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Antiviral effects of *Lactobacillus ruminis* SPM0211 and *Bifidobacterium longum* SPM1205 and SPM1206 on rotavirus-infected Caco-2 cells and a neonatal mouse model

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Rotavirus is worldwide cause of severe gastroenteritis including severe diarrhea and fatal dehydration in infants and young children. There is an available vaccination program for preventing rotavirus infection, but it has limits and restrictions. Probiotics therapy could be an alternative method of antiviral prevention and modulation against rotavirus infection. In this study, we screened the antiviral activity of probiotic bacteria such as 3 *Lactobacillus* spp. and 14 *Bifidobacterium* spp. isolated from young Korean. Three of the bacteria, *Lactobacillus ruminis* SPM0211, *Bifidobacterium longum* SPM1205, and SPM1206, inhibited human strain Wa rotavirus infection in Caco-2 cells. Furthermore, these bacterial strains inhibited rotavirus replication in a rotavirus-infected neonatal mouse model. To clarify the mechanism of inhibition, we investigated gene expression of Interferon (IFN)-signaling components and IFN-inducible antiviral effectors. All 3 probiotics increased IFN-α and IFN-β levels compared with the control. Gene expression of IFN-signaling components and IFN-inducible antiviral effectors also increased. Overall, these results indicate that *L. ruminis* SPM0211, *B. longum* SPM1205 and 1206 efficiently inhibit rotavirus replication *in vitro* and *in vivo*. Especially, the antiviral effect of *Lactobacillus ruminis* SPM0211 is worthy of notice. This is the first report of *L. ruminis* with antiviral activity. Anti-rotaviral effects of the 3 probiotics are likely due to their modulation of the immune response through promoting type I IFNs, which are key regulators in IFN signaling pathway.

Keywords: rotavirus, probiotics, antiviral effects, *Lactobacillus ruminis*, *Bifidobacterium longum*

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Introduction

Rotavirus is a worldwide cause of severe gastroenteritis in infants and young children. It occur a range of clinical symptoms including severe diarrhea, vomiting causing fatal dehydration, shock, and death. The symptoms normally disappear within 3–7 days, but may last for up to 2–3 weeks (Desselberger *et al*., 2009). Antiviral therapy is not currently available for the treatment of rotavirus gastroenteritis; fluid replacement and zinc administration have been used to prevent dehydration and treat severe diarrhea (Chandran *et al*., 2010).

As one of the prevention, rotavirus vaccines are licensed in many countries and are used routinely. The incidence rate of rotavirus disease seems similar all over the world. However, the mortality rate is due to vaccination, fatalities are rare in young children in developed countries (Parashar *et al*., 2009; Chen *et al*., 2012; WHO, 2013). Rotavirus vaccines are safe and efficient for preventing rotavirus gastroenteritis, although they have limits. The vaccination programs have difficulty distribution because of cost. So, new prevention or treatment agents to modulate rotavirus infectious disease are necessary.

Alternative method of antiviral agents is suggested application of probiotic bacteria (Chai *et al*., 2013; Kwak *et al*., 2013; Lee *et al*., 2013; Kim *et al*., 2014; Lehtoranta *et al*., 2014). Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Probiotics, such as *Lactobacillus* spp. and *Bifidobacterium* spp., are reported multiple potential health benefits including blocking gastroenteric pathogens, enhancing immune responses, and neutralizing viruses (Rolfe, 2000; Minocha, 2009; Zhang *et al*., 2013). Preidis *et al*. (2011) and Sur *et al*. (2011) reported that probiotics such as *Lactobacillus rhamnosus*, *L. casei*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus* promote the innate immune response and stimulate cellular multiplication of epithelial cells. Recently, some researchers reported that probiotics are effective in the treatment of rotavirus gastroenteritis (Dubey *et al*., 2008; Maragkoudakis *et al*., 2010; Ventola *et al*., 2012; Vlasova *et al*., 2013).

In this study, we screened the antiviral activity of 3 *Lactobacillus* spp. and 14 *Bifidobacterium* spp. isolated from young Korean. These probiotics were evaluated the antiviral activity with rotavirus-infected Caco-2 cells and a neonatal mouse model. Continuously, we investigated how probiotics suppress rotavirus infection using quantitative real time polymerase chain reaction (RT-qPCR).

Materials and Methods

Cells and viruses

The human colon adenocarcinoma cell line Caco-2 and the rhesus monkey kidney cell line Vero were obtained from the Korea Cell Line Bank. Caco-2 cells were grown in Minimum Essential Medium (MEM, Gibco Invitrogen) containing 10% fetal bovine serum (FBS, Gibco Invitrogen), 100 U/ml penicillin and 100 U/ml streptomycin at 37°C in a humidified 5% CO_2 incubator. Vero cells were grown in Roswell Park Memorial Institute 1640 medium (RPMI 1640, Gibco Invitrogen) containing 10% FBS, 100 U/ml penicillin and 100 U/ml streptomycin at 37°C in a humidified 5% CO_2 incubator.

Human rotavirus Wa strain (HRV, KBPV-VR-47) was obtained from the Korea Bank for Pathogenic Viruses. Viruses were propagated in Vero cells and harvested by freeze-thawing 3 times. HRV were concentrated by ultracentrifugation, and the titer was determined as 1.5×10^5 plaque forming unit (PFU)/ml by plaque assay on Vero cells. The virus stock was stored at -80°C until use. HRV were activated for 50 min by treatment with 5 μl/ml trypsin at 37°C.

Probiotics bacteria strains

Two probiotic bacterial genera were used: 3 *Lactobacillus* spp. and 14 *Bifidobacterium* spp. isolated from young Korean (Table 1). These were cultured at 37°C for 48 h in general anaerobic medium (GAM) broth (Nissui Pharm.) under anaerobic conditions. For the preparation of probiotic bacterial extracts, cells were harvested during the exponential growth phase by centrifugation at 4,000 rpm for 10 min, washed with phosphate buffered saline (PBS), and resuspended in the same buffer. The bacterial suspensions were adjusted to a final concentration of 1×10^9 colony forming unit (CFU)/ml and sonicated for 6 min (amplitude 100%). Cell extracts were used for further experiments after filtration.

Animal experiments

BALB/c dams and their 7 day-old pups were obtained from Koatech and housed in static microisolator cages on a special rack that delivers HEPA filtered air to each cage. Food and water were available ad libitum. This study was approved by the Duksung Women's University Institutional Animal Care and Use Committee (2013-007-003), and the experiments were performed in accordance with the guidelines of Duksung Women's University Policy on Animal Care and Use.

Mice were divided randomly into five groups; the negative control group received PBS, the three test groups received SPM0211, SPM1205 or SPM1206, respectively and the other is the normal group. The mice were inoculated with extract of Wa rotavirus (1×10^9 PFU/ml, 150–200 μl) via oral gavage over 5 consecutive days. The normal control group was segregated from the other animals to prevent infection. Weight was recorded every day until sacrifice. Fecal samples were collected from each mouse and tested for rotavirus infection using a diagnostic kit (SD). Once the virus was detected, the three probiotics, extract of SPM0211, SPM1205, and SPM1206 (1×10^9 CFU/ml, 150–200 μl), were administered orally for 3 days. Animals were sacrificed, feces were carefully removed from the colon and rectum, and the gastrointestinal tract was collected and weighed. Specimens were rinsed twice with sterile PBS and stored at -80°C.

Cytotoxicity assay

The cytotoxicity of the probiotic bacterial strains on cultured cells was assessed using the MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide] (Sigma) assay. Vero cells were seeded on 96-well plates at a density of 1×10^4 cells/well with 10% (v/v) extracts of the 3 *Lactobacillus* spp. and 14 *Bifidobacterium* spp. and incubated for 24 h. Cells with media alone were used as the control group. The incubation medium was removed and 100 μl of MTT solution was added to each well. After incubation for 4 h at 37°C, the MTT solution was removed and 100 μl of dimethyl sulfoxide (DMSO) was added to each well. Viable cells were detected by measuring absorbance at 540 nm.

Antiviral inhibition assay

Vero cells were cultured in six-well plates. Cells were inoculated with trypsin-activated HRV (1.5×10^5 PFU/ml) or 10% (v/v) extracts of the 3 *Lactobacillus* spp. or 14 *Bifidobacterium* spp. for 2 h at 37°C with 5% CO_2. The inoculum was removed, and cells were washed with PBS. The cells were covered with 2 ml of overlay media consisting of 3% agarose, 2.5% FBS and RPMI 1640 media. After the overlay media hardened, the cells were incubated for 48–72 h at 37°C with 5% CO_2. The overlay media were removed, and the cells were stained with crystal violet to observe plaque formation. We selected 3 probiotic bacteria, SPM0211, SPM1205, and SPM1206, based on the results of plaque assay on Vero cells. The three selected probiotic bacteria were used in a plaque assay on Caco-2 cells as described above.

Real Time PCR analysis

Total cellular RNA was extracted from HRV-infected Caco-2 cells using QIAamp Viral RNA Mini Kit and the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The intestinal tissues were homogenized using a tissue homogenizer. Total RNA was isolated from the tissues using RNeasy Mini Kit (Qiagen) and reverse transcribed into cDNA with the Omniscript Reverse Transcription kit (Qiagen) according to with the manufacturer's instructions. The cDNA was used as a template for real-time PCR. A real-time PCR reaction mixture was carried out in 25 μl reaction mixtures consisting of 12.5 μl of master mix, 1 μl of each primer, 2 μl of cDNA template, and 8.5 μl of sterile water. Master mix was prepared using Power Master SYBR® Green PCR Master Mix (Applied Biosystems). The human PCR primers were: Rota VP7: Sense, 5′-GGCTTTAAAAGAGAGAATTTCCG TCTCG-3′; antisense, 5′-GGTCACATCATACAATTCTA ATCTAAG-3′, GAPDH: Sense, 5′-CCATCACCATCTTCC AGGAG-3′; antisense, 5′-CCTGCTTCACCACCTTCTTG-3′. The murin PCR primers were: IFN-α: Sense, 5′-CCTGT GTGATGCAACAGGTC-3′; antisense, 5′-TCACTCCTCC TTGCTCAATC-3′, IFN-β: Sense, 5′-ATGAACAACAGGT GGATCCTCC-3′; antisense, 5′-AGGAGCTCCTGACATT TCCGAA-3′, IFNR: Sense, 5′-CATGTGTGCTTCCCACC ACT-3′; antisense, 5′-TGGAATAGTTGCCCGAGTCC-3′,

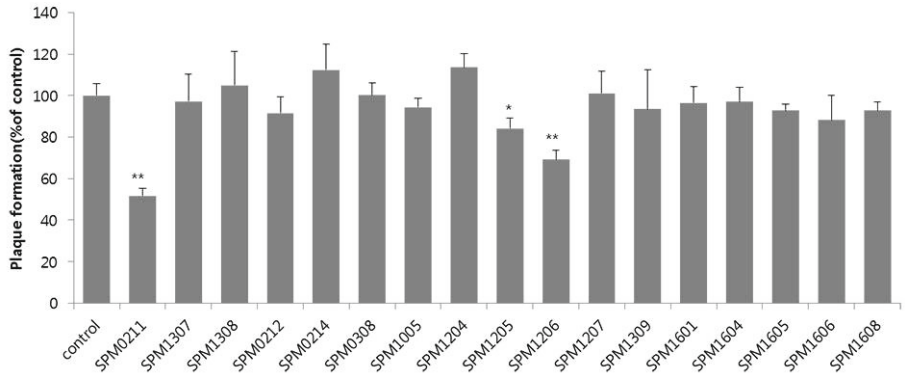

Fig. 1. Antiviral effects of probiotic bacteria isolated from young Korean on rotavirus-infected Vero cells. Vero cells (5×10^6 cell/ml) were simultaneously infected with 400 μl of rotavirus and treated with extract of probiotic bacteria [1×10^9 CFU/ml, 10% (v/v)] for 2 h. Antiviral activity was determined by plaque assay. We repeated the independent experiment at least five times. The highest and lowest values were excluded. Values shown represents the mean ± SD. Significant differences, * P<0.05, ** P<0.01

STAT1: Sense, 5′-CCATGCAAATCAGACAGTACCTGGC-3′; antisense, 5′-CCTTCACATTTCTGACTTTACTGTC-3′, OAS: Sense, 5′-GGATGCCTGGGAGAGAATCG-3′; antisense, 5′-TCGCCTGCTCTTCGAAACTG-3′, Mx1: Sense, 5′-TCTGAGGAGAGCCAGACGAT-3′; antisense, 5′-ACTCTGGTCCCCAATGACAG-3′, PKR: Sense, 5′-GCACCGGGTTTTGT-ATCGA-3′; antisense, 5′-GGAGCACGAAGTACAAGCGC-3′, GAPDH: Sense, 5′-ACGGCCGCATCTTCTTGTGCA-3′; antisense, 5′-ACGGCCAAATCCGTTCACACC-3′. Real-time quantitative PCR was conducted in an Applied biosystems® Step One Plus™ Real-Time PCR Systems (Applied Biosystems). GAPDH which are housekeeping genes, were used as internal controls. We repeated the independent experiment at least five times. The highest and lowest values were excluded. Relative quantification of the target gene was calculated using the DDCT method (Livak and Schmittgen, 2001).

Table 1. Effects of probiotic bacteria isolated from young Korean on the viability in Vero cells

Species	Origin		Cell viability (% of control)[a]
	Sex	Age	
Lactobaillus ruminis			
SPM0211	Female	21	83.04 ± 5.92
SPM1307	Female	21	87.68 ± 2.45
SPM1308	Female	21	83.97 ± 2.31
Bifidobacterium adolescentis			
SPM0212	Female	21	95.54 ± 6.70
SPM0214	Female	21	98.31 ± 0.92
SPM0308	Female	22	79.59 ± 3.81
SPM1005	Male	25	90.90 ± 1.78
SPM1601	Male	20	93.53 ± 0.97
SPM1604	Male	20	98.35 ± 0.33
SPM1605	Male	20	76.69 ± 6.77
SPM1606	Male	20	98.89 ±1.96
SPM1608	Male	20	78.79 ± 4.44
Bifidobacterium longum			
SPM1205	Female	22	98.01 ±2.69
SPM1206	Female	22	87.70 ±3.24
SPM1207	Female	22	81.13 ± 1.02
Bifidobacterium pseudocatenulatum			
SPM1204	Female	21	91.60 ±1.25
SPM1309	Male	24	82.89 ± 2.73

[a]The value represents the mean ± S.D.

Statistical analysis

Values were expressed as mean ± standard deviation (SD). Statistical significance was assessed at $P < 0.05$ for all comparisons. Statistical analysis was performed with Statistical Product and Service Solutions (SPSS) statistical software.

Results

Cell viability

Using the MTT assay, we investigated the cytotoxicity of probiotic bacteria strains on Vero cells. Cell viability after treatment with probiotic extract showed about 80% compared with control group (Table 1).

Screening of probiotics for antiviral effects *in vitro*

Initially, 3 *Lactobacillus* spp. and 14 *Bifidobacterium* spp. isolated from young Korean were used to perform plaque assays on Vero cells to select the probiotic strain with the highest inhibitory effect. Three strains, SPM0211, SPM1205, and SPM1206, suppressed Wa rotavirus infection in Vero cells compared to the control (Fig. 1). *Lactobacillus ruminis* SPM-

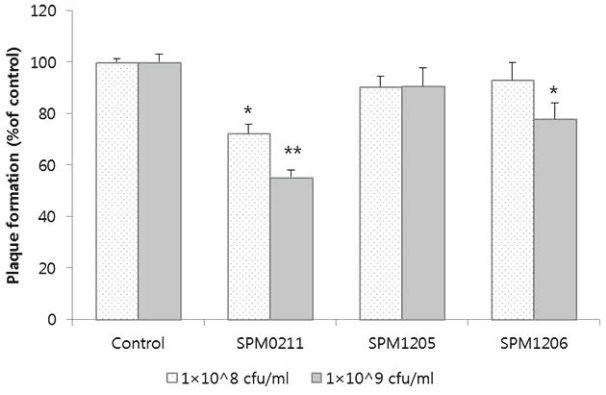

Fig. 2. Antiviral effects of SPM0211, SPM1205, and SPM1206 on rotavirus-infected Caco-2 cells. Caco-2 cells (5×10^6 cell/ml) were simultaneously infected with 400 μl of rotavirus and treated with extract of probiotic bacteria [1×10^9 CFU/ml, 10% (v/v)] for 2 h. Antiviral activity was determined by plaque assay. We repeated the independent experiment at least five times. The highest and lowest values were excluded. Values shown represents the mean ± SD. Significant differences, * P<0.05, ** P<0.01

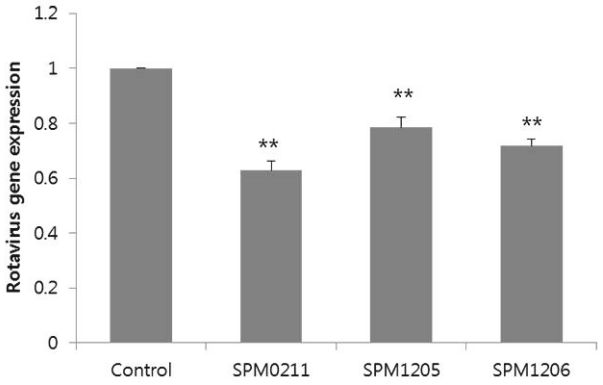

Fig. 3. Effects of SPM0211, SPM1205, and SPM1206 on rotavirus gene expression in rotavirus-infected Caco-2 cells. Caco-2 cells (5×10^6 cell/ml) were infected with 400 μl of rotavirus for 2 h followed by treatment with extract of probiotic bacteria [1×10^9 CFU/ml, 10% (v/v)] for 24 h. We repeated the independent experiment at least five times. The highest and lowest values were excluded. Values shown represents the mean ± SD. Significant differences, * P<0.05, ** P<0.01

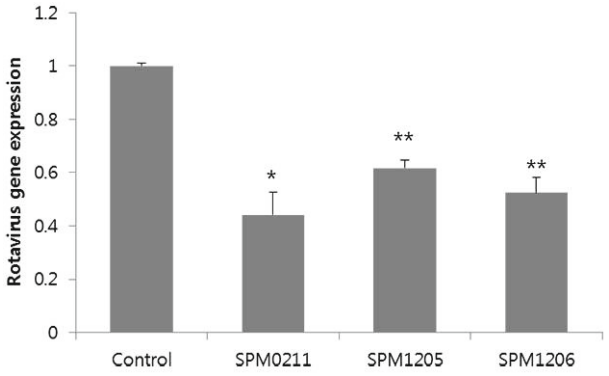

Fig. 5. Effects of SPM0211, SPM1205, and SPM1206 on rotavirus gene expression in a rotavirus-infected neonatal mouse model. Neonatal mice were inoculated with 150–200 μl of rotavirus for 5 days followed by treatment with extract of probiotic bacteria (1×10^9 CFU/ml, 150–200 μl) for 3 days. Values shown represent the mean ± SD. Significant differences, * P<0.05, ** P <0.01

0211 inhibited rotavirus infection by about 50%. *Bifidobacterium longum* SPM1206 and SPM1205 reduced plaque formation to about 32% and 17%, respectively. Based on these results, we selected these 3 probiotic bacteria, SPM0211, SPM1205, and SPM1206.

Antiviral activity of *B. longum* SPM1205, SPM1206, and *L. ruminis* SPM0211 on Caco-2 cells

We carried out plaque assays with *B. longum* SPM1205, SPM1206, and *L. ruminis* SPM0211 on Caco-2 cells as described above using either 1×10^8 CFU/ml or 1×10^9 CFU/ml of each strains. SPM0211, SPM1205, and SPM1206 all demonstrated significant antiviral activity in HRV-infected Caco-2 cells (Fig. 2), with similar results in Vero cells. *L. ruminis* SPM0211 treatment significantly decreased plaque formation. SPM0211 at 1×10^8 CFU/ml dropped plaques on Caco-2 cells by 28% compared to the control, and SPM0211 at 1×10^9 CFU/ml decreased plaque formation a further 17%, indicating that SPM0211 dose-dependently reduced the plaque formation on Caco-2 cells. *B. longum* SPM1206 showed similar results to SPM0211. To determine whether the 3 probiotics had antiviral activity through inhibition of rotavirus replication, we evaluated rotavirus RNA expression in rotavirus-infected Caco-2 cells by RT-qPCR. Rotavirus gene expression reflected similar results in Caco-2 cells treated with 3 probiotics (1×10^9 CFU/ml) after rotavirus infection (Fig. 3). SPM0211 decreased rotavirus gene expression by 40%; SPM1205 and SPM1206 dropped gene expression by 28%, 30%, respectively, compared to control group.

Antiviral activity of 3 probiotics in a Wa rotavirus-infected neonatal mouse model

We observed that treatment with SPM0211, SPM1205, and SPM1206 significantly inhibited rotavirus gene expression in HRV-infected Caco-2 cells. Thus we investigated the anti-

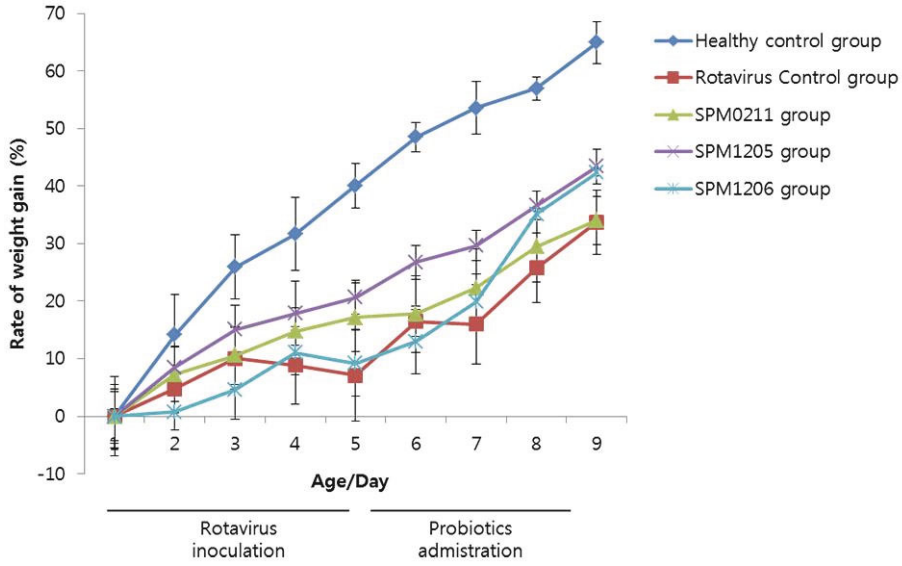

Fig. 4. The change of weight growth in a rotavirus-infected neonatal mouse model.

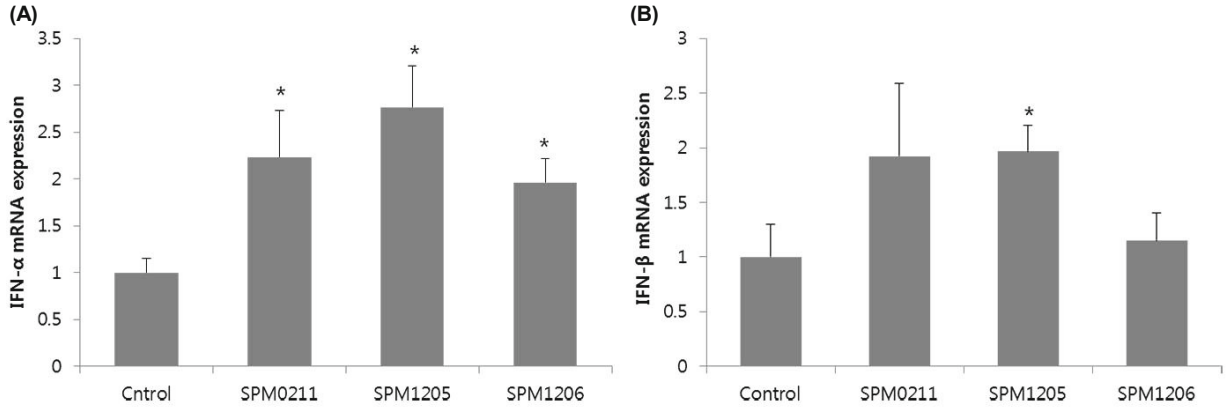

Fig. 6. Effects of SPM0211, SPM1205, and SPM1206 on rotavirus induced IFN-α (A) and IFN-β (B) gene expression in a rotavirus-infected neonatal mouse model. Neonatal mice were inoculated with 150–200 μl of rotavirus for 5 days followed by treatment with extract of probiotic bacteria (1×10^9 CFU/ml, 150–200 μl) for 3 days. Values shown represent the mean ± SD. Significant differences, * $P<0.05$

viral effects of the 3 probiotics using a rotavirus-infected neonatal mouse model. We observed body weight of neonatal mice after rotavirus infection (Fig. 4). The control group lost weight from the 4 day, whereas the normal group steadily increased in weight. These results were in agreement with the incubation period of rotavirus. In the other groups, the weight of the mice was reduced compared to the normal group after an incubation period of rotavirus. After administration of SPM0211, SPM1205, and SPM1206 (1×10^9 CFU/ml, each) in rotavirus-infected neonatal mice, the weights of the 3 groups gradually increased compared to control group.

To assess the potential antiviral activity of the 3 probiotics, we evaluated rotavirus gene expression in the rotavirus-infected neonatal mouse model. SPM0211, SPM1205, and SPM1206 significantly inhibited rotavirus gene expression in HRV-infected neonatal mice, similar to the *in vitro* results (Fig. 5). We found that the SPM0211 group had a 56% decreased in rotavirus gene expression as compare to the control group. SPM1205 and SPM1206 reduced rotavirus gene expression 39% and 47%, respectively. Both groups were increased antiviral activity compare with results of *in vitro*. We confirmed that SPM0211, SPM1205, and SPM1206 inhibited rotavirus replication in a neonatal mouse model.

Mechanism of antiviral activity of the 3 probiotics

We examined whether the 3 probiotics inhibited rotavirus infection through a type I IFN signaling pathway in Wa rotavirus-infected neonatal mice by RT-qPCR. We observed that SPM0211, SPM1205, and SPM1206 significantly increased IFN-α and IFN-β gene expression in rotavirus infected neonatal mice (Fig. 6). IFN-α and IFN-β gene expression were increased more double than control group in SPM0211 and SPM1205 treated groups. In SPM1206 treated HRV-infected mice, IFN-α expression was significantly increased but IFN-β expression was unaffected. We measured IFN receptor and signal transducer and activator of transcription (STAT) 1 gene expression, which is stimulated by type I IFNs (Fig. 7). All 3 probiotics significantly increased the levels of IFN receptor and STAT1 gene expression. SPM0211 showed the most outstanding results. We investigated gene expression of three IFN-inducible antiviral effectors: myxovirus resistance A (MxA), 20,50-oligoadenylate synthetase (OAS) and protein kinase R (PKR) (Fig. 8). MxA and OAS gene expression were increased by treatment with the 3

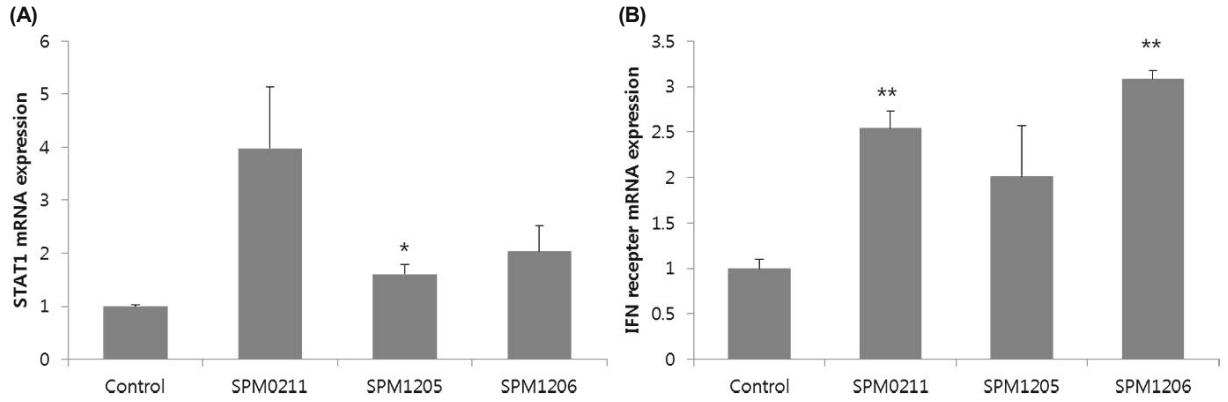

Fig. 7. Effects of SPM0211, SPM1205, and SPM1206 on rotavirus induced STAT1 (A) and IFN-receptor (B) gene expression in a rotavirus-infected neonatal mouse model. Neonatal mice were inoculated with 150–200 μl of rotavirus for 5 days followed by treatment with extract of probiotic bacteria (1×10^9 CFU/ml, 150–200 μl) for 3 days. Values shown represent the mean ± SD. Significant differences, * $P<0.05$, ** $P<0.01$

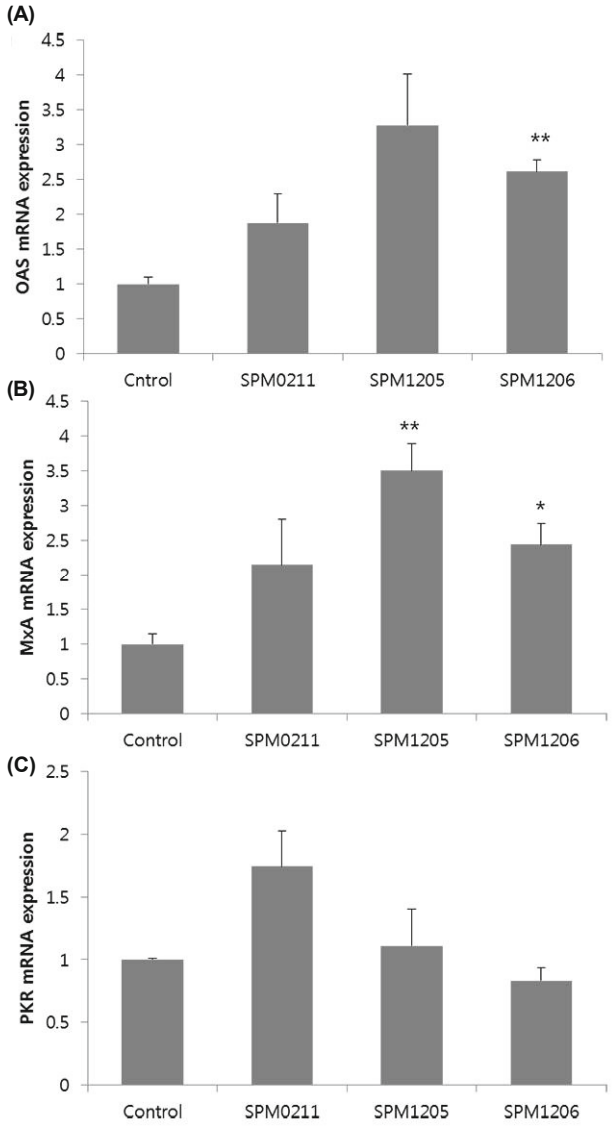

Fig. 8. Effects of SPM0211, SPM1205, and SPM1206 on gene expression levels of rotavirus induced antiviral effectors in a rotavirus-infected neonatal mouse model (A) OAS, (B) MxA, (C) PKR. Neonatal mice were inoculated with 150–200 μl of rotavirus for 5 days followed by treatment with extract of probiotic bacteria (1×10^9 CFU/ml, 150–200 μl) for 3 days. Values shown represent the mean ± SD. Significant differences, * $P<0.05$, ** $P<0.01$

probiotics. PKR expression was increased by SPM0211, but not influenced by SPM1205 or SPM1206.

Discussion

Administration of probiotics is effective as an alternative treatment for moderate rotavirus gastroenteritis as a bacteriotherapy. Recently, Guandalini (2011) and Foye *et al.* (2012) reported that probiotic bacteriotherapy is increasingly recognized to moderate infectious diarrhea and a mechanism to improve intestinal homeostasis. According to Ventola *et al.* (2012) *L. rhamnosus* GG may increase rotavirus clearance from the body and reduce colon swelling. Vlasova *et al.* (2013) reported that *L. rhamnosus* GG and *B. lactis* Bb12 moderate rotavirus–associated diarrhea in neonatal gnotobiotic pigs. A number of pediatric clinical trials were reported that *L. acidophilus*, *L. paracasei*, *L. rhamnosus GG*, *L. reuteri*, *S. thermophilus*, and the probiotic mixture (VSL3) have significant effects on the treatment of diarrhea in children (Guandalini *et al.*, 2000; Sarker *et al.*, 2005; Dubey, 2008; Misra *et al.*, 2009). These studies clearly show that probiotics and its metabolites are good alternatives as low cost prevention and treatment agents.

In this study, we screened the antiviral effects of 3 *Lactobacillus* spp. and 14 *Bifidobacterium* spp. isolated from young Korean against Wa rotavirus in infected Vero cells. We selected 3 probiotic bacteria, *Lactobacillus ruminis* SPM0211, *Bifidobacterium longum* SPM1205 and SPM1206, that showed the highest inhibitory effect against Wa rotavirus based on the results of a plaque assay on rotavirus-infected Vero cells. These probiotics showed significant antiviral activity in Wa rotavirus infected Caco-2 cells. SPM0211 decreased plaque formation by 45% compared to the control group. SPM1205 and 1206 were also inhibited 10% and 23%, respectively. According to Colbère-Garapin *et al.* (2007) probiotics could block viral attachment at the surface of intestinal cells by competitive inhibition during entry. However, these results did not confirm whether the probiotics inhibited rotavirus replication through modulation of the viral cycle. Therefore, we evaluated rotavirus RNA expression by treating Wa-infected Caco-2 cells with each of the 3 probiotics. All 3 probiotics demonstrated antiviral activity through inhibition of rotavirus replication. SPM0211 showed the most beneficial effects (40% inhibition) against rotavirus infection. SPM1205 and SPM1206 dropped gene expression by 28%, 30%, respectively, compared to control group. Taken together, these results revealed that SPM0211, SPM1205, and SPM1206 inhibited Wa rotavirus replication and infection.

We further investigated the antiviral effects of the 3 probiotics against rotavirus using a rotavirus-infected neonatal mouse model. The control group infected rotavirus lost weight from the fourth day after administration of rotavirus, whereas the normal group steadily increased in weight. In the SPM0211 and SPM1205 administrated group, the rate of weight gain gradually increased without weight loss. Although SPM1206 group lost weight from the fifth day, the rate of weight loss was small and the duration was short compared to control. We evaluated rotavirus gene expression to confirm the antiviral activity of the 3 probiotics. The SPM0211 group had a 56% decreased in rotavirus gene expression as compared to the control group. SPM1206 and SPM1205 reduced rotavirus gene expression 39% and 47%, respectively. SPM1205, SPM1206, and SPM0211 had similarly antiviral activity through inhibition of rotavirus replication *in vitro* and *in vivo*. These results indicated that all 3 probiotics might inhibit rotavirus infection and reduce symptom and duration.

Rotavirus induces inflammation by activating the cytokine response in intestinal epithelial cells (Rollo *et al.*, 1999). Interferon is a representative cytokine activated by inflammation. Type I IFNs, including IFN-α and IFN-β, are key components of the host defense against viral infections and possess pow-

erful antiviral properties (Randall and Goodbourn, 2008). IFN-α/β, released by virus-infected cells, binds to their specific IFN receptors and leads to activation of the Jak/STAT signaling pathway. Activation of STAT1 induces an IFN-mediated antiviral response through effector pathways such as the Mx GTPase pathway, the 20,50-oligoadenylate-synthetase-directed ribonuclease L pathway, the PKR pathway. These effector pathways individually block viral transcription, degrade viral RNA, inhibit translation, and modify protein function to control all steps of viral replication (Sadler and Williams, 2008). Recently, it was reported that several rotavirus strains suppress IFN production in rotavirus-infected cells (Pott *et al.*, 2011; Angel *et al.*, 2012; Shen *et al.*, 2013). IFNs have been used as anti-rotavirus agents (Feng *et al.*, 2008; Pott *et al.*, 2011). Similarly, Park *et al.* (2013) reported that *L. plantarum* DK119 exhibits antiviral effects on influenza virus infection by modulating innate immunity. And Lee *et al.* (2013) reported that *B. adolescentis* SPM0212 inhibits hepatitis B virus through an IFN-mediated antiviral response. As mentioned above, probiotics have the ability to moderate the immune response and antiviral activity through activation of various cytokines. To clarify the mechanism of antiviral effect of novel probiotics, we assessed whether *L. ruminis* SPM0211, *B. longum* SPM1205 and SPM1206 inhibited rotavirus infection through an IFN-mediated antiviral response. The results of IFN-α and IFN-β gene expressions were similar to the results of previous studies (Lee *et al.*, 2013; Park *et al.*, 2013). All 3 probiotics significantly increased IFN-α and IFN-β levels. We founded that IFN-signaling components were also significantly upregulated by each of the 3 probiotics. Besides, treatment with SPM0211, SPM1205, and SPM1206 increased gene expression of IFN-inducible antiviral effectors. These results indicated that novel probiotics modulate the immune response by promoting type I IFNs, which are key regulators in the IFN signaling pathway. Interestingly, the antiviral activity of the 3 probiotics was not congruous with the gene expression of IFN-inducible antiviral effectors. The reason of above results is predicted that rotavirus suppressed immune response by various mechanisms during the infection period. Our data were indicated that the 3 novel probiotics have antiviral activity through modulating IFN-mediated innate immunity against the rotavirus infection.

Especially, it is worth noting that *Lactobacillus ruminis* SPM0211 has antiviral effect against rotavirus. *L. ruminis* is known as autochthonous species in the gastrointestinal tract of humans and other mammals. *L. ruminis* exists in various species, but there is lack of research on the biological activity of them relatively to other Lactobacilli. Taweechotipatr *et al.* (2009) and Neville *et al.* (2012) demonstrated that *L. ruminis* exhibited immunomodulatory properties. We previously reported the antimicrobial activity of *L. ruminis* SPM0211 isolated from healthy Koreans (Yun *et al.*, 2005; Lee *et al.*, 2011). Recently, some researchers isolated *L. ruminis* strains from various species and reported the character of them (Forde *et al.*, 2011; Gärtner *et al.*, 2015; O'Donnell *et al.*, 2015). However, there was no report about the antiviral activity of *L. ruminis*. This is the first report concerning the antiviral activity of *L. ruminis*.

Conclusions

Overall, our results indicated that *L. ruminis* SPM0211 and *B. longum* SPM1205 and 1206 efficiently inhibit rotavirus replication in Wa rotavirus-infected Caco-2 cells and in an infected neonatal mouse model. The antiviral effects of the three probiotics are likely due to a modulation of the immune response through promoting type I IFNs, which are key regulators in the IFN signaling pathway. Especially, the antiviral effect of *L. ruminis* SPM0211 is worthy of notice. This is the first report of *L. ruminis* with antiviral activity. Our data suggest *B. longum* SPM1205, 1206, and *L. ruminis* SPM0211 have the potential to be a new prevention and reliever tool against rotavirus gastroenteritis.

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