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Immune responses of BALB/c mice orally immunized with *Salmonella* Typhimurium ghost cells carrying antigens of enterotoxigenic *Escherichia coli*

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ABSTRACT

Salmonella Typhimurium ghost vaccines containing antigens of enterotoxigenic *Escherichia coli* (ETEC) were obtained by a strain harboring pMMP184, which carries a ghost cassette. The immune responses of BALB/c mice orally vaccinated with these ghost vaccines were determined in this study. Total IgG against *S.* Typhimurium were highly detected by the oral immunization route in BALB/c mice. IgGs against ETEC antigens in the ghost cells carrying F41 and intimin were detected at 4 weeks after vaccination. However, FedF elicited a delayed induction of IgGs, whereas FedA immune response failed to or barely induced IgGs after vaccination. Proliferations of CD3e/CD4-T cells were observed in splenocytes of BALB/c mice immunized with *S.* Typhimurium ghost cells carrying FedF. However, CD45R-B220/CD23-B cells were proliferated by ghost cells carrying FedA, F41, and intimin. The immunized BALB/c mice showed 25~50% protection against challenge with wild type *S.* Typhimurium, when compared to control mice. Therefore, it is assumed that oral vaccination of *S.* Typhimurium ghost cells has the potential to protect mice against pathogenic *E. coli*.

Key words: antigen, BALB/c mouse, ghost cells, pMMP184, *S.* Typhimurium, vaccination

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Introduction

Bacterial ghost vaccines have been mainly studied in fish with many improvements (KWON et al., 2006; RA et al., 2009; TU et al., 2010; KIM et al., 2011; CHOI and KIM, 2012). In order to industrialize ghost vaccines, the survived pathogenic bacteria after ghost formation can be completely killed by mechanical procedures, such as freeze-drying or by killing agents such as β-propiolactone (LANGEMANN et al., 2010).

Although polyvalent ghost vaccines have been partially studied in a foreign antigen delivery system, their efficacy has not been yet concluded in previous studies (LANGEMANN et al., 2010). In addition, the protein to be expressed in the ghost vaccine does not have a system for efficient regulation. Moreover, over-expression of foreign antigens in a host bacteria may cause the abnormal physiological state to induce cell death. Furthermore, the ghost vaccines studied have not yet been constructed with an environmentally friendly system. For example, previously constructed ghost vaccines have been applied by antibiotic markers for selection of a strain carrying a ghost cassette. These systems have the risk of disseminating antibiotic resistant genes to the environment (JECHLINGER et al., 2005; MAYR et al., 2005; KWON et al., 2006; RA et al., 2009).

Abuse of antibiotics in livestock breeding causes antibiotic-resistant bacteria through a variety of mechanisms. Since diffusion of antibiotic-resistant bacteria is dangerous to human beings, abuse prohibition of antibiotics in livestock has been enforced by law. Although a number of physiologically active substances have been studied to substitute antibiotics (DONOVAN et al., 2012; THACKER, 2013), it is currently impossible to confirm the effect of the replaced substance in the environment of livestock breeding. In order to overcome this problem, an alternative method is to use polyvalent vaccines (HUR et al., 2012; CHAUDHARI et al., 2013). Since a polyvalent vaccine can protect livestock against currently known pathogens, as well as opportunistic bacteria, the vaccine is capable of protecting livestock against a variety of pathogens. In a previous study, we constructed a plasmid pMMP184 carrying a stringently regulated ghost cassette and foreign antigen delivery system via a host balanced lethal system, based on aspartate semi-aldehyde dehydrogenase (*asd*) (KIM et al., 2016).

In this study, for the purpose of protecting piglets against enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* Typhimurium that cause fatal damage to the productivity of piglets, ghost vaccines were prepared by *Salmonella* Typhimurium carrying these antigens, and the prepared vaccines were administered to mice. In addition, we evaluated the ability of these ghost vaccines to protect mice against challenges with virulent *Salmonella* Typhimurium.

Materials and methods

Bacterial strains, plasmids, and reagents. The bacterial strains and plasmids used in this study are listed in Table 1. *S.* Typhimurium and *E. coli* were grown at 28, 37, or 42 °C in Luria-Bertani (LB) or M9 minimal medium (BERTANI, 1951). Antibiotics were added to the culture media at the following concentrations: ampicillin, 100 μg/mL; streptomycin, 50 μg/mL. When required, L-arabinose and diaminopimellic acid (DAP) were added to final concentrations of 0.2% and 50 μg/mL in the medium, respectively.

Table 1. Bacterial strains and plasmids used in this study

Strains or plasmids	Descriptions	References
E. coli		
Top10	F-mcrA (mrr-hsdRMS-mcrBC) Φ80lacZ*Δ*M15 *Δ*lacX74 nupG recA1 araD139 *Δ*(ara-leu)7697 galE15 galK rpsL (Str^R) endA1	Invitrogen
DH5α	*fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Lab stock
χ6212	*Φ*80d lacZ ΔM15 *deoR Δ(lacZYA-argF)U169 supE44λ- gyrA96 recA1 relA1 endA1 asdA4 Δzhf-2::Tn10 sdR17* ($R^- M^+$)	Lab stock
Salmonella		
MMP13	*S.* Typhimurium JOL401 *asd*A16	This study
S. Typhimurium 3339	wild-type strain SL1344, hisG rpsL	Lab stock
Plasmids		
T-vector	a TA cloning vector	promega
pET28a	an overexpression vector containing pBR322 origin	Novogen
pYA3342	a vector containing pBR origin	Kang et al., 2002
pMMP184	a derivative of pYA3342 carrying cI857 PR E ara and PR *ompA* TM	this study
pMMP187	a derivative of pYA3332 carrying cI857 PR E ara and PR *ompA* TM	this study
pMMP258	A derivative of pMMP184 carrying *fedA* gene	this study
pMMP264	A derivative of pMMP184 carrying *fedF* gene	this study
pMMP272	A derivative of pMMP184 carrying *f41* gene	this study
pMMP273	A derivative of pMMP184 carrying *intimin* gene	this study

General DNA manipulations. DNA manipulations were done by the protocols of SAMBROOK et al. (1989). Plasmids were introduced into *E. coli* competent cells via

heat-shock with $CaCl_2$ treatment, or into *Salmonella* competent cells via electroporation (SAMBROOK et al., 1989). PCR amplification was employed in order to identify *S.* Typhimurium strains, and to clone DNA fragments. Nucleotide sequencing was done using an ABI 373 automatic sequencer (PE Applied Biosystems).

The procedures of DNA manipulation for the novel repressor system and antisense RNA regulation were performed by the method of KIM et al. (2016). The procedure to clone ETEC antigens was also done by the method of KIM et al. (2016).

Growth curve of ghost bacteria, formation of ghost cells, and measurement of viable cells. Bacterial strains carrying the ghost cassette were inoculated into 100 mL LB medium (with 1% pre-cultured cells) containing 0.2% arabinose. The inoculated media were cultured at 28 °C until 1.5 or higher at 600 nm (OD_{600}). These cells were then harvested by centrifugation at 6,000×g for 5 min. The cells were washed three times with PBS buffer (pH 7.4). The washed cells were resuspended in PBS buffer (pH 7.4) and M9 medium at the same volume of the original culture. Ghost cells were then obtained after incubation at 42 °C. When surviving cells after ghost formation were reduced to $\leq 10^3$ CFU/mL, ghost formation was forcibly terminated by washing with distilled water. The obtained ghost cells were then resuspended in distilled water to an appropriate cell mass. The resuspended ghost cells were freeze-dried and stored until use. The prepared ghost cells were injected into mice after appropriate dilution with PBS buffer containing 0.1% gelatin (PBSG buffer).

SDS-PAGE and Immunoblot. Ghost cells formed at 42 ºC were harvested by centrifugation at 5,000×g for 10 min. The recovered cells were washed twice with 0.85% NaCl and then resuspended in 10 mM Tris HCl (pH 7.5). The suspended cells were mixed with SDS gel loading buffer, boiled for 5 min, and centrifuged at 12,000× *g* for 5 min. The supernatant was recovered as a crude protein solution for SDS-PAGE. Protein samples (65 μg) from total cell lysates were separated via SDS-PAGE. Bands were visualized by Coomassie staining (SAMBROOK et al., 1989). For immunoblotting, proteins separated by SDS-PAGE were electrophoretically transferred onto nitrocellulose membranes. Immunoblotting was conducted in accordance with the protocols established by SAMBROOK et al. (1989). Each antigen was recognized specifically by antibodies elicited from rabbits against each antigen. Goat anti-rabbit IgG:horseradish peroxidase (HRP)-conjugated antibody was used as the secondary antibody. 4-choloro-1-naphthol was used as the HRP substrate.

Mouse vaccination with S. Typhimurium *ghost cells.* The properly diluted S. Typhimurium ghost cells were administered via oral route into 10 BALB/c mice, acclimatized for 1 week. The inoculated dosage was adjusted to 1×10^7 CFU. Food and water were prohibited for 4 h prior to the immunization of ghost cells. At 1 h post immunization, mice were provided *ad libitum* access to food and water. The 2nd and 3rd

immunizations were done at a 2 week interval with the same vaccine candidates and doses. The vaccinated mice were observed for 10 weeks after immunization. Peripheral blood samples were collected at 0 to 10 weeks post immunization to determine the IgG levels.

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed according to the method of KANG et al. (2002). Briefly, LPS or each antigen, including FedA, FedF, intimin, and F41 (0.2 mg/mL), was dissolved in 0.05 M carbonate buffer (pH 9.6) and coated onto microtiter plate at 4 °C overnight. The coating solution was removed from the treated microtiter plate (SPL) and washed 4~6 times with PBS buffer (pH 7.4). The treated plate was blocked with 0.1% skim milk in PBS at 37 °C for 1 h. Wells were then reacted with plasma (10-fold serially diluted) at 37 °C for 2 h. After washing the wells three times with PBS, the wells were then incubated with goat anti-mouse IgG-horseradish peroxidase (HRP)-conjugate (Southern Biotech, 1:5,000 dilution) at 37 °C for 2 h. Bound HRP was reacted with 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, Sigma-Aldrich) for 10~90 min. After stopping the reaction with 0.1% SDS, antibody titers were determined by measuring the absorbance value at wavelength of 405 nm on an ELISA reader (Dynex, USA).

Fluorescence activated cell sorting (FACS). The spleens of the mice were removed at 5 weeks post immunization, and splenocytes were separated from the spleens. The separated splenocytes (1.0×10^6) were transferred into E-tubes, mixed with fluorescent dye-conjugated antibodies (BD Pharmingen), and incubated at 4 °C for 30 min. The antibody cocktail for detection of B lymphocytes was composed of fluorescently labeled PE-CD45R/B220, PE CD23 (FcεRII), and APC sIgM. The antibody cocktail for detecting T lymphocytes was composed of PE-CD3e, PE CD4, and FITC CD8 (BD Pharmingen). The treated solutions were centrifuged at 3,000×g for 10 min at 4 °C, and then the supernatant was completely removed. The precipitated pellet was dissolved in 2 mL PBS (pH 7.4), transferred into a FACS tube, and observed by FACS (BD Biosciences). FACS analysis of 20,000 events was performed using CellQuest software (BD Biosciences).

Protection efficacy of S. Typhimurium *ghost cells against virulent S.* Typhimurium *after oral vaccination in mice.* In order to evaluate the protection efficacy of *S.* Typhimurium ghost cells against virulent *S.* Typhimurium after oral vaccination in mice, a challenge test was performed as follows: The ghost cells carrying each antigen were administered via oral route (1×10^7 CFU dose) to 10 mice. Mice were booster-vaccinated (2^{nd} and 3^{rd} vaccinations) at a 2 week interval using the same dose and route. The challenge test was performed by the oral route in 5 mice, using 3.6×10^4 CFU of *S.* Typhimurium χ3339 two weeks after the last booster vaccination. Food and water were prohibited for 4 h before the administration of ghost cells or challenge with the virulent *S.* Typhimurium. At 1 h post immunization, the mice were supplied with food and water *ad libitum*. The treated

mice were observed for 4 weeks. The animal experiments in this study were approved by the Animal Ethics Committee of Gyeongnam National University of Science and Technology, in accordance with the guidelines of the Korean Council on Animal Care (AEC-20100730-0002).

Statistical analysis. All data are expressed as means ± standard deviation (SD) unless otherwise specified. An independent sample *t*-test was used to analyze statistical differences in immune responses between immunized groups and the non-immunized control group. Differences were considered to be statistically significant when the P-values were ≤0.05 or ≤0.01. All analyses were performed using the SAS statistical software package (version 9.1, SAS Inst., Inc., USA).

Results

S. Typhimurium *ghost cells elicited immune responses in orally immunized mice.* In order to examine the immune responses in sera of BALB/c mice immunized orally by *S.* Typhimurium ghost cells, total IgGs against *S.* Typhimurium LPS and ETEC antigens, including FedA, FedF, F41, and intimin, were analyzed by the sera of the mice obtained from 0 to 10 weeks after vaccination with the antigens. The ETEC antigens were cloned into pMMP184 after PCR amplification, in which the antigens were designed to be harbored on the ghost cells after expression. Since the cloned antigen genes were controlled under the cI857 P_R system, the genes were derepressed by temperature increase to 42°C. The expressed antigens were then anchored onto the envelope of the ghost cells due to a signal peptide. When the anchored antigens were analyzed by western blotting, F41 maintained the highest amount, whereas FedA exhibited the lowest amount (Fig. 1). When the obtained ghost cells were administered into mice via the oral route, immune response (total IgG) to *S.* typhimurium LPS was elicited at 2 weeks after the mice were treated with the ghost cells containing FedA. The IgG continuously maintained a high level from the 4^{th} to the 10^{th} week after immunization (Fig. 2). The immune responses (IgGs) to LPS after immunization of the ghost cells, carrying other antigens such as FedF, F41, and intimin, were detected at 4 weeks post-immunization of the ghost cells. They also maintained high levels until 10 weeks after vaccination.

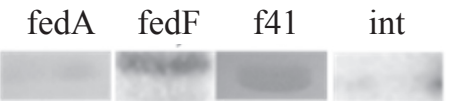

Fig. 1. Expressions of FedA, FedF, F41 and intimin on the ghost cells. Detection of each antigen was done by immunoblot. Primary antibodies to each of the tested antigens were obtained from rabbits, while goat anti-rabbit IgGs conjugated with HRP were used as secondary antibodies.

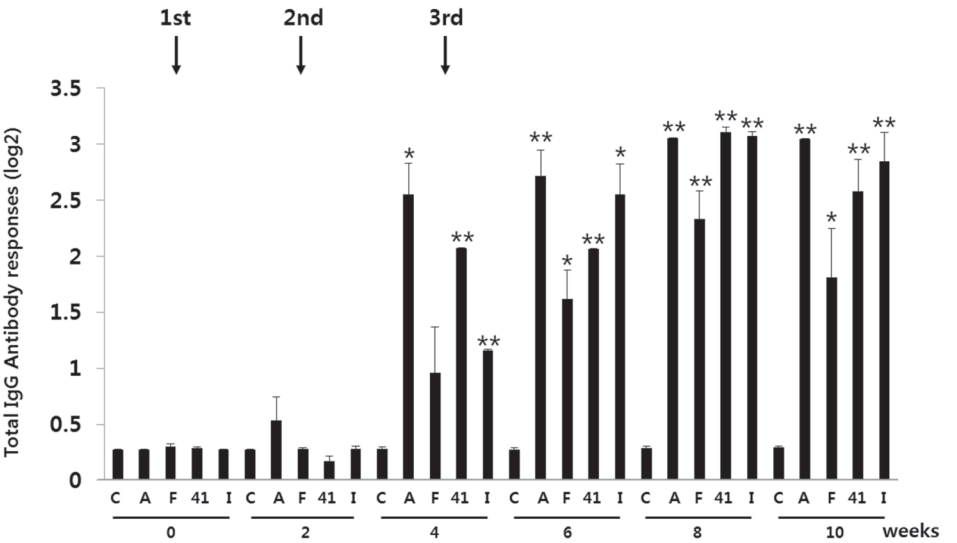

Fig. 2. Analyses of total IgG to LPS from serum after immunization of ghost vaccines via oral route. The microtiter plate was treated with 0.2 mg/mL LPS, serially diluted sera and goat anti-mouse IgG-horseradish peroxidase (HRP)-conjugate. The treated solutions were measured at 405 nm by an ELISA reader. X- and Y-axes indicate week post injection and immune response by log value, respectively. C, only PBS; A, fedA; F, fedF; 41, f41; I, intimin. The 1st, 2nd, and 3rd immunizations were done at a 2 week interval with vaccine candidates in 1×10^7 CFU doses.

The immune responses to the applied antigens showed differential eliciting times and total IgG contents depending on the ghost vaccines (Fig. 3). Although immune responses to F41 and intimin were detected from 4 weeks after vaccination, an immune response to FedF was detected at 8 weeks after vaccination with a high level. However, the immune response to FedA was detected at lower levels in all the tested weeks when compared to immune responses after vaccination with the ghost cells carrying other antigens. Although the immune responses according to the antigens exhibited differential patterns, we suggest that the ghost cells carrying pathogenic *E. coli* antigens elicit immune responses against these antigens.

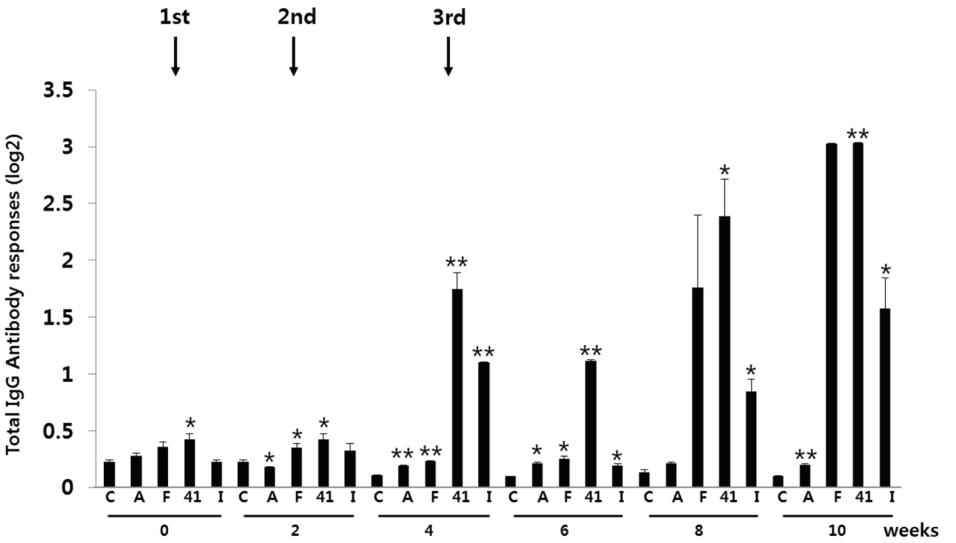

Fig. 3. Analyses of total IgG to each antigen from serum after immunization of ghost vaccines via oral route. Each antigen was treated by 0.2 mg/mL, serially diluted sera and goat anti-mouse IgG-horseradish peroxidase (HRP)-conjugate. The treated solutions were measured at 405 nm by ELISA reader. X- and Y-axes indicate week post injection and immune response by log value, respectively. C, only PBS; A, fedA; F, fedF; 41, f41; I, intimin. The 1st, 2nd, and 3rd immunizations were done at a 2 week interval with vaccine candidates in 1×10^7 CFU doses.

Orally administered ghost cells induced proliferations of differential T-cell and B-cell subtypes. Since orally administered ghost cells elicited immune responses of total IgG, we investigated differentiations of T-cells and B-cells via FACS. In order to examine the proliferation of T- and B-cells, we analyzed the distribution patterns of T- and B-cells for splenocytes collected from the spleen of mice treated with different ghost cells. As shown in Fig. 4A and 4B, the splenocytes treated with ghost cells carrying FedF exhibited higher CD4 and CD3e amounts than those of the control. However, treatments with other ghost cells did not show any significant differences. B-cells in mice treated with FedA, F41, and intimin, but not FedF, were differentiated into CD45R/B220 and CD23 of various amounts (Fig. 4C and 4D).

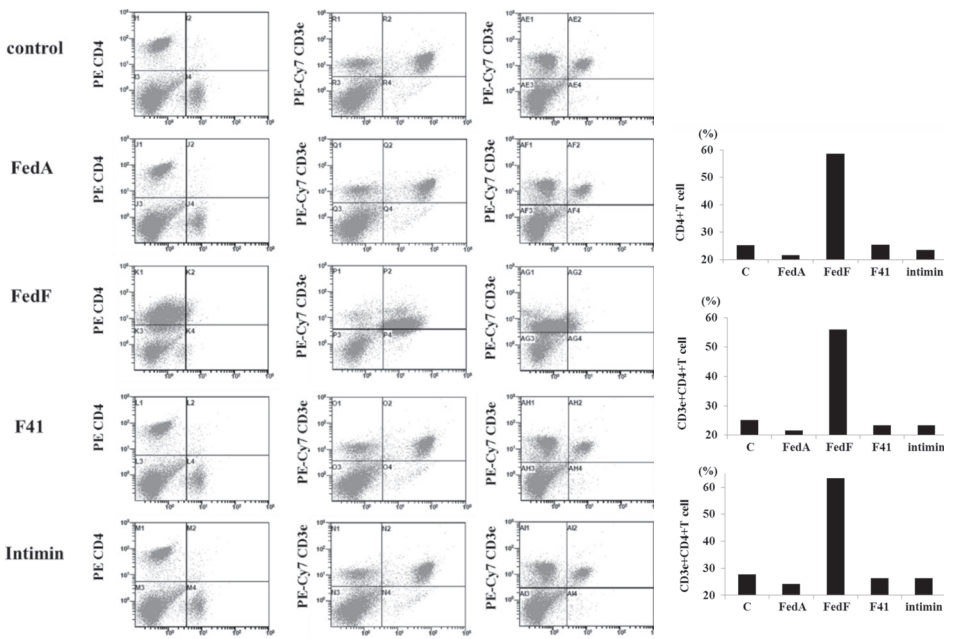

Fig. 4. A-B. Distributions of T-and B-cells isolated from splenocytes of mice after immunization of ghost vaccine via oral route. Results of FACS to T- and B-cells (left panels of A and C) and histograms of FACS results (B and D). Splenocytes were isolated from mice at 5 weeks post administration of ghost cells. On the left panels in A, the X- and Y-axes indicate PE CD4 and PE-Cy7 CD3e, respectively. On the middle panels, the X- and Y-axes indicate FITC CD8a and PE-Cy7 CD3e, respectively. On the right panels, the X- and Y-axes indicate FITC CD8a and PE CD4, respectively. On the left panels in C, the X- and Y-axes indicate APC sIgM and PE-Cy7 CD45R/B220, respectively. On the right panels, the X- and Y-axes indicate APC sIgM and PE CD23, respectively.

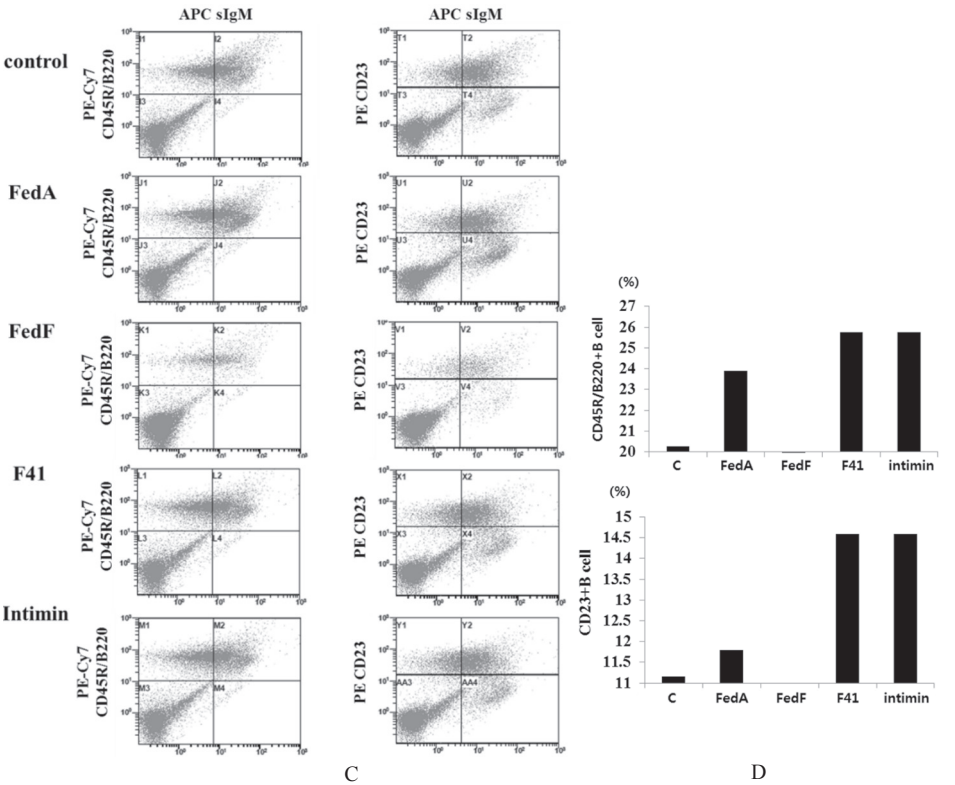

Fig. 4. C-D. Distributions of T-and B-cells isolated from splenocytes of mice after immunization of ghost vaccine via oral route. Results of FACS to T- and B-cells (left panels of A and C) and histograms of FACS results (B and D). Splenocytes were isolated from mice at 5 weeks post administration of ghost cells. On the left panels in A, the X- and Y-axes indicate PE CD4 and PE-Cy7 CD3e, respectively. On the middle panels, the X- and Y-axes indicate FITC CD8a and PE-Cy7 CD3e, respectively. On the right panels, the X- and Y-axes indicate FITC CD8a and PE CD4, respectively. On the left panels in C, the X- and Y-axes indicate APC sIgM and PE-Cy7 CD45R/B220, respectively. On the right panels, the X- and Y-axes indicate APC sIgM and PE CD23, respectively.

Ghost cells offered partial protection to BALB/c mice against virulent S. *Typhimurium after oral vaccination.* The protective effect of ghost cells in BALB/c mice against virulent *S.* Typhimurium was evaluated after oral vaccination. Virulent *S.* Typhimurium χ3339 was grown in LB broth and applied for challenge (3.6×10^4 CFU) of mice at 10 weeks post vaccination via the oral route. As shown in Fig. 5, mice in the control group

started to die at 9 days post challenge. All the mice in the control group had died at 16 days. However, the mice vaccinated with ghost cells containing FedA and F41 antigens showed a survival rate of 25%, whereas mice vaccinated with ghost cells containing FedF and intimin antigens exhibited a higher survival rate (50%).

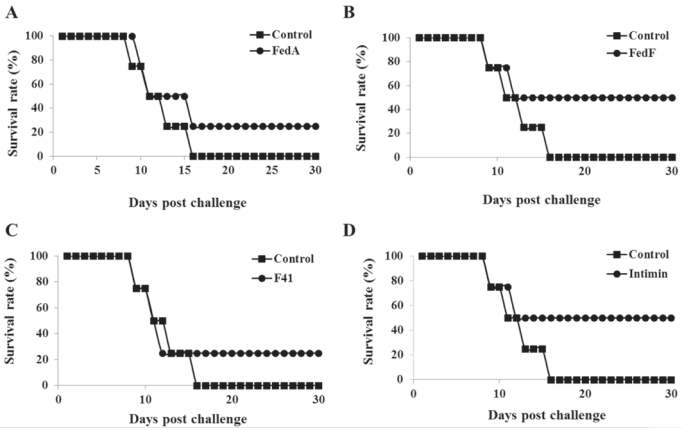

Fig. 5. Protection assay of BALB/c mice immunized by ghost cells and challenged with wild type *S*. Typhimurium. (A) FedA, (B) FedF, (C) F41, and (D) intimin. The X- and Y-axes indicate days post challenge by wild type *S.* Typhimurium χ3339 and survival rate, respectively. Control, PBS immunization; each antigen, vaccination by double injection of each antigen of 1×10^7 CFU dose (FedA, FedF, F41, Intimin).

Discussion

In order to stringently regulate expression of the *E* gene, we prepared the ghost cassette system carrying *cI*857 and anti-sense RNA (KIM et al., 2016). The *E* gene in the system is under the control of cI857, which acts as a temperature-dependent repressor at transcriptional level. The anti-sense RNA regulated by the ara system interacts with E mRNA at the translational level. Therefore, the *E* gene is repressed by anti-sense RNA when supplemented with arabinose in medium. Plasmid pMMP184 carrying the stringently regulated *E* gene is originated from the backbone of pYA3342 which includes pBR ori, multicloning site, and *asd* gene (KANG et al., 2002).

In this study, total IgG immune responses were well-elicited against *S.* Typhimurium and the ETEC antigens. When the ghost cells were administered into mice via the oral route, humoral and cellular immune responses were elicited due to various antigens, including lipopolysaccharides (LPS), monophosphoryl lipid A, peptidoglycan flagella, and cellular surface protein of ghost cell envelopes (JONES and FALKOW, 1996, RIEDMANN et al., 2007). On the other hand, since these components are recognized by toll-like receptors

(TLR), these factors elicit innate immune responses primarily (NOREEN et al., 2012; QIAN and CAO, 2013).

CD4 (cluster of differentiation 4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. CD4+ T helper cells are white blood cells that are essential to the human immune system. CD3e is a component of the T cell receptor-CD3 complex that plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways (KUHNS and BADGANDI, 2012; ZHU et al., 2013). In addition, epsilon polypeptide plays an essential role in T-cell development (FISCHER et al., 2005). Therefore, it is expected that FedF can induce defense mechanisms and stimulate an immune response associated with CD3e and CD4 in splenocytes.

CD45R/B220 is expressed in lytically active NK cells, some activated or apoptotic T cells, and some non-B-lineage hematopoietic progenitors (DRIVER et al., 2001). B-1 cells characteristically express high levels of sIgM and the B cell isoform of CD45R/B200 (GHOSN et al., 2008). B1 cells are a sub-class of B cell lymphocytes involved in the humoral immune response (ROTHSTEIN et al., 2013). CD23, the low affinity IgE Fc receptor, is expressed in mature resting conventional B cells, but not by B-1 cells (CD5+B lymophocytes), T lymphocytes, or mast cells. LPS induces transcriptional activation of CD23. CD23 production is regulated by a number of cytokines including IL-4, IL-5, IL-9, IL-13, GM-CSF, INF-γ, and CD40 (LAPA et al., 2000; ROSENWASSER and MENG, 2005; JACKSON et al., 2009). Taken together, our results suggest that ghost cells can promote the differentiation of cells associated with cell-mediated, humoral, and innate immune responses, according to the ETEC antigens.

When non-living *Salmonella* vaccines, prepared by acetone, heat, or phenol treatments, are parenterally administered to mice, the treated mice are protected from 0 to 100% against various inoculums of virulent *Salmonella* (ROBSON and VAS, 1972; EISENSTEIN et al., 1984; HARRISON et al., 1997; JAZANI et al., 2005). The results of this study are in good agreement with these related to previously known non-living *Salmonella* vaccines.

Conclusions

In our previous study, the *S.* Typhimurium ghost system was prepared by using an *E* gene to maintain a dual regulation system. The pathogenic *E. coli* antigens were normally expressed by a foreign antigen delivery system in the ghost vaccine system. When *S.* Typhimurium ghost cells were administered via the oral route to BALB/c mice, immunoglobulin Gs (IgGs) from sera were elicited by LPS and antigens. Such induction was associated with the differentiation of CD4 or CD4-T-cells in mice treated with ghost cells containing FedF antigen, whereas the remaining antigens tested in this study were

associated with CD23-B cells. Since *S*. Typhimurium ghost cells in this study protected mice at the rate of 25~50% against virulent *S*. Typhimurium, we suggest that the ghost cells have a potential as a vaccine to protect livestock against *S*. Typhimurium infection.

Acknowledgements

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SAŽETAK

Salmonella Typhimurium rekombinantna cjepiva što sadrže antigene enterotoksigenih sojeva *Escherichia coli* (ETEC) proizvedena su od soja koji posjeduje pMMP184 i nosi rekombinantnu kasetu. Istražen je imunosni odgovor BALB/c miševa oralno cijepljenih tim rekombinantnim cjepivima. Ustanovljena je visoka razina ukupnih IgG za *S.* Typhimurium nakon oralne imunizacije miševa BALB/c. Imunoglobulini IgG za antigene ETEC u rekombinantnom cjepivu koje sadrži F41 i intimin bili su dokazani četiri tjedna nakon cijepljenja. Ipak, FedF je potaknuo kasnu tvorbu imunoglobulina IgG, dok FedA nije potaknuo ili je potaknuo slab imunosni odgovor nakon cijepljenja. Proliferacija CD3e/CD4-T stanica bila je dokazana u splenocitima miševa BALB/c imuniziranih rekombinantnim cjepivom *S.* Typhimurium s ugrađenim FedF. Međutim, stanice CD45R-B220/CD23-B proliferirale su nakon cijepljenja rekombinantom FedA, F41 i intimin. Imunizirani miševi BALB/c pokazivali su 25~50% zaštitu nakon izazivačke infekcije serovarom *S.* Typhimurium u usporedbi s necijepljenim kontrolnim miševima. Stoga se pretpostavlja da oralno cijepljenje s rekombinantnim stanicama serovara *S.* Typhimurium ima potencijal da zaštiti miševe od infekcije patogenim sojevima *E. coli*.

Ključne riječi: antigen, miš BALB/c, rekombinantne stanice, pMMP184, *S.* Typhimurium, cijepljenje