J_Infection and Immunity

J-1

The Inhibitory Effect of Behenic Acid on Biofilm Formation of *Staphylococcus aureus*

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*Staphylococcus aure*us is opportunistic pathogen which presents on skin, nose, and respiratory of human. *S. aureus* in our body forms a biofilm composed of proteins and polysaccharide on the biotic or abiotic surface to survive in environmental stress. The cells in the biofilm are 50 to 500 times more resistant to antimicrobial agents than planktonic cells. Therefore, the biofilm formation is a reason for chronic infections. *S. aureus* is the main pathogenic bacteria of infection in diabetic foot ulcers, which 15-25% of diabetic patients suffer from, and the higher the blood sugar level, the more severe the symptoms of infection. In this study, we evaluated the effect of behenic acid on biofilm formation of *S. aureus* in range of normal blood sugar level (0.1%) and diabetes level (0.2% ~ 0.6%). The biofilm formation was quantitatively analyzed by crystal violet assay. Behenic acid inhibited the biofilm formation by a concentration-dependent manner at all tested glucose concentration. Especially, behenic acid showed the higher biofilm inhibition with 0.1% glucose than with 0.2 ~ 0.6% glucose. We propose that behenic acid is an inhibitor against the biofilm formation of *S. aureus* in the human body.

Keywords : *Staphylococcus aureus* biofilm, behenic acid, diabetic foot ulcer

J-2

Optimal Method for Identification of *Staphylococcus* spp.

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Staphylococcus is a genus of Gram-positive facultative anaerobic organisms and under the microscope, they appear round, and form in grape-like clusters. The staphylococcus infection can cause a wide variety of diseases in humans and animals through either toxin production or penetration. These bacteria commonly inhabit the skin and nose where they are innocuous, but may enter the body through cuts or abrasions which may be nearly invisible. And, *Staphylococcus* spp. is zoonosis, which is easily infected from humans to animals. Animal experiments are essential to biological and medical research. High quality laboratory animal is most important in the experiment for get reliability and reproducibility data. However, most of the infections in laboratory animals are closely related to the hygiene of breeders and/or researcher. However, it is not easy to identify bacterial of similar colony morphology and morphology diagnosis is subjective. In this study, we comparing conventional diagnosis methods for objectively identification of *Staphylococcus* spp. Our PCR assay will be used to improve quality control in laboratory animals and laboratory animal facilities.

Keywords : Staphylococci, PCR assay, quality control

J-3

Effects of *Pediococcus pentosaceus* Strains Isolated from Three Different Types of Kimchi in ICR Mice Infected with *Escherichia coli* or *Salmonella* Typhimurium

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One hundred twenty ICR male mouse with initial body weight of 26±2 g(5 weeks old) were randomly assigned to six treatments for 2 week feeding trial to determine the effect of *Pediococcus pentosaceus* strains which are isolated from three different types of Kimchi in ICR mice infected with *Escherichia coli* or *Salmonella* Typhimurium. Six groups were Normal control group without *E. coli* and *S.* Typhimurium orally administrated (NC-; n = 20), Normal control group with *E. coli* or *S.* Typhimurium orally administrated (NC+; n = 20), *L. plantarum* orally administrated group after *E. coli* or *S.* Typhimurium orally administrated (LP; n = 20), *P. pentosaceus* strain A orally administrated group after *E. coli* or *S.* Typhimurium orally administrated (PSA; n = 20), *P. pentosaceus* strain B orally administrated group after *E. coli* or *S.* Typhimurium orally administrated (PSB; n = 20), *P. pentosaceus* Strain C orally administrated group after *E. coli* or *S.* Typhimurium orally administrated (PSC; n = 20) on each *E. coli* infected groups and *S.* Typhimurium infected groups. LP and PSC had significantly($p < 0.05$) improved in growth performance compared with other groups except for NC- in *E.coli* infected mice group. NC+ were significantly lower ($p < 0.05$) growth performance compared with other groups except for NC- in S.Typhimurium infected mice groups. In *E.coli* and *Salmonella* count in intestine, LP and PSC groups had significantly lower($p < 0.05$) counts than NC+, and PSB groups. In conclusion, LP and PSC strains are isolated from kimchi can act as probiotics by inhibiting *E. coli* and *S.* Typhimurium.

Keywords : Kimchi, probiotic, intestinal microorganisms

J-4

Efficacy of Novel Oil Adjuvant CAvant®SOE for Foot and Mouth Disease Vaccine

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Foot and Mouth Disease (FMD) is a notifiable contagious disease of cloven-hoofed mammals. A high potency vaccine that stimulates the host immune response is the foremost strategy used to prevent disease persistence in endemic regions. FMD vaccines comprise inactivated virus antigens whose immunogenicity is potentiated by immunogenic adjuvants. Oil-based adjuvants have clear advantages over traditional adjuvant vaccines; however, there is potential to develop novel adjuvants to increase the potency of FMD vaccines. Thus, we aimed to evaluate the efficacy of a novel water-in-oil emulsion, called CAvant®SOE, as a novel vaccine adjuvant for use with inactivated FMD vaccines. We found that inactivated A22 Iraq virus plus CAvant®SOE induced better antigen-specific humoral and cell-mediated immune responses in mice than a commercial vaccine. Moreover, A22 Iraq-CAvant®SOE induced slightly higher production of neutralizing antibodies than the commercial vaccine. Intramuscular immunization of pigs using A22 Iraq-CAvant®SOE also led to higher and longer-lasting virus-neutralizing antibody titers than immunization with the reference adjuvant. A single dose of A22 Iraq-CAvant®SOE elicited effective control of virus shedding, with no detectable clinical symptoms; it also protected against challenge with heterologous FMDV. Levels of protection correlated strongly with vaccine-induced neutralizing antibody titers. Collectively, these results indicate that CAvant®SOE-adjuvanted vaccine is a promising candidate for control of FMD in pigs.

Keywords : Foot and mouth disease virus, adjuvant, CAvant®SOE

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J-5

Toll-like Receptor 7 Agonist GS-9620 as an Effective Adjuvant in Influenza Vaccines

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GS-9620 is a synthetic dihydropteridinone derivative that was identified as a TLR7 specific small-molecule agonist. GS-9620 has previously been shown to suppress hepatitis B virus (HBV) in various animal models. Though published knowledge of GS-9620 fulfils the criteria as a potent immune-stimulant, there is no documented usage of immune-stimulant for the vaccine adjuvant currently. In this study, we evaluate the mode of action and efficacy of GS-9620 as an immune-stimulant for the vaccine adjuvant. In vitro, GS-9620 showed the induction of TLR7-mediated robust cytokine production and immune response gene expression in human and murine immune cells. In vivo, the effect of GS-9620 as an immune-stimulant was examined with influenza recombinant protein sM2HA2 and inactivated A/Puerto Rico/8/34 virus (iPR8). Consequently, intramuscular administration of sM2HA2 and intranasal administration of iPR8 with GS-9620 induced significantly higher antigen-specific humoral and cell-mediated immune responses than well-known TLR7 agonist Imiquimod. Further, it enhanced the lung virus clearance and protective efficacy of the sM2HA2 subunit antigen and iPR8 vaccine against the lethal challenge of divergent influenza subtypes (H5N2, H9N2, H1N1 and PR8) in a murine model. Our results collectively suggest that GS-9620 can provide an effective mucosal and systemic immune-stimulant for adjuvant of both subunit and inactivated whole virus vaccine.

Keywords : Toll-like receptor 7, GS-9620, adjuvant

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J-6

CAvant WO-60 as an Effective Immunological Adjuvant for Avian Influenza and Newcastle Disease Vaccine

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Despite current vaccines used in poultry being immunogenic, the number of pathogens including avian influenza virus (AIV) and Newcastle disease virus (NDV) causes enormous economic losses to the global poultry industry. To solve this problem, vaccine efficacy can be further improved with the introduction of effective adjuvants. Here, we developed a new adjuvant, CAvant® WO-60, that safely and effectively enhances both the immunogenicity of conserved influenza antigen sM2HA2 and inactivated whole H9N2 antigen (iH9N2): it induced both humoral and cell-mediated immunity in mice and provided 100% protection from challenge with 10 LD50 of A/Aquatic bird/Korea/W81/2005(H5N2) and A/Chicken/Korea/116/2004 (H9N2) AIV. Importantly, immunization of chickens with CAvant® WO-60 emulsified iH9N2+inactivated NDV LaSota (iNDV) bivalent inactivated vaccine induced seroprotective levels of antigen-specific antibody responses. Thus, the new adjuvant CAvant® WO-60 would be a promising adjuvant for poultry vaccines.

Keywords : CAvant® WO-60, adjuvant, Water-in-Oil

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J-7

Efficacy of Recombinant Subunit Vaccine Candidate against Foot-and-Mouth Disease Virus Serotype O and A in Pig

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Foot-and-mouth disease virus (FMDV) causes an acute, severe and highly contagious disease of cloven-hoofed animals whose control relies on efficient vaccination. Although currently, available inactivated virus vaccines have proved to be effective in FMD control, concerns regarding their safety have been raised during the vaccine formulation process. Therefore, it is necessary to develop safe and effective alternative vaccine to replace the traditional inactivated vaccine. In this study, we developed two multi-epitope recombinant protein OVM and AVM, OVM antigen comprised of tandem repeats of antigenic site of two South Korea isolates O/Andong/SKR/2010 and O/Jincheon/SKR/2014 with classical vaccine strain O1/Manisa/Turkey/69, AVM antigen comprised of tandem repeats of antigenic site of South Korea isolates A/Pocheon/KOR/2010 with two classical vaccine strain A22/Iraq/24/64 and A/Malaysia/97 and evaluated their efficacy with immunogenic adjuvant ISA201. In the mouse model, OVM-AVM vaccine candidate induced effective antigen-specific humoral and cell-mediated immune responses and effectively protected from mouse-adapted O/Jincheon/SKR/2014, O/VET/2013 and A/Malaysia/97 virus challenge. Moreover, intramuscular immunization of pigs with OVM-AVM vaccine candidate effectively protected from O/Jincheon/SKR/2014 and A/SKR/4/2018 challenge. Together, our results suggested that the developed OVM-AVM vaccine candidate provides opportunities for safer and effective vaccine production that may replace the traditional inactivated vaccine for the prevention and control of FMD in pigs in the future.

Keywords : FMDV, OVM-AVM, multi-epitope recombinant protein

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J-8

E3 Ubiquitin Ligase RNF 172 Negatively Regulates Innate Immune Signal by Targeting Nuclear Factor-κB Essential Modulator

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Nuclear Factor-κB Essential Modulator (NEMO) is a key regulatory protein that functions during NF-κB and Interferon-mediated signaling in response to extracellular stimuli as well as pathogen infection. Smooth regulation of NEMO signaling is essential for the host innate immune responses and maintenance of homeostasis. In this study, we report that the E3 ligase RNF 172 (also known as membrane associated RING-CH2 or MARCH2) is a novel negative regulator of NEMO-mediated signaling upon bacterial or viral infection. RNF 172 directly interacted with NEMO during the late time of infection and catalyzed the K-48-linked ubiquitination of Lys326 on NEMO, which then resulted in its proteasomal degradation. Ultimately, CRISPR/Cas9 mediated deletion of RNF 172 exhibited a marked resistance along with increased innate immune responses against viral or bacterial infections both *in vitro* and *in vivo*. In addition, RNF $172^{-/-}$ mice were more susceptible to a LPS challenge due to the massive production of cytokines. Collectively, these findings provide new insights into the molecular regulation of NEMO and suggest an important role for RNF 172 in homeostatic control of innate immune responses.

Keywords : RNF 172, NEMO, ubiquitination

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J-9

Differentially Expressed Genes in a Bacterial Wilt Resistant Tomato Plant Transplanted with Two Different Soil Microbial Fractions

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Bacterial wilt (BW) caused by *Ralstonia solanacearum* greatly reduces the production of *Solanaceae* crops including tomato plant. In the previous study, BW-resistant tomato cultivar transplanted with upland soil microbial fraction (UpMF) showed the strong resistance to BW compared to the control, while the BW-resistance was completely abolished by forest soil microbial fraction (FoMF) transplant. To compare differentially expressed genes (DEGs) between two different microbiota transplant, RNA-Seq was performed using Illumina HiSeq system. Interestingly, most of genes involved in plant-microbe interactions was equally expressed irrespective of microbiota transplant. However, DEG analysis identified a total of 32 DEGs that showed robust differential expression under two different MFs and at three different time points. Most of these DEGs were associated with signaling pathway in tomato plants. Among 32 genes, 15 and 17 genes showed the increased and decreased expression in UpMF transplant compared to FoMF transplant, respectively. To compare the expression of DEGs, quantitative reverse transcriptional PCR is under investigation using RNAs isolated from the tomato plants transplanted with UpMF and FoMF. This result suggests that microbiota-specific signaling occurs in tomato plant to modulate BW resistance in a BW-resistant tomato plant.

Keywords : Bacterial wilt, tomato plant, differentially expressed genes

J-10

Oral Immunization with Cell Extracts of SARS-CoV-2 Spike Protein Antigen-Expressing Recombinant *Lactococcus lactis* in mice

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began to spread around the world and became a global pandemic named coronavirus disease (COVID-19). At present, many COVID-19 vaccines are being developed, and most of these vaccines are administered by intramuscular injection. The aim of the study is to develop an oral vaccine for COVID-19 by using SARS-CoV-2 spike (S) protein antigen-expressing recombinant *Lactococcus lactis* and test it in mice. SARS-CoV-2 S protein receptor binding domain (RBD) S1 subunit as an antigen and it was transformed into wild type *L. lactis* IL1403, and its expression was confirmed by western blot. Cell extracts of recombinant *L. lactis* were orally administered into mice. Western blot experiment detected intracellular target antigen of the recombinant *L. lactis*, however, extracellular target antigen was not detected. After immunization with priming, 1st and 2nd boosting, antigen specific serum IgG and fecal IgA were measured and the immunization group was 1.5-fold ($p = 0.002$) and 1.4-fold ($p = 0.016$) higher than control group, respectively. Our results indicate that cell extracts of SARS-CoV-2 S protein antigen-expressing recombinant *Lactococcus lactis* induces mice to produce antigen-specific antibody. This strategy may potentially be used in development of oral vaccines.

Keywords : Coronavirus, *L. lactis,* oral vaccine

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J-11

Anti-Inflammatory and Anti-Fibrotic Effects of *Nocardiopsis* sp. 13G027 Extract in Transforming Growth Factor β1-Induced Nasal Polyp-Derived Fibroblasts

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Nocardiopsis species produce bioactive compounds such as antimicrobial agents, anti-cancer substances, and toxins. However, no report has described their anti-inflammatory and anti-fibrotic effects during nasal polyp (NP) formation. In the present study, we investigated the anti-inflammatory and anti-fibrotic effects of *Nocardiopsis* sp. 13G027 extract in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages and transforming growth factor (TGF)-β1-stimulated nasal polyp-derived fibroblasts (NPDFs). Nitric oxide (NO) production was analyzed using the Griess reaction. Prostaglandin E_2 (PGE_2) release was determined using sandwich enzyme-linked immunosorbent assay. The expression of mitogen-activated protein kinases (MAPKs) and protein kinase B (Akt) in LPS-induced RAW 264.7 cells and α-smooth muscle actin (α-SMA), type I collagen (Col-1), fibronectin, and phosphorylated small mothers against decapentaplegic 2/3 (Smad2/3) in NPDFs was measured using Western blotting. Treatment with the 13G027 extract significantly suppressed NO and PGE_2 production. The extract suppressed the LPS-induced the phosphorylation of MAPKs and Akt and the DNA-binding activity of activator protein-1 (AP-1). The expression of pro-fibrotic components such as α-SMA, Col-1, fibronectin, and Smad2/3 was inhibited in TGF-β1-exposed NPDFs. Therefore, the current findings suggested that *Nocardiopsis* sp. 13G027 is a valuable candidate for the treatment of inflammatory disorders such as NP formation.

Keywords : Nasal polyp, *Nocardiopsis* sp. 13G027, anti-inflammatory and anti-fibrotic

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J-12

Comparative Analysis of Chikungunya Virus for Reliable and Sensitive Detection Using qPCR, ddPCR and RPA

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Background: Chikungunya virus (CHIKV) is the pathogen of re-emerging epidemic disease accompanying abrupt febrile illness. Until now, millions of people have been infected worldwide. However, vaccines and drugs against CHIKV are not yet available. In the absence of vaccines and drugs against CHIKV, the reliable and sensitive assays for CHIKV diagnostics are crucial for the clinical decision. Methods: For reliable and accurate detection of CHIKV, quantitative PCR (qPCR), droplet digital PCR (ddPCR) and Recombinase Polymerase Amplification (RPA) assays targeting to Capsid protein (C) and Envelop protein 2 (E2) genes were developed. Both qPCR and ddPCR assay used same probes and primer pairs. The primer pairs and probes for RPA was designed and tested according to manufacturer's instruction. Results: All assays were tested using viral RNA (or cDNA) in low concentration. qPCR and ddPCR show the similar limit of detection. Both qPCR and ddPCR assay can detect few copies of CHIKV genomes. The RPA assay can detect CHIKV genomes of low copy number within 15 minutes. Conclusions: Both qPCR and ddPCR for CHIKV were very sensitive and reliable for the detection of CHIKV. The RPA assay is more rapid than PCR-based assay though the sensitivity of RPA assay was lower than that of PCR-based assay.

Keywords : Chikungunya virus, quantitative PCR and droplet digital PCR, Recombinase Polymerase Amplification

J-13

Comparison of Sensitivity and Quantification of SARS-CoV-2 using Two PCR-Based Methods

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World Health Organization (WHO) announced that Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had become a pandemic on March 11. SARS-CoV-2 is a newly discovered human coronavirus and highly transmittable and has rapidly spreading worldwide, highlighting the essential role of diagnosis in preventing the spread of the epidemic. Currently, the diagnosis of SARS-CoV-2 infection is mainly done by the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) methods. Although the RT-qPCR method is regarded as a gold standard test on emergency situations, the result of RT-qPCR is relative quantification and Ct value can be varied by primer pairs, reaction mixture and etc. To assess the methods, comparison of RT-qPCR and ddPCR with various primer pairs were done. The cultured viral genomic RNA and the extracted RNA from clinical samples were used as templates for the assays. The Ct values of RT-qPCR assay with various primer pairs were varied with the same template, while the results of ddPCR were similar regardless to primer pairs, indicating ddPCR assay can be used for the diagnosis and quantification of SARS-CoV-2 genomic RNA.

Keywords : Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Reverse transcription-quantitative polymerase chain reaction (RT-qPCR), Droplet digital PCR (ddPCR)

J-14

Pathogenic Bacterial Shiga Toxins Involve Pro-Inflammatory Cytokine Production Via p38 MAPK/MK2/TTP Pathways

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Multi-functional bacterial exoprotein Shiga toxins (Stxs) produced by *Shigella dysenteriae* serotype 1 and certain *Escherichia coli* are responsible for causing hemorrhagic colitis that may progressively lead to hemolytic uremic syndrome (HUS) and central nervous system (CNS). The precise pathophysiological mechanism between Stxs and the toxin-induced inflammation has not been completely understood. Numerous studies have defined the p38 mitogen-activated protein kinase (MAPK) and its downstream target MAPK-activated protein kinase 2 (MK2) signaling pathway in a variety of cell types. We identified previously unknown role of Tristetraprolin (TTP) as MK2 substrate in Stxs-intoxicated cells. We have observed that Stxs induce phosphorylation of the MK2 at residue Thr334 and TTP in the toxin receptor Gb_3-positive cells including macrophage-like differentiated THP-1 (D-THP-1) and human proximal tubule epithelial cell line HK-2 while not in the Gb_3-negative human T84 colon carcinoma cells. Thus, Stxs selectively mediate MK2 and TTP activation in a Gb_3-dependent manner. TTP-knockdown by using targeted siRNA in the D-THP-1 cells treated with Stx2a upregulate the expression of TNF-α, IL-8, MCP-1 and MIP-1 α at transcriptional and translational levels. In conclusion, the MK2-TTP signaling pathway regulates Stx-mediated inflammatory response.

Keywords : Shiga toxin, MAPK-activated protein kinase 2 (MK2), Tristetraprolin(TTP)

J-15

A Murine $CD8^+$ T Cell Epitope Identified in the Receptor-Binding Domain of the SARS-CoV-2 Spike Protein

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The ongoing COVID-19 pandemic caused by SARS-CoV-2 has posed a devastating threat world-wide. The receptor-binding domain (RBD) of the spike protein is one of the most important antignes for SARS-CoV-2 vaccines, while the analysis of CD8 cytotoxic T lymphocyte activity in preclinical studies using mouse models is critical for evaluating vaccine efficacy. Here, we immunized C57BL/6 wild-type mice and transgenic mice expressing human angiotensin-converting enzyme 2 (ACE2) with the SARS-CoV-2 RBD protein to evaluate the IFN-γ-producing T cells in the splenocytes of the immunized mice using an overlapping peptide pool by an enzyme-linked immunospot assay and flow cytometry. We identified SARS-CoV-2 $S_{395-404}$ as a major histocompatibility complex (MHC) class I-restricted epitope for the RBD-specific CD8 T cell responses in C57BL/6 mice.

Keywords : SARS-CoV-2, CD8 cytotoxic T lymphocyte, epitope