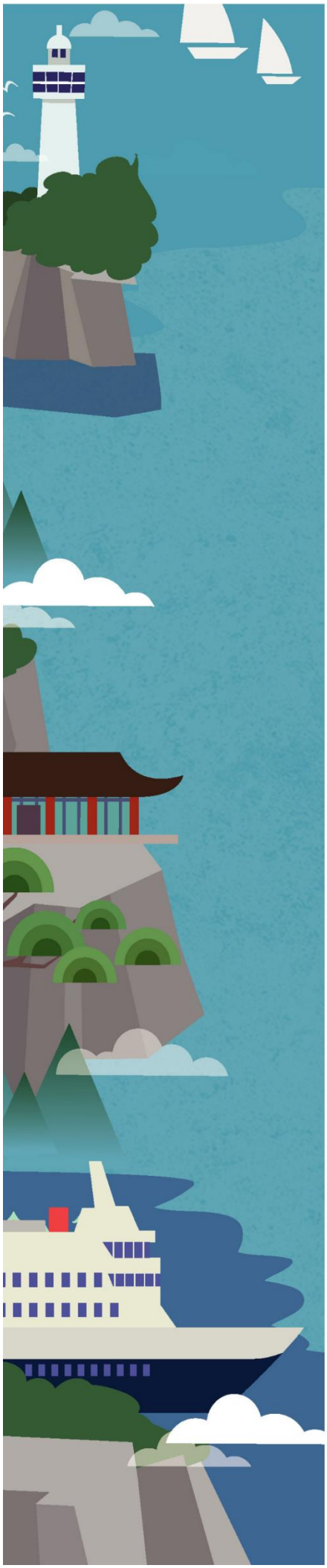


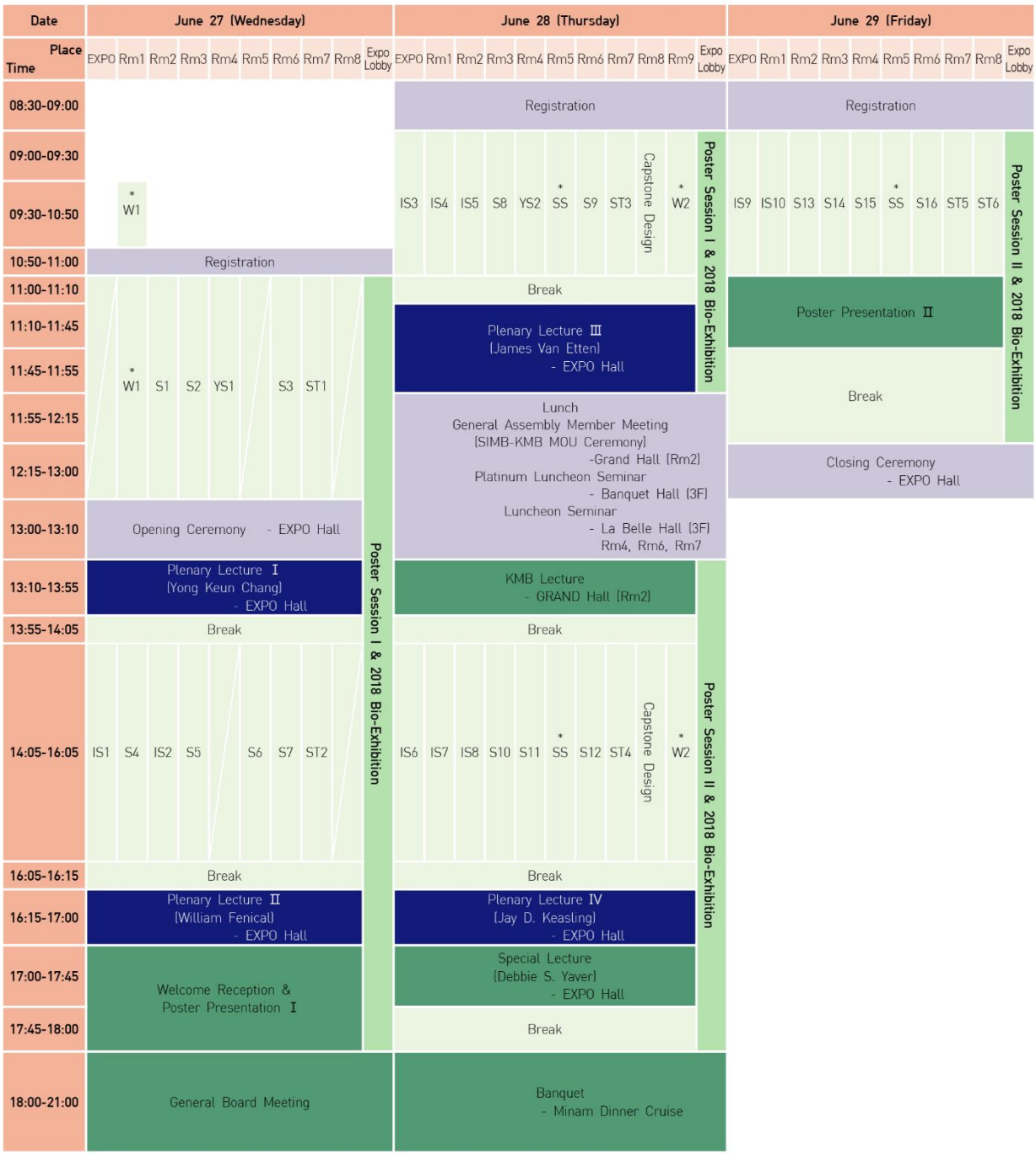
KMB 2018

45th Annual Meeting & International Symposium

Poster Session

Schedule

June 27-29, 2018
Yeosu EXPO Convention Center, Korea



IS: International Symposium, S: Symposium, YS: Young Scientist Presentation, ST: Student Presentation, W: Workshop, SS: Special Session
* Closed Door Session

P-33

The Regulatory Function of FAF1 in Production of NADPH Oxidase-Induced ROS

Tae-Hwan Kim, Hyun-Cheol Lee, C.Y.Hewawaduge, Kiramage Chathuranga, Thilina U.B. Herath, W.A. Gayan Chathuranga, Eun-Seo Lee, Pathum Ekanayaka, H.M.S.M. Wijerathne, Dung Tan Huynh, Chul-Joong Kim, Jong-Soo Lee*

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FAF1 has been reported to potentially interact with a diverse proteins and function as negative and/or positive regulator in variety of cellular possesses. Recently, it was revealed that FAF1 functions as a positive regulator of type I signaling pathway. However, it was still poorly understood how FAF1 modulates the inflammatory responses against bacterial infection. Here, we show that FAF1 has a pivotal role in activation of NADPH oxidase 2 in macrophages upon TLR2 or bacterial infection. $FAF1^{gt/gt}$ mice showed reduced inflammatory responses, resulting in the increase of bacterial replication upon *Listeria monocytogenes* infection compared with wild-type mice. Consistently, deficiency of FAF1 reduces the production of pro-inflammatory cytokines depending on NADPH oxidase-mediated ROS in macrophages upon TLR2 stimulation *in vitro*. Moreover, we demonstrated that FAF1 interacts with p67phox which is an activator of NOX2 in macrophages, thus enhances the activity of NADPH oxidase 2 upon *L. monocytogenes* infection. Collectively, these results indicate FAF1 is important for host protection against bacterial infection.

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P-35

NQO1 Inhibits Type I Interferon Secretion by Reducing Self-Association of TBK1

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TANK-binding kinase 1 (TBK1) is a key component of innate immunity that phosphorylates transcriptional factors to activate induction of antiviral interferons and inflammatory cytokines upon virus infection. Here we report that the NAD(P)H:quinone oxidoreductase 1 (NQO1) regulates the innate immunity by reducing self-association of TBK1, the essential step for its activation. Deficiency of NQO1 enhanced cytokine secretion, causing reduced replication of virus in vivo and in vitro. We also demonstrated that this regulation of innate immunity was mediated by the interaction between NQO1 and TBK1. NQO1 reduced oligomerization of TBK1 and finally inhibited activation of TBK1 characterized by the phosphorylation on serine 172. Additionally, we show that TBK1 is responsible for the phosphorylation of serine on NQO1. Taken together, our study suggests that TBK1 and NQO1 form negative feedback loop for regulation of antiviral innate immunity.

[The Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Grant no. 318039-3)]

P-34

Foot-and-Mouth Disease Virus VP1 Point Mutation Negatively Regulates Type I Interferon Pathway

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Foot and mouth disease (FMD) is a highly infectious virus and serves as a causative agent of an acute vesicular disease affecting pigs, cattle and other domestic, and wild animals worldwide. Upon virus infections, host defense mechanisms, specially Type-I interferon (IFN) pathway plays a key role to protect hosts from virus infections through producing pro-inflammatory cytokines. However, based on the recent research findings, as a highly infectious virus, FMD virus possess different cellular invasion strategies to invade host cells by the suppression of host defense mechanisms through its structural and non-structural proteins. In this study, we evaluate the Type-I IFN pathway suppression strategy of FMD virus structural protein VP1 and its K83E point mutation. Based on the results, overexpression of VP1 K83E point mutation alleviates the IFN-β and IFN-α suppression in 293T and PK15 cells, respectively, while reducing the Vesicular stomatitis virus (VSV) replication than VP1 wild-type (WT) transfected cells. Additionally, similar to IFN-β and IFN-α results, the same pattern can be seen with the other antiviral gene expressions. Furthermore, luciferase reporter assay results suggest that VP1 WT binds with the MAVS but not the mutant and finally, the binding assay with MAVS, VP1 WT and its mutant support above hypothesis. These data thus suggest that VP1 binds with MAVS and blocks the MAVS, TRAF-3 binding resulting inhibition of Type-I IFN pathway activation as a one of cellular invasion strategy of FMD virus and K83E point mutation of VP1 is strong enough to inhibit that suppressive mechanism.

[The Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Grant no. 315044031, 316043-3)]

P-36

Tryptophanyl-tRNA-Synthetase Functions as a Cytokine on Virus Infection

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The ARSs are the essential enzymes in translation that catalyze specific amino acid to its cognate tRNA. However, some ARS in higher eukaryotes possess non-canonical function to regulate additional cell metabolisms. As a part of the non-canonical functions of ARS, here we report the new ability of WRS in antiviral innate immune system. We show rapid secretion of WRS in response to virus infection and the extracellular function of WRS to priming innate immunity. This stimulation enhances the level of cytokines and finally mediates inhibition of virus replication in vitro and in vivo. Thus, we subjoin the WRS to the antiviral innate immune system as a new factor provoking the defence responses. This study suggests that secreted full-length WRS has a non-canonical function in activating and mediating innate immune responses to the virus infection and the WRS is able to be utilized for application in potential.

[The Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Grant no. 315044031, 316043-3, 318039-3)]

P-40

Autophagy Protein Rubicon Regulates Type I IFN Signaling by Targeting IRF3 Dimerization

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Rubicon is a part of a Beclin-1-Vps34-containing autophagy complex. Rubicon induces antimicrobial responses upon TLR stimulation, and functions as a feedback inhibitor to prevent unbalanced proinflammatory responses depending on dectin-1 signaling. However, the role played by Rubicon during antiviral immune responses, particularly the type I interferon responses, remains largely unknown. Here, we report that Rubicon acts as a negative regulator for virus-triggered IFN signaling. Knockdown of Rubicon promoted type I interferon signaling and inhibited virus replication, while overexpression of Rubicon has the opposite effect. Rubicon specifically interacts with the IRF association domain (IAD) of IRF3, and this interaction leads to inhibition of the dimerization of IRF3, which negatively regulates IFN-mediated antiviral response. Thus, our findings suggest that the novel additional role of Rubicon as a negative regulator that inhibits the IFN signaling and cellular antiviral responses, providing a novel cellular mechanism of IRF3 inhibition.

[The National Research Foundation of Korea (Grant no. 2015020957)]

N-50

Coptidis Rhizoma Extract Inhibits Respiratory Syncytial Virus in vitro and in vivo

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Huang Lian (*Coptidis Rhizoma*) is derived from thedried rhizome of Ranunculaceous plants and is frequently used in many traditional formulas forproperties of "clearing damp-heat, quenching fire and counteracting poison" inAsia for centuries. However underlying mechanism of *Coptidis Rhizoma* extract (CRE) exhibited antiviral activity against RSV has not been reported in detail. In here, we investigated the antiviral activities of CRE against RSV in human respiratory tract cell line (HEp2) and BALB/c mice. An effective dose of CRE significantly reduces the replication of RSV in HEp2 cells and reduces the RSV-induced cell death. This anti-RSV activity was through the induction of type I interferon-related signaling and the antiviral state inHEp2 cells. Furthermore, oral inoculation of CRE exhibited prophylactic effects in BALB/c mice against RSV. Compounds present in the aqueous fractions of CRE confirmed by HPLC analysis and among them; we confirmed that palmatine was related to the antiviral properties and immune-modulation effect. Collectively, CRE and its components exhibit promising immunomodulatory role with prophylactic effect thus, could be a potential source as promising natural antivirals that can confer protection to RSV.

[The Ministry for Food, Agriculture, Forestryand Fisheries, Republic of Korea (Grant no. 315044031, 316043-3)]

N-51

Inhibition of Respiratory Syncytial Virus Replication in vitro and in vivo by *Plantago asiatica* and *Clerodendron trichotomum*

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Plantago asiatica (PA) and *Clerodendron trichotomum* (CT) are common herbal medicine, native to East Asia. *PA* seed is renowned for its diuretic, antipyretic, antiphlogistic, and defecation-facilitating properties. Meanwhile, the dried leaf and stem of CA contains miscellaneous pharmacological activities including antihypertensive, sedative, analgesic, and anti-inflammatory effects. Although both herbs are used for its many therapeutic effects, antiviral activity against RSV has not been reported in detail. The present study discovered the antiviral activities of *Plantago asiatica* extract (PAE) and *Clerodendron trichotomum* extract (CTE) against Respiratory Syncytial Virus (RSV) in human respiratory tract cell line (HEp2) and BALB/c mice. An effective dose of both herb extracts significantly reduce the replication of RSV in HEp2 cells and subsequent cell death. Moreover, herb treatments diminish syncytial formation after RSV infection in HEp2 cells significantly. Time dependent treatment of PAE and CTE after RSV infection in HEp2 cells expressed 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. More importantly, in vivo results illustrate oral administration of PAE and CTE exhibited prophylactic effects in BALB/c mice against RSV. Taken together, *PA* and CT extracts will be a potential source as promising natural antivirals that can confer protection to RSV.

[The Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Grant no. 315044031, Grant No. 316043-3)]

N-52

Recombinant FMDV O Type Multi-VP1e Can Induces Protection against Lethal FMDV Challenge

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Development of new vaccines consider as the only option to prevent foot and mouth disease (FMD) cause by newly immerging FMD virus (FMDV) serotypes. An effective antigen-adjuvant combination is also critically affect to the efficacy of vaccine. In this study, we evaluated a recombinant subunit vaccine candidate with effective immunogenic adjuvant for FMDV. To construct multi-epitope-based antigen, weselected epitope sites containing G-H loop and C-terminus known as majorimmunogenic sites in VP1 region from three serotypesof FMDVs, O/Jincheon/SKR/2014, O/Andong/SKR/2010 and O1/Manisa/Turkey/69 and conjugatedas a O type VP1 multi-epitope (OVM). OVM protein was expressed in soluble form and purified byFPLC and IMAC system. Intramuscular (IM) administration of OVM with ISA 201 induced humoral and cellular immunity and completely protect from lethal infection of FMDV O/Jincheon/SKR/2014 in mice. Additionally, OVM vaccine induced the production of sufficient neutralizing antibodies against each three serotypes of FMDV and virus neutralizing titer in pigs. Furthermore, weevaluated adjuvant effect of recombinant Tryptophanyl-tRNA Synthetase (WRS) protein. OVM with ISA 201 and WRS administrated mice show higher humoral andcellular immunity compare to the OVM with ISA 201 administrated group. Together,our findings demonstrate OVM recombinant subunit vaccine with ISA 201 and WRS may be a promising vaccine candidate against diverse FMDVs.

[TheMinistry for Food, Agriculture, Forestry and Fisheries, Republic of Korea(Grant no. 315044031, 318039-3)]

N-53

Influenza Vaccine Candidates Emulsified with CAWIO Induces Protection against Lethal Influenza Challenge

Eun-Seo Lee, Hyun-Cheol Lee, Gayan Chathuranga, Tae-Hwan Kim, Kiramage Chathuranga, Pathum Ekanayaka, H.M.S.M. Wijerathne, Dung Tan Huynh, Jong-Soo Lee*

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Avian influenza virus (AIV) can infect poultry,mammals, and other hosts and causes enormous economic losses to the global poultry industry. Many centuries are preparing avian influenza vaccine which provide the good protection efficacy and also developing the new adjuvant foravian. In this study, we evaluated that the new adjuvant CAWIO (CAVAC, Korea) is safe and effective to enhance the immunogenicity of Influenza antigens and compared with ISA70 (SEPPIC, France). As antigens, we used the conserved Influenza antigen (sM2HA2) which studied previously and inactivated H9N2 antigen. As results, CAWIO mice groups which immunized with recombinant conserved antigens (sM2HA2) or inactivated H9N2 antigen can induce higher level of anti-serum IgGand better secretion of IFN-γ and IL-4 from splenocytes comparative to ISA70 groups. Moreover, in challenge tests with 10 MLD50 of A/Aquaticbird/Korea/W81/2005 (H5N2) or A/Chicken/Korea/116/2004(H9N2) viruses in BALB/cmice, CAWIO mice groups provided better protection of BALB/c mice compared with ISA70 groups. These results indicate that CAWIO is a promising adjuvant foravian influenza vaccines.

[The Ministry for Food,Agriculture, Forestry and Fisheries (Grant no. 316043-3)]

N-54

GRA7 Has the Antiviral Activity in vitro and in vivo by Inducing Antiviral State

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Dense Granule Protein-7 (GRA-7) is an excretory protein of *T. gondii*. However, the roles of GRA-7 against virus infection are not completely investigated. Here, we identified the antiviral properties of GRA-7 in vitro and in vivo against diverse viruses. Non-toxic amounts of GRA-7 antigen significantly inhibited the replication of Influenza PR8, VSV, and HSV on RAW264.7 cells. GRA-7 also displayed antiviral activity in epithelial (HEK293T and HELA) cells against VSV, PR8, HSV, and Adenoviruses. For the detail explanation of these antiviral activities, we checked the induction of type I interferon and pro-inflammatory cytokines both in mRNA and cytokine levels upon treatment of GRA-7 in vitro. Moreover, intra-nasal inoculation of GRA-7 protected BALB/c mice from lethal infection with Influenza A virus (H1N1). Furthermore, intra-nasal administration of GRA-7 in BALB/c mice showed lower lung viral titer, when challenge with RSV. Therefore, these results clearly showed that GRA-7 has an antiviral activity both in vitro and in vivo suggesting the potential use of GRA-7 antigen as a candidate drug for prophylactic purposes. [The Ministry for Food, Agriculture, Forestry and Fisheries (Grant No. 315044031, 316043-3)]

N-55

Mucosal Administrations of *Lactobacillus casei* Surface-Displayed HA1 Domain Protect Avian Influenza Virus Infection (H5N2) in Mice

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A avian influenza virus have induced severe economic losses in poultry industry in Korea. Especially, a low pathogenic H5N2 strain becomes the main causative agent. To develop an efficacious mucosal vaccine against the LPAI H5N2 virus, a recombinant *Lactobacillus casei* strain expressing the hemagglutinin HA1 domain of H5N2 on the surface was constructed (HA1/*L.casei*). We have investigated the protective efficacy of HA1/*L.casei*, in a murine model, and become a promising mucosal vaccine candidate against LPAI H5N2 or not. Mice were mucosally inoculated through oral and nasal routes with PBS; pgsA/*L.casei* and pgsA-HA1/*L.casei*. Both nasal and oral vaccination induced humoral immune response in the serum and mucosal areas. Nasal administration of HA1/*L.casei* induced higher prominently antibody responses in both mucosal secretions (IgA) and serum (IgG) than those induced by oral administration. Interestingly, HA1/*L.casei* induced a mixed T_H1/T_H2 type of immune response with a significant induction of IgG1 as well as IgG2a antibodies. Moreover, the cell-mediated immune responses were evoked in both nasal and oral administration elucidated by the production of IFN-γ and IL-4 in splenocytes. Most importantly, mucosal administration of HA1/*L.casei* triggered a complete protection against a lethal dose challenge of LPAI H5N2 viruses ($1xLD_{50}$). Briefly, the results suggest that mucosal inoculation of HA1/*L.casei* can be used as a protective vaccine candidate against LPAI H5N2 virus.
[Ministry of Health and Welfare (Grant No. HI16C1032)]

N-56

Lactobacillus-Displayed CTA1-Conjugated PEDSe Antigen Induces Protective Immune Responses against PED Virus

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Porcine epidemic diarrhea (PED) causes significant economic impact throughout the swine-raising countries including Korea. PED virus (PEDV), the causative agent of PED belongs to the member of family Coronaviridae, and COE, SS2 and SS6 regions of PEDV spike protein plays a role in neutralizing antibody production against PEDV. We used PEDSe multi-epitope (Spike multi-epitope, Se=COE, SS2, SS6) with or without non-toxic mucosal immunogenic adjuvant, cholera toxin subunit A1 (CTA1) to develop an effective and safe mucosal vaccine against PEDV, from the recently circulating strain in Korea. Our target antigens PEDSe and CTA1 were expressed on surface of *Lactobacillus casei*. Surface localization of the fusion proteins on *L.casei* was verified by cellular fractionation and FACS. Oral and intranasal inoculations of *rL.casei* into mice generated high serum IgG and mucosal IgA levels. Interestingly, r*L.casei* conjugated with CTA1 induced more potent mucosal, humoral and cell-mediated immune responses than pgsA-PEDSe/ *L.casei*. Mucosal immunization with r*L.casei* stimulated cellular immune responses, provided long-lasting immune responses and produced neutralizing antibodies against PEDV infection. In short, these results suggest that mucosal immunization with r*L.casei*-displayed CTA1-conjugated-PEDSe antigen could be a promising vaccine candidate against currently circulating PEDV.

[The Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (Grant no. 315044031, 316043-3)]