Effect of the Lactobacillus rhamnosus strain GG and tagatose as a synbiotic combination in a dextran sulfate sodium-induced colitis murine model

S. J. Son,1,2 J. H. Koh,3 M. R. Park,1 S. Ryu,1 W. J. Lee,1 B. Yun,1 J.-H. Lee,4 S. Oh,5† and Y. Kim6†

1Department of Animal Science and Institute of Milk Genomics, Chonbuk National University, Jeonju 54896, Republic of Korea
2Agency of National Food Cluster, Iksan-si, Jeollabuk-do 54576, Republic of Korea
3R&D, CJ Cheil Jedang Corporation, Seoul 04560, Republic of Korea
4Department of Bioenvironmental Chemistry, Chonbuk National University, Jeonju 54896, Republic of Korea
5Department of Functional Food and Biotechnology, Jeonju University, Jeonju 55069, Republic of Korea
6Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea

ABSTRACT

Synbiotics, a combination of prebiotics and probiotics, produce synergistic effects to promote gastrointestinal health. Herein, we investigated the synbiotic interaction between the Lactobacillus rhamnosus strain GG (LGG; a probiotic strain) and tagatose (a prebiotic) in a dextran sulfate sodium (DSS)-induced colitis murine model. Initially, body weight, food intake, and clinical features were dramatically decreased after treatment with DSS, and the addition of LGG, tagatose, or both ameliorated these effects. In our pyrosequencing analysis of fecal microbiota, DSS treatment increased the abundance of Proteobacteria and decreased that of Firmicutes. When LGG and tagatose were administered as synbiotics, the gut microbiota composition recovered from the dysbiosis caused by DSS treatment. In particular, the abundance of Bacteroides, Lactobacillus, and Akkermansia was significantly associated with probiotic, prebiotic, and synbiotic treatments. Taken together, our results suggest that LGG and tagatose as synbiotics can alleviate colitis, and synbiotics could be applied as dietary supplements in dairy foods such as yogurt and cheese.

Key words: Lactobacillus rhamnosus GG, tagatose, synbiotic, DSS-induced colitis, gut microbiota

INTRODUCTION

In 1908, Elie Metchnikoff described probiotics as live microorganisms that, when consumed in adequate amounts, can confer health benefits in hosts (Guarner and Schaafsma, 1998). Many studies have highlighted the key functions of probiotics, such as blocking intestinal pathogenic microbes (Servin, 2004; Cotter et al., 2005; Song et al., 2015) and enhancing mucosal integrity (Ewaschuk et al., 2008, Shen et al., 2006). Among probiotic strains, the Lactobacillus rhamnosus strain GG (LGG) is generally recognized as safe (GRAS) and is relatively resistant to acid and bile, adheres to epithelial and intestinal mucosal cells (Tuomola and Salminen, 1998; Ouwehand et al., 1999), exerts antimicrobial activity (Silva et al., 1987), and colonizes the human intestine (Goldin et al., 1992; Alander et al., 1997). Although previous studies (Isolauri et al., 1991; Bennett et al., 1996; Hilton et al., 1997), including clinical trials (Szajewska et al., 2001; Kuisma et al., 2003), have shown how LGG prevents diarrhea, multifaceted analyses of the protective effects of LGG in colitis induced animal model have been unsubstantiated.

Prebiotics are indigestible carbohydrates that beneficially affect the host by selectively stimulating growth and activity of specific bacterial species in the colon to improve host health (Glenn and Roberfroid, 1995; Schrezenmeir and de Vrese, 2001). Importantly, tagatose is a naturally occurring monosaccharide as well as an isomer of galactose and stereoisomer of fructose. It has a low energy status (38% of the calories of sucrose; 1.5 kcal/g) but sweetness of up to 90% of sucrose (Cheetham and Wootton, 1993; Armstrong et al., 2009). Since 2001, the FAO and WHO registered tagatose as GRAS for use in food and beverages as a low-calorie ingredient with putative health-promoting benefits. However, few studies have assessed whether probiotic bacteria can utilize tagatose as a prebiotic in treating colitis in an in vivo animal model.

Although probiotics and prebiotics are administered individually, a combination of probiotics and prebiotics (i.e., a synbiotic) may also be beneficial (de Vreese 2018).
MATERIALS AND METHODS

**Bacterial Strains and Culture Conditions**

*Lactobacillus rhamnosus* strain GG (ATCC 53103; LGG) was obtained from stock cultures maintained by the Institute of Milk Genomics, Chonbuk National University (Jeonju, Korea). The LGG strain was grown in de Man, Rogosa, and Sharpe broth (Difco Laboratories, Detroit, MI) at 37°C for 18 h. For long-term storage, the stock cultures were maintained at −80°C using 50% glycerol as a cryoprotectant.

**Tagatose**

Tagatose, provided by CJ CheilJedang Corp. (Seoul, Korea), was used as a food-grade prebiotic substrate in this study. Tagatose was dissolved in PBS (Welgene Inc., Daegu, Korea) and administered orally to mice.

**Animal Experiments**

All animal experiments were approved by the Animal Care and Use Committee of Chonbuk University (Jeonju, Korea) and performed in accordance with the University’s Guidelines for the Care and Use of Laboratory Animals (2015–05). Figure 1A represents a schematic of the experimental plan. All mice were 6-wk-old female BALB/c mice purchased from Orient Bio Inc. (Seongnam, Korea). Groups of 7 or 8 mice were housed in isolator cages (JD-C-07, Jeungdo Bio & Plant Co., Seoul, Korea) with sterilized bedding under the following auto-controlled conditions: lighting (12 h light, 12 h dark), temperature (24°C), and relative humidity (55%). The acclimation period was 14 d. After acclimation, mice were placed in one of the following groups: (1) DSS-control (DSS-C), (2) prebiotic (PRE; tagatose), (3) probiotic (PRO; LGG), or (4) synbiotics (SYN; combination of tagatose and LGG). These mice were administered, respectively, PBS (200 µL of suspension per mouse), tagatose (25 mg), LGG (10⁹ cfu/mL), or LGG and tagatose (10⁹ cfu/mL of LGG and 25 mg of tagatose) every other day for 3 wk. Suspensions were given by oral gavage (Fuchigami Kikai, Kyoto, Japan). We also had a no-DSS control group (CON; mice administered PBS only for 21 days; no DSS induction of colitis). In addition, mice placed in an antibiotics (ANT) treatment group were orally administered 100 µL of sulfasalazine (Sigma Aldrich, St. Louis, MO) every other day for 2 wk, with this treatment initiated 1 wk after the other treatments began (d 8).

To induce experimental acute colitis, mice in the 4 treatment groups (DSS-C, PRE, PRO, SYN) plus the ANT group were provided drinking water containing low-molecular-weight DSS (5% DSS with a molecular weight of 36,000–50,000 Da, MP Biomedicals, Solon, OH; Okayasu et al., 1990; Martinez et al., 2006) for an additional week after the 2-wk study period. During the 3-wk period, daily clinical assessment of DSS-induced colitis was performed, including measuring BW and food intake and noting rectal bleeding, stool conditions, and blood in stool using the disease activity index (DAI) scoring system (Chassaing et al., 2014). The DAI score was calculated as the scores of BW loss (0 = none; 1 = 1 to 5%; 2 = 6 to 10%; 3 = 11 to 20%; 4 = >20%), stool consistency (0 = normal; 2 = loose stool; 4 = diarrhea), and stool blood (0 = negative; 2 = fecal occult blood test positive; 4 = gross bleeding). After 3 wk, all mice were killed for downstream experiments.

**Morphological and Biological Analyses of Colon and Serum Samples**

Mice were anesthetized with isoflurane (Hana Pharm, Seoul, Korea) using an inhalation narcosis control (JD-C-107A, Jeungdo Bio & Plant Co., Seoul, Korea), and the everted sac method was used as previously described (Enokizono et al., 2007). Mice were immediately dissected and everted colon (below the cecum) sacs were prepared by quickly removing the small intestine. Colon sacs were dissected and washed with ice-cold PBS to measure the length. Afterward, colon samples were fixed in 10% neutral buffered formalin solution (Sigma Aldrich, St. Louis, MO) and embedded in paraffin for histological assessments. Sections (4 µm)
of colonic mucosa were stained with hematoxylin and eosin (H&E; Rees, 1998).

Blood samples were collected in BD Vacutainer blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) after cardiac puncture and then centrifuged at 2,000 × g for 10 min to separate serum and plasma. The expression of proinflammatory cytokines, including IL-6, IL-10, and tumor necrosis factor (TNF)-α, from serum samples was determined using a magnetic Luminex screening assay according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN) on a Bio-Plex 200 analyzer (Bio-Rad, Hercules, CA).

**Pyrosequencing Analysis of Fecal Microbiota**

Fecal samples for microbiota analysis were collected using sterile forceps every other day for 3 wk
and stored at −80°C until further use. Total DNA was extracted from the collected fecal samples and subjected to pyrosequencing of the V1–V3 region of the bacterial 16S rRNA gene using a barcoded primer set (27F: 5'-GAGTTTGATCMTGGCTCAG-3' and 800R: 5'-TACCCAGGGTATCTAATCC-3') and the GS-FLX sequencing system (Roche, Basel, Switzerland). The raw sequence data were analyzed using Mothur software, version 1.39.5 (Schloss et al., 2009). Sequencing errors and chimeras were removed according to previous procedures (Schloss et al., 2011; Kozich et al., 2013). Low-quality reads and undesirable sequences, such as those of chloroplast, mitochondria, and Archaea, were removed, and chimera sequences were screened using UCHIME (Edgar et al., 2011). Sequences were aligned and classified against the SILVA release 128, non-redundant SSU Ref data set (Quast et al., 2013). Sequences were classified to operational taxonomic units (OTU) at 3% dissimilarity, and the sequence number was normalized by subsampling randomly for the downstream analyses of α-diversity, as assessed by phylogenetic information, Chao 1, Shannon, and Simpson, and β-diversity, assessed by principal coordinates analysis (PCoA).

**Statistical Analysis**

 Differences in values from each experiment were determined by using the Student’s t-test. Each result is representative of at least 3 independent biological replicates. A P-value of < 0.05 in all replicate experiments was considered statistically significant.

**RESULTS AND DISCUSSION**

**Changes in BW, Food Intake, and Diarrhea Score**

To assess the protective effects of LGG and tagatose in DSS-induced mice, we measured BW, food intake, and diarrhea score of mice every other day. As shown in Figure 1B, BW was significantly lower in the DSS-C group than in the other groups at d 21 (P < 0.05). Interestingly, the BW of mice in the SYN group was higher than that of mice in the PRO and PRE groups during periods of DSS treatment except at d 21. Moreover, the SYN group had the largest difference in BW measured between d 1 and 21 (2.30 ± 0.03 g). The control (PBS only) DSS mice showed the smallest difference in BW between d 1 and 21 (−0.09 ± 0.06 g). Moreover, food intake was significantly lower in the DSS-C group than in the other groups (P < 0.05; data not shown). Oral administration of DSS successfully induced colitis, which was first manifested as diarrhea and then as bloody stools. Consistent with our BW observations, the diarrhea (DAI) scores of the SYN, PRE, and PRO groups were significantly lower than those in the DSS-C groups (P < 0.05; Figure 1C). Mice in the SYN group were less susceptible to colitis than mice in other treated groups throughout the experimental periods. There were no differences among diarrhea scores of the SYN (1.42 ± 0.58), PRE (1.33 ± 0.82), and PRO (1.00 ± 0.71) groups at d 21. Taken together, these results indicate that LGG and tagatose, as a synbiotic, effectively alleviated BW loss, increased food intake, and decreased diarrhea scores. Thus, synbiotics may help in preventing severe weight loss in colitis cases.

**Expression of Proinflammatory Cytokines in Serum**

Cytokines are involved in colitis pathology and several cytokines induced by lactic acid bacteria play key roles in immune regulation (Lee et al., 2011; Kim et al., 2015). We investigated the expression of proinflammatory cytokines IL-6, IL-10, and TNF-α in mice with DSS-induced colitis. These proinflammatory cytokines...
Figure 2. Effects of oral administration of *Lactobacillus rhamnosus* strain GG (LGG) and tagatose as synbiotics on colon length (A) and intestinal damage (B). Comparison of colon lengths (mean ± SD) among tested groups with or without dextran sulfate sodium (DSS) treatment. Mice were in placed in one of the following groups: (1) DSS-control (DSS-C), (2) prebiotics (PRE; tagatose), (3) probiotics (PRO; LGG), (4) synbiotics (SYN; combination of tagatose and LGG) or (5) antibiotics only (ANT); CON is a no-DSS control. Asterisks indicate a statistically significant difference compared with DSS-C (*P* < 0.05). (C) Hematoxylin and eosin staining of colon tissue sections, 200× magnification; scale bar = 200 µm.
play a crucial role in creating an immunogenic microenvironment and function in the response against inflammation (Mantovani et al., 1992). The abundance of these cytokines increased in DSS-treated mice (Figure 3). As expected, the PRO, PRE, and SYN treatments significantly inhibited the expression of IL-6 and IL-10. The expression of TNF-α was significantly decreased only in the SYN group. Interestingly, the level of TNF-α in the PRO group was numerically slightly higher than that of DSS-C group but not significantly ($P > 0.05$). Thus, the SYN treatment was most effective in inhibiting the transcription of proinflammatory cytokine genes (Figure 3). Several studies have reported that specific Lactobacillus strains can induce the production of IL-6, IL-10, TNF-α, and IFN-γ, as well as anti-inflammatory cytokines such as IL-10 and transforming growth factor β (TGF-β; Christensen et al., 2002). We observed that LGG and tagatose as synbiotics inhibited the regulation of proinflammatory cytokines and prevented initiation of the inflammatory response.

**Analysis of Gut Microbiota**

We performed amplicon sequencing on fecal samples. Under OTU with 3% dissimilarity, a variable number of sequences were normalized to 1,709 per group by random subsampling, and we found that the subsampled sequences sufficiently represented the microbial communities with an average Good’s coverage of 98.6 ± 1.9% from all treatment groups. The observed bacterial OTU number was larger in the control group (145.1) than in the other groups by about 4- to 12-fold, as supported by Chao1 and the abundance-based coverage estimator, which are counts for species. For diversity, including richness and evenness by the Shannon and inverse Simpson indices, the DSS-induced microbial community showed higher diversity compared with that in the other groups (data not shown).

As expected, the gut microbiota community in mice with DSS-induced colitis showed an increased abundance of Proteobacteria (47.0% in DSS) compared with that in the CON group (1 out of 2,970 sequences for CON; Figure 4A). All Proteobacteria in the DSS-induced group were from the Enterobacteriaceae family, including Cosenzaea, Escherichia, Proteus, and Shigella. Enterobacteriaceae were also found in the ANT group (19.7%), yet the Bacteroidaceae was the dominant family observed (70.5%; Figure 4A). In the CON group, the majority of the bacterial community was Bacteroidetes (57.5%), followed by Firmicutes (41.7%). Bacteroidetes were predominant in the PRE, PRO, and SYN groups (97.8, 96.9, and 86.8%, respectively), with the remainder of bacterial diversity attributed to the Firmicutes phylum. Bacteroides was most common across the treatment groups. Interestingly, the PRO group had 3.1% Firmicutes bacteria, composed of the Lactobacillus genus and the Clostridiales order (Figure 4B).

Bacterial diversity was compared between the groups using the calculated Jaccard index, theta YC (Yue and Clayton) distance (Yue and Clayton, 2005), and weighted UniFrac analysis (Lozupone and Knight, 2005). Dendrograms clustered using the Jaccard index, theta YC, and weighted UniFrac indicated groupings for the PRO, PRE, and SYN groups, whereas the DSS-induced group showed a substantial separation from the rest of the groups (Figure 4C). Nonmetric multidimensional scaling (NMDS) analysis was performed to visualize the theta YC distances (Figure 4D). The

---

**Figure 3.** Protein levels (mean ± SD) of secreted proinflammatory cytokines: IL-6 (A), IL-10 (B), and tumor necrosis factor (TNF)-α (C), in blood serum samples as quantified by ELISA. Mice were in placed in one of the following groups: (1) DSS-control (DSS-C), (2) prebiotics (PRE; tagatose), (3) probiotics (PRO; LGG), or (4) synbiotics (SYN; combination of tagatose and LGG). Asterisks indicate a statistically significant difference compared with DSS-C ($P < 0.05$).
NMDS diagram showed that the communities of the PRO, PRE, SYN, and ANT groups were separated from those of the DSS-C group. In addition, bacterial abundances at the genus level were presented on a heatmap that showed correlations among taxa and the groups depicted by dendrograms based on the Bray–Curtis dissimilarity matrix (Figure 4E). Communities of the SYN group showed a relatively well-grouped cluster, which was similar to the above-described dendrograms in Figure 4C.

From the PCoA on the weighted (quantitative) UniFrac distance metric, we found a similar pattern to that of the NMDS analysis of the theta YC distance (Figure 5). It seemed that the responsible OTU, according to the Spearman rank correlation coefficient, were similar to those in the NMDS analysis. Notably, the DSS-C

![Figure 4. Pyrosequencing of gut microbiota. Relative sequence abundance of bacterial phyla (A) and families (B); dendrogram between the communities by unweighted pair group method with arithmetic means (UPGMA; C); nonmetric multidimensional scaling diagram based on theta YC distances (stress value of 0.066 and R² of 0.99) (D); and heatmap indicating abundances at genus level, with the removal of genera that were <1% across the samples (dendrograms represent similarities between the samples and corresponding taxa, based on the Bray–Curtis dissimilarity matrix; E). Mice were in placed in one of the following groups: (1) DSS-control (DSS-C), (2) prebiotics (PRE; tagatose), (3) probiotics (PRO; LGG), (4) synbiotics (SYN; combination of tagatose and LGG) or (5) antibiotics only (ANT); CON is a no-DSS control.](image-url)
group and the PRO, PRE, and SYN groups were found to be significantly different ($P < 0.001$). Interestingly, OTU 1 (*Bacteroides*), OTU 3 (*Lactobacillus*), and OTU 12 (*Akkermansia*) were mostly responsible for moving group positions in a negative and positive direction, respectively, along the horizontal axis (PCoA1). The appearance of these OTU was significantly associated with probiotic, prebiotic, or synbiotic treatment. Inflammation is intimately connected to the gut microbiota and the immune system (Schwabe and Jobin, 2013). A recent report illustrated that *Lactobacillus casei* dramatically increased the abundance of *Bifidobacterium*, *Lactobacillus*, and short-chain fatty acid (SCFA)-producing bacteria, including *Bacteroides*, in a murine model (Wang et al., 2017). Moreover, others reported that detrimental microbial metabolites, such as secondary bile acids, promote carcinogenesis, whereas major bacterial fermentation products (i.e., SCFA), such as butyrate, propionate, and acetate, have health-promoting activity in the colon (Flint et al., 2012). *Akkermansia* is a mucin-degrading bacterium commonly found in the gut. It has been reported that *Akkermansia* is associated with inflammation and metabolic disorders, even though its function is debatable. The presence of enough fiber improves the function of *Akkermansia* in the development and progression of metabolic diseases (Desai et al., 2016). In addition, oral administration of common prebiotic substrates promotes the growth of *Akkermansia* in the intestinal tract (Zhou, 2017); hence, we considered that synbiotics could regulate the diversity of potential probiotic bacteria in the gut and possibly reduce the incidence of DSS-induced colitis. However, the OTU responsible for moving along the vertical axis (PCoA2) in the negative direction included OTU 2 (*Escherichia–Shigella*), OTU 7 (*Escherichia–Shigella*), OTU 11 (*Proteus*), OTU 13 (*Enterobacteriaceae_unclassified*), OTU 19 (*Erysipelotrichia*), OTU 22 (*Enterococcus*), and OTU 23 (*Escherichia–Shigella*). Importantly, the DSS group was separated from the other groups, mostly due to OTU that caused shifts to the vertical direction. Overall, the results suggest that the intestinal bacterial community

---

**Figure 5.** Two-dimensional principal coordinate analysis (PCoA) of weighted UniFrac distance. The first and second axes represent 22.6 and 15.8% of the variation for the distances, respectively (total 38.4%). The arrows show operational taxonomic units (OTU) responsible for shifting samples along the 2 axes; OTU 1 and 3 were mostly responsible for moving sample positions in a negative and positive direction, respectively, along the horizontal axis (PCoA1). The OTU responsible for moving along the vertical axis (PCoA2) included OTU 2, 7, 11, 13, 19, 22, and 23 to the negative direction. Mice were in placed in one of the following groups: (1) DSS-control (DSS-C), (2) prebiotics (PRE; tagatose), (3) probiotics (PRO; LGG), (4) synbiotics (SYN; combination of tagatose and LGG) or (5) antibiotics only (ANT); CON is a no-DSS control. The dextran sulfate sodium (DSS) sample was separated from the other samples mostly due to OTU that shifted to the vertical direction.
was affected by DSS-induced colitis. These community members were in turn restored by the administration of LGG and tagatose as synbiotics.

**CONCLUSIONS**

Herein, we illustrated that LGG and tagatose as synbiotics may alleviate colitis symptoms in a DSS-induced mouse model. Therefore, the protective functions and roles of synbiotics in human colon health may shed light on novel applications in the dairy food industry.

**ACKNOWLEDGMENTS**

This research was supported by the Ministry of Trade, Industry & Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) and Establishment of Infrastructure for Industrialization of Korean Useful Microbes (R0004073) and the Cooperative Research Program for Agriculture Science & Technology Development, Rural Development Administration, Republic of Korea (Project No. PJ01197801).

**REFERENCES**


