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Pseudomonas aeruginosa is an opportunistic pathogen that causes life-threatening infection in immunocompromised individuals. Patients infected with P. aeruginosa often develop serious pathological symptoms, such as pneumonia and sepsis. P. aeruginosa possesses intrinsic resistance to many antibiotics and proactively acquires resistance by genetic mutations during antibiotic stress. In this study, we sought to understand genomic and transcriptomic landscapes of P. aeruginosa clinical isolates that are highly resistant to multiple antibiotics. To achieve this goal, we are in the process of sequencing whole genomes of three multidrug resistant(MDR) P. aeruginosa strains, Y71, Y82, Y89 and one susceptible clinical isolate, Y31. Furthermore, RNA-seq analysis is to be performed to understand transcriptomic landscapes in the presence vs. absence of antibiotic stresses. Bacterial responses explored at the genomic and transcriptomic levels will help us understand key and conserved mechanisms leading to antibiotics resistance.

Keywords: Autophagy, ROS, AMPK

N-3 Sequence Variations in the Non-coding Sequence of CTX Phages in Vibrio cholerae
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Vibrio cholerae O1 7th cholera pandemic Wave 3 atypical El Tor strains contain a mosaic CTX phage that harbor classical type ctxB, and a potential molecular mechanism of generation of the mosaic CTX-3 – CTX-6 was demonstrated in a strain that contained two different CTX phages (a strain V212-1 that contained a CTX-1 on the chromosome 1 and a tandem repeat of CTX-2 on the chromosome 2). This study focused on the sequence variations in the non-coding sequences between ctxB and rstR of various CTX phages. The non-coding sequences of CTX-1 and CTX-ela are phage type-specific. The length of the non-coding region of CTX-1 and CTX-ela are 601 and 730 nucleotides, respectively. The non-coding sequences of CTX-2 and CTX-ela are homologous indicating the non-coding sequence of CTX-2 is derived from CTX-ela. The non-coding sequence of CTX phage could be divided into three regions. There is a phage type-specific Variable region between two homologous Common regions (Common region 1 and 2). The non-coding sequence of RSI element is similar to CTX-1 except that the common region 1 is replaced by a short RSI-specific sequence. The non-coding region of CTX-O139 is similar to CTX-ela and CTX-2, however it contains phage type-specific extra sequence between the Common region 2 and rstR.

Keywords: Vibrio cholerae

N-4 The Prevalence and Characteristics of Bacteria Causing Acute Diarrhea in Korea, 2012-2015
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This study was performed to determine the characteristics of the diarrheal causing pathogens according to season, isolated regions, patient’s age and sex and to provide useful data for the prevention of diarrheal disease. 70,406 stool specimens were collected from patients with diarrhea to identify the pathogenic bacteria from 2012 to 2015 in Korea. From the 70,406 stool specimens, 11,875 pathogenic bacteria were isolated and analyzed according to season, isolated regions, patients’ age and sex. The proportions of isolated pathogenic bacteria were Salmonella spp. 1,750 (14.7%), pathogenic E. coli 3,040 (25.6%), V. parahaemolyticus 87 (0.7%), Shigella spp. 59 (0.5%), Campylobacter spp. 716 (6.0%), C. perfringens 1,455 (12.3%), S. aureus 3,706 (31.2%), B. cereus 1,002 (8.4%), L. monocytogenes 12 (0.1%) and Y. enterocolitica 48 (0.4%). Isolation rate for most of pathogenic bacteria showed highest ratio in summer season, from June to August. Isolation rate of pathogenic bacteria by patients’ age showed highest ratio at 0 to 19 year for most of pathogenic bacteria. And Isolation rate by region, 59.6% isolated from cities and 40.4% isolated from rural provinces.

Keywords: Surveillance, Diarrhea-causing bacteria, Enter-Net

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Evidence-based Cosmeceutical Ingredients: Poly-γ-glutamic Acid and γ-Oligopeptide
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High-molecular-weight poly-γ-glutamic acid (γ-PGA) and γ-oligopeptide derive enhanced skin hydration through both extrinsic and intrinsic modes of hydrating action. The hydration provides both immediate and long-term moisturizing effects on the skin. Furthermore, γ-PGA not effectively only inhibits hyaluronidase activity with its very low concentrations but also help boost the level of endogenous hyaluronic acid. γ-PGA affects on the expression of epidermal matrix proteins, such as TG1 and TG3. It may contribute to enhanced structural integrity of the skin, indicates that γ-PGA can support strengthening of the skin against physical injuries. In addition, γ-PGA can normalize some of the undesirable immune responses in the skin by the reduction of inflammation and the increase of collagen. γ-PGA with evidence-based medicinal benefits, continue to revolutionize the world of hair, lip and skin care by offering safe and natural ingredients for consumer’s personal use. [This research was supported by the Ministry of Trade, Industry & Energy(MOTIE), Korea Institute for Advancement of Technology(KIAT) through the Encouragement Program for The Industries of Economic Cooperation Region]

Keywords: Cosmeceutical ingredients, Poly-γ-glutamic acid, γ-oligopeptide

N-7

A Novel Loop-Mediated Isothermal Amplification Assay for Serogroup Identification of Neisseria meningitidis in Cerebrospinal Fluid
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We have developed a novel Neisseria meningitidis serogroup-specific loop-mediated isothermal amplification (LAMP) assay for six of the most common meningococcal serogroups (A,B,C,W,X, and Y). The assay was evaluated using a set of 31 meningococcal LAMP assay positive cerebro spinal fluid (CSF) specimens from 1574 children with suspected meningitis identified in prospective surveillance between 1998 and 2002 in Vietnam, China, and Korea. Primer specificity was validated using 15 N.meningitidis strains (including serogroups A,B,C,E,W,X,Y, and Z) and 19 non-N.meningitidis species. The N.meningitidis serogroup LAMP detected down to ten copies and 100 colony-forming units per reaction. Twenty-nine CSF had N.meningitidis serogroup identified by LAMP compared with two CSF in which N.meningitidis serogroup was identified by culture and multi-locus sequence typing. This is the first report of a serogroup-specific identification assay for N.meningitidis using the LAMP method. Our results suggest that this assay will be a rapid, sensitive, and uniquely serogroup-specific assay with potential for application in clinical laboratories and public health surveillance systems.

Keywords: LAMP

N-8

Formation of Prion Protein Aggregates Using Recombinant Prion Proteins Prepared in a Large-scale
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The aims of this investigation are: 1) to generate a large-scale recombinant prion protein (PrP) used for the assay and 2) to establish the optimal conditions for PrP aggregate formation assay. For generation of four different types of recombinant PrPs, the human and mouse genes of full length and truncated PrPs were cloned in bacterial expression vectors. Each bacterial clone was mass-cultured with in situ PrP expression induction in the liquid fermenter. For isolation and purification of PrPs, three serial preparative chromatography steps (affinity, ion-exchange, and reverse chromatography) were established. Using optimized procedures, four types of recombinant PrPs with a high purity were prepared. By investigating the reaction buffer composition, the optimal conditions for aggregate formation of four different PrPs were established. The PrPs with N-terminal truncation were the more efficient substrates than the full length PrPs in generating aggregates. This suggests that the N-terminal region of PrP interferes with the efficient formation of PrP aggregates. The results of the current study will be applied for the diagnostic purpose in establishing the technical base of PrP aggregate formation assay.

Keywords: Prion protein (PrP), PrP aggregate
The Effect of CMA-3 Antimicrobial Peptide Action Mechanism from Substitute of Amino Acid on the CA-MA Hybrid Peptide
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CA-MA is a hybrid antimicrobial peptide (AMP) derived from two naturally occurring AMPs, cecropin A and magainin 2. CA-MA shows strong antimicrobial activity against Gram-negative and Gram-positive bacteria but also exhibit s cytotoxicity toward mammalian cells. Our objective was to identify CA-MA analogues with reduced cytotoxicity by systematic replacement of amino acids with positively charged R groups, aliphatic R groups, or p-ol R groups. CMA3 appeared to act by inducing pore formation (toroidal model) in the bacterial membrane. Additionally, no fluorescence was released from small or giant unilamellar vesicles exposed to 60 MCMA3 for 80 s, whereas fluorescence was released within 35 s upon exposure to CA-MA. Finally, in a mouse model of septic shock, CMA3 reduced the levels of proinflammatory factors, released within 35 s upon exposure to CA-MA. This study suggests that CMA3 is an antimicrobial/antiendotoxin peptide that could serve as the basis for the development of anti-inflammatory and/or antimicrobial agents with low cytotoxicity.

Antibacterial Activity of Curcumin via Apoptosis-Like Response in Escherichia coli
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Curcumin, a naturally occurring phenolic compound, has been shown to exhibit antimicrobial activity against Candida albicans, Escherichia coli, Pseudomonas aeruginosa, etc. but the mechanism still remains unclear. The present study was designed to investigate the novel antibacterial mechanism of curcumin that shows an apoptosis-like response in E. coli. We first found that curcumin induces membrane damage at relatively high concentrations, but there was no effect at the minimum inhibitory concentration (MIC). At the MIC, curcumin-treated cells displayed various apoptotic markers such as reactive oxygen species (ROS), accumulation, membrane depolarization, and Ca2+ influx. Expression of RecA protein that is related with a bacterial apoptosis-like response, was also increased by curcumin. In order to evaluate the influence of RecA on the appearance of other apoptotic markers, phosphatidylserine (PS) exposure and DNA fragmentation were examined and compared with a RecA deletion strain (ΔRecA). These markers were detected in E. coli wildtype cells, but not in ΔRecA cells. In conclusion, our data demonstrate that curcumin induces an apoptosis-like response in E. coli that involves RecA protein.

Keywords: Apoptosis-like response, Curcumin, RecA

Genetic Analysis of Cpe-carrying Clostridium perfringens Strains Isolated in Korea
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Clostridium perfringens is ubiquitous in the environment and intestinal tracts of most mammals, but this organism can cause gas gangrene and enteritis in human. Multiple Locus Sequence Typing (MLST) is a useful and powerful tool for molecular epidemiology. In this study, C. perfringens isolated from food poisoning and sporadic case were investigated to identify the genetic variations in this organism. MLST performed on 84 C. perfringens strains obtained from food poisoning isolates (34) and sporadic diarrhea isolates (50) in Korea. Also, toxin genes (cpa, cpe, cph, etx, and iap), and all variants of cph2 (the gene encoding for the beta 2 toxin) were analyzed by PCR. MLST analyses of the 84 isolates generated 19 different STs (sequence types) and among the 19 STs, 15 was newly found. MLST gene fragments varied in length from 739 to 554 bps, with average p distances of 0.006 (gene fragment) to 0.037 (SSD gene fragment). ST-41 (36 isolates) were dominant in this study. Food poisoning isolates group and sporadic diarrhea isolates group can be distinguished by Neighbor-joining tree constructed from STs. And among the PCR tested toxins, only cpa and cpe toxin detected. These results revealed genetic differences between the groups of food poisoning isolates and sporadic diarrhea isolates.

Keywords: Clostridium perfringens, Multiple Locus Sequence Typing (MLST)

Relevance of Plasmin Activity in Prion Disease and Control of Its Activity in Vitro
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The cellular prion protein (PrPc) undergoes conformational conversion to the infectious prion protein (PrPSc). When PrPc is cleaved by plasmin, PrPc in a proper cleavage state is depleted for PrPSc generation. We hypothesized that the plasmin activity is altered during the progress of prion disease. To test this hypothesis, we measured plasmin activity of the brain samples collected from RML prion-infected and control mice at the terminal stage of disease. Ten% brain homogenate samples did not show the difference in plasmin activity. However, the membrane fraction prepared from 10% brain homogenate significantly increased plasmin activity in the prion-infected group. Time course experiment demonstrated that plasmin activity in the membrane fraction gradually increased as disease progressed toward the terminal stage. Because the control of plasmin activity is considered as a potential target to interfere with the PrPSc formation, we investigated a way to increase the plasmin activity. We found that detergent molecules can enhance plasmin activity in vitro. Non-ionic detergents, such as NP-40, sodium deoxycholate (DOC), Triton X-100, and Tween-20 increased plasmin enzyme activities. However, an ionic detergent sodium dodecyl sulfate inhibited enzymatic activities.

Keywords: Prion, Detergents, Plasmin activity, Membrane fraction

Antibacterial Activity of Curcumin via Apoptosis-Like Response in Escherichia coli
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Curcumin, a naturally occurring phenolic compound, has been shown to exhibit antimicrobial activity against Candida albicans, Escherichia coli, Pseudomonas aeruginosa, etc. but the mechanism still remains unclear. The present study was designed to investigate the novel antibacterial mechanism of curcumin that shows an apoptosis-like response in E. coli. We first found that curcumin induces membrane damage at relatively high concentrations, but there was no effect at the minimum inhibitory concentration (MIC). At the MIC, curcumin-treated cells displayed various apoptotic markers such as reactive oxygen species (ROS) accumulation, membrane depolarization, and Ca2+ influx. Expression of RecA protein that is related with a bacterial apoptosis-like response, was also increased by curcumin. In order to evaluate the influence of RecA on the appearance of other apoptotic markers, phosphatidylserine (PS) exposure and DNA fragmentation were examined and compared with a RecA deletion strain (ΔRecA). These markers were detected in E. coli wildtype cells, but not in ΔRecA cells. In conclusion, our data demonstrate that curcumin induces an apoptosis-like response in E. coli that involves RecA protein.

Keywords: Apoptosis-like response, Curcumin, RecA
Designation of a Novel Antibacterial Nanoparticle for Preventing Pathogenic Bacteria

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Keywords: ROS, Drug-resistant bacteria, Nanoparticle

In this study, we report the translational potential of ferrocene-loaded PCAE micelles as novel antibacterial agents using culture models of E. coli and P. aeruginosa and a mouse model of drug resistant P. aeruginosa infection.

Keywords: ROS, Drug-resistant bacteria, Nanoparticle

Antimicrobial Effect of Prodigiosin from Serratia sp.

PDGS120915 Against Intestinal Pathogens

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Prodigiosin (4-methoxy-5-[[2Z]-[4-methyl-5-phenyl-2H-pyrral-2-ylidene)]methyl]-1H,1'H-2,2'-bipyrrrole) is a red pigment which has unique tripyrrrole chemical structure. Prodigiosin is a secondary metabolite of bacteria, such as Serratia marcescens, Vibrio psychroerythrus, and Hahella chejuensis. In this study, Serratia sp. PDGS120915 was isolated from slightly contaminated stream water. Isolated strain was identified and purified by HPLC method. Its antimicrobial activity against Listeria monocytogenes, Bacillus cereus, Pseudomonas aeruginosa, Salmonella typhimurium, and Vibrio parahaemolyticus showed with inhibition zone 44, 37, 30, 28, and 26 mm respectively. In addition, this pigment showing its antimicrobial activity potential against L. monocytogenes, P. aeruginosa, V. parahaemolyticus, B. cereus, and S. typhimurium with MIC at 0.03, 0.06, 0.06, 0.07, and 0.07 mg/mL respectively.

Keywords: Antimicrobial, Prodigiosin, Serratia

Structural Characteristics of Chemically Sulfated-Hyaluronic Acid from Streptococcus dysgalactiae

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Hyaluronic acid (HA) is a natural and linear polymer composed of repeating disaccharide units of β-1, 3-N-acetyl glucosamine and β-1, 4-glucuronic acid. HA was produced from a fed-batch fermentation process using Streptococcus dysgalactiae in a 5 l bioreactor. HA was isolated water-soluble form (HA-WS) and water-insoluble form (HA-WI) from culture medium, and was obtained chemically sulfated derivative (S-HA) that resulted in a 90% yield from HA-WI. The structural features of the sulfated-HA (S-HA) were investigated by FT-IR and 1H/13C-NMR spectroscopy. Results of FT-IR and 1H/13C-NMR indicated that sulfonfyl groups were inserted on the HA. The FT-IR and NMR patterns revealed the similarity in both the FT-IR spectrum as well as NMR spectrum of both reference standard and purified HA from S. dysgalactiae. Furthermore, the binding structure analysis using 1H/13C-NMR showed N-acetyl-glucosamine and glucuronic acid.

Keywords: Structural characteristics, Streptococcus dysgalactiae, Sulfated-hyaluronic acid
Phytol Exhibits the Antibacterial Property by Inducing Oxidative Stress Response in Pseudomonas aeruginosa
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Phytol, isolated from Aster yomena, is widely distributed as a constituent of chlorophyll. In the present study, we examined the antibacterial activity and mechanism of phytol inducing cell death by oxidative stress in Pseudomonas aeruginosa. It was first confirmed that phytol elevated intracellular reactive oxygen species (ROS) level. The observed transient NADH depletion in phytol-treated cells indicated that the increase of ROS is triggered via hyperactivation of electron transport chain. The generated ROS also caused oxidative stress through a disturbance in the balance between the phytol-induced ROS and antioxidant defenses. We identified oxidative stress was stimulated by ROS generation through the decrease of GSH level. The ROS-caused oxidative stress contributed to DNA damage leading to the bacterial cell death. However, the DNA damage was alleviated by pretreatment of antioxidant N-acetylcysteine (NAC), and it supports that phytol-triggered oxidative stress is related with the DNA damage. Furthermore, cell filamentation and membrane depolarization were also observed in the phytol-induced cell death responses. In conclusion, we suggest that phytol has the antibacterial activity through oxidative stress-mediated cell damage in P. aeruginosa.

Keywords: Phytol, Pseudomonas aeruginosa, Oxidative stress

Identification and Mechanism of Myxinidin and Its Analogs with Potent Antimicrobial Activity and Anti-biofilm against both Gram-positive and Gram-negative Bacteria
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Cationic antimicrobial peptides (AMPs) are essential components of the innate immune system, offering protection against invading pathogenic bacteria. In nature, AMPs serve as antibiotics with broad-spectrum antimicrobial and anti-biofilm properties. However, low observed stability in high-salt environments and physiological instability in biological membranes limit the naturally occurring AMPs as novel therapeutics. We therefore designed short synthetic cationic peptides by substituting key residues in myxinidin, an AMP derived from the epidermis of hagfish, with lysine, arginine, and tryptophan. These peptides showed high binding affinity for both lipopolysaccharides and lipoteichoic acids and inhibited biofilm formation by most bacteria, but did not cause significant lysis of human red blood cells and were not cytotoxic to normal human keratinocytes. Circular dichroism analysis revealed that myxinidin and its analogs assumed α-helical or β-sheet structures within artificial liposomes and bacterial membranes. Taken together, these findings suggest myxinidin analogs may be promising candidate antibiotic agents for therapeutic application against antibiotic-resistant bacteria.

Keywords: Antimicrobial peptide, Membrane permeation, Anti-biofilm

Antimicrobial Actions Designed Peptides in Pathogenic Bacteria
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To overcome a drug-resistant, we designed novel antimicrobial peptides with repeated sequences, composed of lysine, leucine, arginine, and tryptophan. Two, three and four repeated XYWX (X: cationic amino acids and Y; hydrophobic amino acid) motifs showed a significant antibacterial effects against drug-susceptible and drug-resistant pathogenic bacteria. All peptides exerted a membranolytic actions in bacterial membrane, consisted of PE/PG lipid bilayers. We also investigated anti-inflammatory activity of selected peptides in immune cell.

Keywords: Peptide, Bacteria, Drug-resistant

Antimicrobial Peptide with Antibacterial Activity and Anti-biofilm Activity against Antibiotics-resistant Bacteria
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Bacterial drug resistance is a major problem in human health as it has led to the reduced efficacy of conventional antibiotics. Thus the identification of novel antibiotics is essential. Antimicrobial peptides (AMP) are found in various living organisms, including plant, insects, amphibians and mammals where the play important roles in defense. AMP have been increasing interest as alternative to conventional antibiotics due to their broad spectrum antimicrobial activity and reduced possibility for the development of bacterial drug resistance. A novel antimicrobial peptide named MAC was isolated from the venom of the solitary bee. MAC exhibited antimicrobial activity and anti-biofilm activity against both Gram-positive, Gram-negative bacteria and multidrug resistance bacteria strains and did not cause significant lysis of human red blood cells and were not cytotoxic to HaCaT cell. The CD spectra of MAC measured in the presence of trifluoroethanol and sodium dodecyl sulfate showed a high content α-helices. Furthermore, results of NPN uptake assay and DSC3(5) assay indicated that peptide killed microbial cells by increasing membrane permeability and damaging membrane. Collectively, the results suggest that peptide have potential for use as novel antimicrobial agents.

Keywords: Antimicrobial peptide, Anti biofilm activity

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**Beta-glucan Encapsulated Silica Nanoparticles Delivering Anti-tuberculosis drug Molecules**
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Tuberculosis is one of the most infectious disease caused by mycobacterium tuberculosis. In result, great number of deaths is occurring in developing countries. Even though antibiotics have developed but the growth rate of infected people and bacterial resistance against antibiotics are not decreased yet. In this study, we developed and analyzed ultra-small INH encapsulated silica nanoparticles in size less than 5nm as well as conjugated them on beta-glucan. we developed a new approach of drug delivery system that anti-tuberculosis drug (Isonizid, INH) is encapsulated at silica nanoparticles which conjugated on beta-glucan(glu/INH/NSNPs).

Beta-glucan enhanced cellular immunity via being taken up by macrophage. We expected both INH encapsulated silica nanoparticles and activated macrophage stimulate anti-bacterial effect against tuberculosis on animal model. Thus activated immune system and nanoparticle based drug delivery system could amplify drug effectiveness.

**Clinical Evaluation of a Natural Polymer, Poly-γ-glutamic acid for Cervical Intraepithelial Neoplasia**

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Poly-γ-glutamic acid (γ-PGA) is known as a natural polypeptide consisting of only glutamic acid with gamma-amide linkages. Previously, accumulated evidences indicated that the γ-PGA induces innate immune response including NK cells activation and interferon secretion through TLR4. Currently, we have initiated a phase II trial to determine the efficacy and safety of γ-PGA compared with placebo in patients with cervical intraepithelial neoplasia grade 1 (CIN1). There is still possibility that CIN1 progressed to invasive cervical cancer. Therefore there are many need for a curative treatment for CIN1. Total 200 patients with CIN1 were administered with γ-PGA or placebo for 4 weeks followed by 8 weeks observation and compared the regression rate of CIN1. So far there was no serious adverse event (SAE) during phase II study. The primary efficacy analysis in the ITT groups and PP groups showed that each p-value between γ-PGA and placebo was 0.0247 and 0.0455, respectively. Taken together, the oral administration of γ-PGA can serve as treatment option that are safe and clinical efficacy via inducing NK cell activity and antiviral effect. [This study was supported by a grant of the National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea[0720510]].

Keywords: Poly gamma glutamic acid, CIN1, Clinical trial phase 2b

**Poly-L-arginine Inhibits PrPSc in Prion Infected Cell Lines**

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**Acute gastroenteritis is prevalent in a child less than 5 years old, and most important etiological agents are norovirus(NoV), group A rotavirus(RoV), enteric adenovirus(AdV), astrovirus(AsV) and sapovirus(SaV). In this study, we analyzed the laboratory surveillance of acute diarrhea induced by viral pathogens sporadically in Korea, 2013-2015. The surveillance investigation process consisted of a Korea National Research Institute of Health (KNIIH) protocol. Among the 17,890 specimens tested, 5,400 (30.2%) of samples were confirmed as positive of enteric virus. NoV was predominant in winter season (Nov-Feb) but RoV was peaked in spring season (Jan-May). NoV GII.4 was the most prevalent genotype and that occupied 59.8% of the NoV GII strains. The major genotype of AdV, AsV and SaV were type1, type1a and GI.1 respectively. NoV GII.4 was major cause of viral acute gastroenteritis in children. However, NoV GII.6 and GII.17 also main caused in winter season (January 2013, December 2014). Type5 of astrovirus was very rare serotype in the world but the prevalence was increased and outbreak also occurred in Korea, 2014. In this reason, the comprehensive and continuous surveillance is needed to identify the prevalence of different acute gastroenteritis pathogens.

Keywords: Acute gastroenteritis, Enteric virus, Laboratory surveillance
**N-25**

Biochemical and Endotoxic Characterization of NaOH-Induced *Vibrio parahaemolyticus* Bacterial Ghosts as a Potential Vaccine Candidate

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_Vibrio parahaemolyticus_ bacterial ghosts (VPGs) are non-living and empty bacterial cell envelopes that were generated by chemically induced lysis. The method is based on MIC (mg/ml) of sodium hydroxide; NaOH (3.125), acetic acid; CH₃COOH(6.25), boric acid; BH₃O₃(< 50.0), citric acid; C₆H₈O₇(25.0), maleic acid; C₄H₄O₄(6.25), hydrochloric acid; HCl (1.56) and sulfuric acid; H₂SO₄(0.781). Real-time PCR showed that only NaOH-induced VPGs were completely DNA-free. SDS-PAGE and agarose gel electrophoresis supported that cytolytic proteins and genomic DNA released from NaOH-induced VPGs to culture medium through the tunnel structure. The formation of the trans-membrane lysis tunnels was observed by SEM. Furthermore, they have low endotoxin content which was analyzed qualitatively by SDS-PAGE and quantitatively by limulus amoebocyte lysate assay. To further characterize the endotoxic potential, they were transfection into murine macrophage. Cytokine mRNA expression of TNF-α, IL-1β, IL-2, IL-6, IL-10 and IL-12 was determined by RT-PCR and real-time qPCR. [This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea(2015035949)].

Keywords: Bacterial Ghost, _Vibrio parahaemolyticus_, Vaccine candidate

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**N-26**

Effect of Antimicrobial Peptides on Oral Pathogens in both Planktonic and Polymicrobial Biofilm States with Low Cytotoxicity

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Dental caries and periodontitis are common bacterial mouth infections. Oral biofilms are multispecies microbial communities that exhibit high resistance to antibiotics. Antimicrobial peptides (AMP) are emerging as promising antimicrobial agents due to their rapid bactericidal activities. This study describes AMPs exhibits a broad spectrum of bactericidal activity with eradicating capability on oral pathogens and respective biofilm. It is also stable in saline solution, saliva. We further compared the antimicrobial activity kinetics of AMP with chlorhexidine. Flow cytometry was used to test if AMP could permeabilize the membrane of oral pathogens. A significant proportion of oral pathogens treated with antimicrobial peptide displayed propidium iodide (PI) fluorescent signal and the number of the bacteria cells with fluorescent signal increased with the dose dependent manner. Additionally, these did not cause lysis of human blood cell and were non cytotoxic to KB epithelial cell. This study provides a proof of concept in applying antimicrobial peptides in the clinical perspective.

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**N-27**

Microbial Mechanism of Antimicrobial Peptide Scolopendin from *Scolopendra subspinipes mutilans*

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Scolopendin, a novel antimicrobial peptide (AMP), was identified from the centipede _Scolopendra subspinipes mutilans_ by RNA sequencing. The molecular mechanisms underlying its antimicrobial activity was investigated. Our findings showed that scolopendin had antimicrobial activity against several pathogenic microorganisms, but did not induce hemolysis of human erythrocytes. Furthermore, disturbances in the cell membrane potential, induction of potassium release from the cytosol, and increased membrane permeability of the microbes _Candida albicans_ and _Escherichia coli_ O157 were detected by the use of 3,3'-dipropylthiacycyanine iodide [DSC(5)] dye, potassium leakage assay, and propidium iodide influx assay, respectively, following scolopendin treatment. Further evidence to support the membrane-targeted action of scolopendin was obtained using liposomes as artificial models of the cell membrane. Use of calcine and FITC-labeled dextran leakage assays from scolopendin-treated giant unilamellar vesicles and large unilamellar vesicles showed that scolopendin has a pore-forming action on microbial membrane, with an estimated pore radius of 2.3-3.3 nm. In conclusion, scolopendin has a membrane-targeted mechanism of action.

Keywords: Scolopendin, _Candida albicans_, Membrane disruption

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**N-28**

Comparison of Antibacterial and Anti-inflammatory Effects of Two Chlorin Derivatives

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Photodynamic therapy (PDT) uses a photosensitizer along with light source in the presence of O₃, which generates cytotoxic reactive oxygen species via excitation of photosensitizer. PDT has been applied for the treatment of wide range of neoplastic and non-neoplastic diseases through cell death and tissue destruction. Chlorin e6(Ce6) is a promising representative of the chlorin platform with many advantages including low toxicity, easy synthesis, fast and sufficiently selective accumulation in target tissue, and higher photosensitizing efficacy over porphyrins or photofrin. For these reasons, many chlorine derivatives have been prepared to utilize Ce6 alone and with different carriers for PDT. The effect of Ce6 and Tin-Ce6-mediated PDT in vitro in the presence of halogen light, focusing on the antibacterial and anti-inflammatory effect was investigated in this study. [This study was supported by the grant received from Korea Healthcare Technology R and D Project, Ministry of Health and Welfare, Republic of Korea. (Grant No.: HN12C0059).]

Keywords: Antibacterial effect, Anti-inflammatory effect, Chlorine derivatives
The Expression of C-C motif Ligand 5 (CCL5) Gene in the Brains of Senescence-Accelerated Mouse (SAM)

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In our previous study, we reported that accelerated aging observed in senescence-accelerated mouse prone 8 (SAMP8) would be due to the expression of endogenous avk marine leukemia virus (MuLV). Retrovirus infection in the central nervous system can induce neurological diseases in animals and lead to upregulation of chemokines. In the present study, in order to further understand the mechanism of neuropathological changes by avk MuLV found in SAMP8 mice, we examined the expression of C-C motif ligand 5 (CCL5) gene in the brains of SAMP8 mice compared with senescence-accelerated mouse resistant 1 (SAMR1), in which avk wasn’t expressed. By RT-PCR analysis, CCL5 mRNA levels in whole brain were significantly higher in 12 month-old SAMP8 mice than in 12-month-old SAMR1 mice. To compare the levels of CCL5 mRNA in SAMP8 mice, quantification of mRNA expression was performed using real-time PCR analysis. The expression of CCL5 mRNA in SAMP8 mice increased approximately a 4.72-fold compared to that of SAMR1 mice. Furthermore, CCL5 protein by western blot also increased markedly in SAMP8 mice compared with SAMR1 mice. Immunohistochemically, CCL5 protein was upregulated in active astrocytes of the hippocampal region of SAMP8 mice compared to the minimal amount of staining in the SAMR1 mice.

Keywords: CCL5, Aging, MuLV

Development of Novel Polyene Antifungal Antibiotics via Characterization of the NPP Post-PKS Enzymes

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Polyene macrolide group including nystatin A1 and amphotericin B is a key class of antifungal drugs to which resistance does not arise readily. This benefit, however, often occurs extreme dose-limiting toxicity. We previously identified that NPP (Nystatin-like Pseudonocardia Polyene) produced by Pseudonocardia autotrophica KCTC9441 exhibits remarkably higher aqueous solubility and significantly enhanced hemolytic toxicity because of harboring a unique additional sugar moiety, N-acetyl-glucosamine. For development of a clinically valuable antifungal agent, post-PKS modification steps of NPP biosynthesis were elucidated that aglycone was glycosylated by two separate glycosyltransferases (NppDi and NppY) in order, and hydroxylated by one cytochrome P450 hydroxylase (NplP). In addition, molecular dissections of NPP-specific biosynthetic enzymes (NppY and NplP) enable to deduce its characteristic and substrate specificity. We are expecting that these results contribute to set the stage for the biotechnological application for biosynthesis of novel polyene compounds.

Keywords: Antifungal antibiotics, Polyene macrolide, Post-PKS enzymes

Identification of a Novel Short PiAMP as Antibacterial and Anti-Inflammatory Agent against Human Pathogens

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Numerous antimicrobial peptides (AMPs) from a natural substance have been identified, isolated and characterized. We evaluated antimicrobial activities of PiAMP, a novel antimicrobial peptide, in vitro. The novel peptide shows strong antimicrobial activity against gram-negative and gram-positive bacteria but also exhibits no cytotoxicity toward mammalian cells. PiAMP appeared to act in the bacterial membrane. In cytotoxicity assays, PiAMP showed no cytotoxicity toward RAW264.7 and HaCaT cell. Additionally, binding of lipopolysaccharide to macrophages results in pro-inflammatory cytokine secretion. The peptide was investigated for their ability to inhibit LPS-mediated cytokine release from RAW264.7 to bind LPS in solution, and when LPS is already bound to macrophages. Finally, we conclude that PiAMP is an antimicrobial and anti-endotoxin peptide that could serve as the basis for the development of anti-inflammatory and/or antimicrobial agents with low cytotoxicity. This study suggests that PiAMP acts as host defense molecules that exert antimicrobial effects by targeting the LPS of gram-negative bacteria. Moreover, it has been reduced production of pro-inflammatory mediators and correspondingly reduced pulmonary disease such as cystic fibrosis.
Anti-prion function of inhibiting compounds of DNMTs
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Currently, various therapeutic chemical compounds for prion disease focus on inhibiting to change conformation structure of prion protein. Here, we found that SGI-1027 and SGI-1027 meta/meta analogue (M/M) known as inhibitors for DNA methyltransferase (DNMTs), eliminated PrPSc in prion infected cells. Unlike function of inhibitor for DNMT proteins, effect of SGI-1027 to eliminate PrPSc was more efficient than M/M. In mechanism study, both SGI-1027 and M/M directly interacted with recombinant human truncated PrPC (hPrPC) and oligomeric fibril formation from PrPC was inhibited by presence of both compounds. However, PrP gene expression was not repressed under SGI-1027 or M/M. Thus, direct interaction of both compounds with PrPC resulted in elimination of PrPSc in prion infected cells via increasing stability of PrPC structure but not epigenetic control of both compounds such as reduction of PrP gene expression. Also, inhibitory function of SGI-1027 for formation of PrPSc was feasible for protection of cell from prion infection by RML derived pathogenic prion. Along our results, the novel anti-prion function of SGI-1027 and M/M via direct interaction with PrPc can be considered as therapeutic reagents for prion disease.

Keywords: Prion, Interaction, Therapeutic compounds, Inhibition, Epigenetic regulation

A Novel Antifungal Mechanism of Food Preservative Propionic Acid
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Propionic acid (PPA) has been used as food preservative because it revealed the inhibitory effect of microorganisms. We investigated the novel antifungal mechanism of PPA, showing apoptotic features. Reactive oxygen species (ROS) generation and metacaspase activation were observed following exposure of PPA. These results indicated that PPA caused an oxidative stress through ROS generation and activated metacaspase, which can promote apoptosis signaling. Then, phosphatidylserine externalization (an early apoptosis marker) and DNA and nuclear fragmentation (late apoptosis markers) were also examined. Consequently, PPA exerts the antifungal effect via apoptotic cell death. Moreover, the results of three additional mitochondrial experiments: mitochondrial membrane depolarization, calcium accumulation, and cytochrome c release demonstrated that PPA-induced apoptosis pathway is mediated by mitochondria. Thus, PPA induces fungal cell death through mitochondria-mediated apoptosis.

Keywords: Food preservative, Propionic acid, Fungal cell death

Anti-inflammatory Activities of Chemically Sulfated-Hyaluronic Acid from Streptococcus dysgalactiae
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Hyaluronic acid (HA) is an important macromolecule in medical and pharmaceutical fields. HA was produced from a fed-batch fermentation process using Streptococcus dysgalactia in a 5 l bioreactor. HA was isolated water-soluble form (HA-HS) and water-insoluble form (HA-WI) from culture medium, and was obtained chemically sulfated-derivative (S-HA) that resulted in a 90% yield from HA-WI. This work aimed to confirm the anti-inflammatory activities of HA and chemically sulfated-HA. The anti-inflammatory activities of HA and S-HA were examined on LPS-induced RAW 264.7 cells. S-HA was significantly inhibited production of pro-inflammatory mediators such as nitric oxide (NO) and PGE2, and the gene levels of iNOS and COX-2, which are responsible for the production of NO and PGE2, respectively. Furthermore, S-HA also suppressed the overproduction of pro-inflammatory cytokine TNF-α (~80 pg/ml) and IL-6 (~100 pg/ml) compared to that of HA-WI. The present study clearly demonstrates that HA-S exhibits anti-inflammatory activities in LPS-stimulated RAW 264.7 macrophage cells.

Keywords: Anti-inflammatory activity, Streptococcus dysgalactiae, Sulfated-hyaluronic acid

Inhibitory Effect of Aptamer on Streptococcus mutans Biofilm
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Dental biofilms are produced by bacterial communities with large biodiversity. The Streptococcus mutans bacterial species plays a leading role in the initiation of dental caries. Here we developed multifunctional aptamer for growth inhibition and detection of Streptococcus mutans. We collected DNA aptamer that bind to S. mutans and developed the aptamer-based detection assay for specific detection of the cell. Moreover, several of aptamers inhibit cell growth of S. mutans more than control. This assay with high specificity can be used as an alternative method for the therapy and detection of oral disease. This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project title: Risk Assessment Research and Development of Rapid Diagnostic Method for Biological, Chemical and Environmental Animal Disease, Project No: PJ01052301)” Rural Development Administration, Republic of Korea. This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea(NRF-2015H1C1A1035921).

Keywords: DNA aptamer, Streptococcus mutans, Oral disease
The Orphan Nuclear Receptor Small Heterodimer Partner is Essential for the Regulation of Danger Signal-mediated Inflammatory Responses in Acute Colitis

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Small heterodimer partner (SHP, NR0B2) is an orphan member of the nuclear receptor superfamily which has been known as a corepressor in various metabolic and endocrine conditions. However, its function in the regulation of immune responses during enterocolitis has been largely uncharacterized. We previously showed that SHP is essentially required for regulation of NLRP3 inflammasome activation in macrophages. In this study, we investigated the function of SHP in acute gastrointestinal inflammation model using dextran sulfate sodium (DSS). When colon homogenates and peritoneal macrophages were collected from SHP+/- and SHP+- mice provoked in acute colitis, the DSS-induced levels of interleukin (IL)-1β and IL-18 were significantly increased in SHP-/- mice than SHP+/- mice. The survival rate of 3% DSS-treated SHP-/- mice was markedly decreased, when compared with that of SHP+/- mice. Moreover, in SHP-/- mice, the body weight was decreased, whereas the DAI score were significantly increased, than that of the SHP+/- mice 4 days after DSS treatment. These results uncover a novel role by which SHP regulates inflammatory responses during enterocolitis through inhibiting excessive inflammatory responses.

Keywords: SHP, NLRP3, DSS

Influences of Cell Surface Hydrophobicity on Adhesion and Biofilm Formation in Several Bacteria and Candida albicans.

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Hydrophobic properties are believed to associate with microbial adherence on biotic and abiotic surfaces. To investigate the correlation of cell surface hydrophobicity (CSH) to adhesion, adherence to HeLa cell and biofilm formation on polystyrene were assayed with clinical isolates of cell surface hydrophobicity (CSH) to adhesion, adherence to HeLa cell and biofilm formation on polystyrene. The CSH of the bacteria were tested against Acinetobacter baumannii. Among the 31 clinical isolates of Acinetobacter baumannii, the positive correlation was observed between CSH and cell adhesion (r=0.681) or biofilm formation (r=0.941). In C. albicans, CSH may play a important role of adhesion and biofilm formation.

Keywords: Cell surface hydrophobicity, Adherence, Biofilm formation

Antimicrobial peptides are important molecules of the host defense system against invading pathogen. Antimicrobial peptides are small molecules containing amino acids with positive charge, which exhibit antimicrobial activity against Gram negative bacteria, Gram positive bacteria, yeasts and fungi and viruses. In this study, antimicrobial peptide SH was identified from the scorpion, which is an amphiphatic α-helical structure and antimicrobial activity. Based on this patent peptide, we designed analogue peptide SH1 by truncation and substitution. The antimicrobial activity, anti-biofilm activities of the five antimicrobial peptides were tested against Acinetobacter baumannii. Moreover, these peptides were found to be resistant to proteolytic degradation, but did not completely exhibit hemolytic activity except for parental peptide. Antimicrobial peptides showed α-helical or β-sheet structures within bacterial membranes by Circular dichroism analysis. Furthermore, we carried out bacterial killing and membrane penetration experiments whether designed peptides penetrated membrane of bacteria. Collectively, our finding indicated its analogs may be attractive candidate antibiotic agents.

Keywords: Antimicrobial Peptide, Acinetobacter baumannii


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The emergence of multidrug-resistant Acinetobacter baumannii is recently becoming increasingly important as nosocomial infection and has affected people with compromised immune system. Thus, effective novel antimicrobial agents are urgently required. Cationic antimicrobial peptides are thought to play an important self-defense role in many organisms. Because of their ability to disrupt the bacteria membrane, leading to cytoplasmic disruption and cell death, so AMPs provide an alternative to existing antibiotics. We therefore designed cationic antimicrobial peptides by substituting residues in truncated parental peptide. The antimicrobial activities, anti-biofilm activities of the five antimicrobial peptides were tested against Acinetobacter baumannii. Moreover, these peptides were found to be resistant to proteolytic degradation, but did not completely exhibit hemolytic activity except for parental peptide. Antimicrobial peptides appeared α-helical or β-sheet structures within bacterial membranes by Circular dichroism analysis. Furthermore, we carried out bacterial killing and membrane penetration experiments whether designed peptides penetrated membrane of bacteria. Collectively, our finding indicated its analogs may be attractive candidate antibiotic agents.

Keywords: Antimicrobial Peptide, Acinetobacter baumannii

Antimicrobial Activity and Action Mechanism of against Pseudomonas aeruginosa Strains

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Antimicrobial peptides are important molecules of the host defense system against invading pathogen. Antimicrobial peptides are small molecules containing amino acids with positive charge, which exhibit antimicrobial activity against Gram negative bacteria, Gram positive bacteria, yeasts and fungi and viruses. In this study, antimicrobial peptide SH was identified from the scorpion, which is an amphiphatic α-helical structure and antimicrobial activity. Based on this patent peptide, we designed analogue peptide SH1 by truncation and substitution. The SH1 have strong antimicrobial activity against Pseudomonas aeruginosa. The secondary structure of these peptide were mainly composed by α-helical as determined by CD measurement. SH1 exhibits the biofilm inhibitory activity. These peptide a little hemolytic activity on red blood cell and low cytotoxicity of HaCaT cell. Furthermore, the gel retardation experiment showed that analogue peptide SH1 bound to DNA strongly than SH. Taken together, this study indicate that the SH and analogue peptide SH1 may have antimicrobial activity and effective therapeutic agent against multidrug-resistant Pseudomonas aeruginosa.
Development of HPV Therapeutic Vaccine using Lactobacilli-Displaying Human Papillomavirus Type16 E7

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Immunotherapy is one of the biggest early-stage trends in the world. We have developed the Lactobacillus surface display system using PgsA protein as anchoring motif for the immunotherapy. HPV is mainly transmitted through sexual contact and most people are infected with HPV shortly after the onset of sexual activity. Cervical cancer is caused by sexually acquired infection with certain types of HPV. Two HPV types (16, 18) cause 70% of cervical cancers and precancerous cervical lesions. We expressed the HPV type 16 E7 antigen on the surface of Lactobacillus casei by employing a display system in which the poly-γ-glutamic acid synthetase A as an anchoring motif. Previously, we confirmed that the oral administration of L. casei-E7 induced humoral and cell mediated immunity as well as antitumor effects in mice. We also confirmed the safety of vaccine through preclinical studies for clinical trials. According to the phase 1 study in Korea, no adverse events due to the vaccine was confirmed. Collectively, our results indicate that L. casei-E7 may be more effective and safe as novel therapeutic vaccine against high grade squamous intraepithelial lesion (HSIL).

Keywords: HPV, Lactobacillus casei, Surface display system

Multilocus Sequence Typing (MLST) of Vibrio parahaemolyticus Strains Isolated in Korea.

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Vibrio parahaemolyticus is a leading cause of food-borne outbreaks and acute gastroenteritis worldwide. In this study, 104 clinical isolates were used to examine the genetic relationship and diversity in Korea during 3 years (2013-2015). Multilocus sequence typing (MLST) method was used to study prevalence and population structure of clinical isolated strains. The 104 tested isolates fell into 11 sequence types (STs), of which two STs were new to the MLST database. The prevalence of STs was ST-3(36.5%), ST-332(26.0%), ST-8(14.4%), ST-50(6.7%), ST-610(1.9%) and the rest of the STs were represented by a single strain. eBURST algorithm analysis showed that tested strains were not so much related to each other, only 66 STs out of V. parahaemolyticus MLST database were formed 5 clonal complexes(CCs), 3 groups, and 3 singletons with our 11 STs. Also, phylogenetic tree analysis showed similar results with eBURST analysis. Only ST-3, the most prevalent ST in Korea is also showed international distributions. The clinical strains from Korea were not clustered by region distributions. From these, distribution of V. parahaemolyticus of Korea seem to not so much different to other countries, suggesting genetic diversity.

Keywords: Vibrio parahaemolyticus, MLST

Generation and Characterization of Chemically-induced Listeria monocytogenes Bacterial Ghosts (LMGs) as a Potential Vaccine Candidate

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Listeria monocytogenes bacterial ghosts (LMGs) are non-living and empty bacterial cell envelopes that were generated by chemically induced lysis. The method is based on MIC (mg/ml) of sodium hydroxide; NaOH (6.25), acetic acid; CH3COOH (6.26), boracic acid; BH3O3 (< 50), citric acid; C6H8O7 (25), maleic acid; C4H4O4 (12.5), hydrochloric acid; HCl (6.25) and sulphuric acid; H2SO4 (12.5). Among those, the lysis efficiency of L. monocytogenes cells reached 100% at 5, 10, 15 and 30 min after treatment of H2SO4, NaOH, HCl and C4H4O4, respectively. The formation of the trans-membrane lysis tunnels in respective chemicals induced LMGs which was observed by SEM.

Keywords: Listeria monocytogenes, Bacterial ghosts, Vaccine candidate

Vibrio vulnificus is gram-negative, motile, nonspore-forming opportunistic pathogen that causes food-borne illness associated with the consumption of contaminated seafood. In order to characterize overall genetic properties and virulence factors of V. vulnificus, V. vulnificus FORC_016 was completely sequenced. The genomic analysis of FORC_016 revealed that the genome consists of two circular DNA chromosomes, and contains 4,461 predicted ORFs, 129 tRNAs, and 34 rRNA genes. V. vulnificus FORC_016 has major virulence genes such as RTX, cytolsin, and metalloproteases. To identify differentially expressed genes of V. vulnificus exposed to crab, the transcriptomic profiles of V. vulnificus FORC_016 exposed or unexposed to crab for 1 or 4 h were analyzed using a RNA-sequencing. 1,327 and 791 genes were identified as differentially expressed when exposed to crab for 1 h or 4 h, respectively (P < 0.05, 2-fold). The genes related to energy production, cell growth, oligopeptide transport, and glucose metabolism were up-regulated, while genes associated with amino acid biosynthesis, nitrogen metabolism were down-regulated. This report provides an extended understanding on V. vulnificus and would be helpful for rapid detection, and prevention of food-borne outbreak in South Korea.

Keywords: Vibrio vulnificus, Genomics, Transcriptomics

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