ABSTRACT

Probiotics can ferment nondigestible carbohydrates and produce short-chain fatty acids (SCFA; acetate, propionate, and butyrate) in the human colon. In this study, the levels of SCFA were determined in the following yogurts fermented with different combinations of probiotics: (1) cocultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (control, C); (2) *S. thermophilus*, *L. bulgaricus*, and *Bifidobacterium bifidum* (C-Bb); (3) *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus acidophilus* (C-La); and (4) *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus gasseri* (C-Lg). Results showed that the acetate levels were significantly higher in C-Bb, C-La, and C-Lg yogurts than in C yogurt. Fermentation and physicochemical characteristics of all yogurts were identical. Treatment of mucus-secreting colon epithelial cells (HT29-MTX) with C-Bb, C-La, and C-Lg yogurt supernatants resulted in an increase in the expression of *MUC2* and *CDX2* and the production of mucin proteins. The adhesion of probiotics onto HT29-MTX cells increased following treatment with C-Bb, C-La, and C-Lg yogurt supernatants. Our data suggest that a yogurt diet rich in acetate improves the protective function of the intestinal epithelium.

Key words: probiotics, short-chain fatty acids, mucin, HT29-MTX

INTRODUCTION

Yogurt is one of the most popular dairy foods produced by fermentation using starter culture bacteria with or without probiotics (Prasanna et al., 2013). Probiotics in yogurt may prevent inflammatory bowel disease and diarrhea and enhance gut immunity (Linares et al., 2017). For instance, yogurt was shown to enhance the intestinal barrier function by alleviating IL-1β-induced intestinal inflammation (Zhai et al., 2019). *Lactobacillus* and *Bifidobacterium* are commonly used probiotics for yogurt preparation that produce various health-promoting compounds during fermentation, including vitamins, γ-aminobutyric acid, bioactive peptides, bacteriocins, conjugated linoleic acid, and exopolysaccharides (Linares et al., 2017). In addition, *Lactobacillus* and *Bifidobacterium* species can produce short-chain fatty acids (SCFA) through the fermentation of pyruvate (Pessione, 2012). Probiotics such as *Bifidobacterium bifidum* MF 20/5 and *Lactobacillus gasseri* PA 16/8 produce more acetate and propionate than *Lactobacillus rhamnosus* GG in de Man, Rogosa, and Sharpe (MRS) medium (LeBlanc et al., 2017). In addition, *Lactobacillus acidophilus* ATCC 11975 produced SCFA in MRS medium in the presence of prebiotics (Fernando et al., 2010; Farooq et al., 2013).

Short-chain fatty acids are microbial metabolites naturally produced in the human colon via bacterial fermentation of nondigestible carbohydrates through several pathways (Feng et al., 2018). Short-chain fatty acids are important regulators of gut homeostasis and epithelial barrier maintenance (Spiljar et al., 2017). The SCFA also stimulate the secretion of mucus that acts as a physicochemical barrier to cover and protect the underlying epithelium from pathogens, toxins, and endogenous substances (Volstatova et al., 2019). For instance, butyrate was reported to stimulate colonic mucus secretion in Sprague-Dawley rats (Shimotoyodome et al., 2000). The main constituents of the mucus layer are mucins, which are high-molecular-weight epithelial glycoproteins encoded by more than 20 different mucin genes such as *MUC2* and *MUC5AC* (Byrd and Bresalier, 2004; Boegh and Nielsen, 2015). In particular, *MUC2* is the most abundant mucin found in the intestinal mucus secreted by goblet cells (Rodríguez-Piñeiro et al., 2013). Genes such as *CDX2* and *TFF3* are known to be related to mucin expression (Gao et al., 2018).

The aim of this study was to evaluate the properties of yogurt fermented with SCFA-producing probiotics. Acidification kinetics, physicochemical characteristics,
and composition of SCFA in yogurt were measured. In addition, the bioactive properties of yogurt, including mucin expression and adhesion ability of probiotics, were determined in human colon epithelial cells (HT29-MTX goblet cells).

### MATERIALS AND METHODS

#### Lactic Acid Bacteria Strains

For their application in yogurt production, *Streptococcus salivarius* ssp. *thermophilus* KCTC 3779, *Lactobacillus delbrueckii* ssp. *bulgaricus* KCTC 3635, *L. acidophilus* KCTC 3171, *L. gasseri* KCTC 3163, and *B. bifidum* KCTC 3202 were obtained from the Korean Collection for Type Cultures (Jeongeup, Korea). Two lactic acid bacteria, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, were selected as conventional starters. Three probiotics, *B. bifidum*, *L. acidophilus*, and *L. gasseri*, were selected as potential probiotics known to produce high amounts of SCFA (Fernando et al., 2010; LeBlanc et al., 2017). *Streptococcus thermophilus*, *L. bulgaricus*, *L. acidophilus*, and *L. gasseri* were maintained on MRS agar and subcultured every 2 wk. A colony was obtained from the agar plate, inoculated into lactobacilli MRS broth (BD Difco), and incubated for 16 h at 37°C. *Bifidobacterium bifidum* was maintained on MRS agar supplemented with 0.05% cysteine in an anaerobic jar with AnaeroPack (Mitsubishi Gas Chemical) and subcultured every 2 wk. A colony was inoculated into MRS broth supplemented with 0.05% cysteine and incubated twice for 48 h in an anaerobic jar at 37°C.

#### Yogurt Production

Yogurt was prepared as previously described with some modifications (Rutella et al., 2016; Mousavi et al., 2019; Kim et al., 2020). Market milk [Seoul Dairy Cooperative, Seoul, Korea; 4% (wt/vol) fat] was pasteurized at 85°C for 30 min and then cooled to 42°C. Probiotics were inoculated into 30 mL of cooled milk, which was then incubated at 42°C until the pH reached 4.6; the milk was then stored at 4°C overnight. This experiment comprised 4 different yogurt samples fermented by different organisms as follows: (1) co-cultivation of *S. thermophilus* and *L. bulgaricus*, as seen in conventional yogurt (C; 1.5 × 10⁶ cfu/mL of each starter); (2) *S. thermophilus*, *L. bulgaricus*, and *B. bifidum* (C-Bb; 1 × 10⁶ cfu/mL of each starter); (3) *S. thermophilus*, *L. bulgaricus*, and *L. acidophilus* (C-La; 1 × 10⁷ cfu/mL of each starter), and (4) *S. thermophilus*, *L. bulgaricus*, and *L. gasseri* (C-Lg; 1 × 10⁷ cfu/mL of each starter).

#### Yogurt Supernatant Separation

For GC and cell culture studies, we separated supernatants by centrifugation of yogurt samples (10 g) at 3,000 × g for 20 min at 4°C and then at 10,000 × g for 30 min. The yogurt supernatants were filtered through a 0.45-µm syringe filter (Advantec). In addition, to remove residual proteins from the supernatants, 1 mL of yogurt supernatants were mixed with 200 µL of 25% meta-phosphoric acid and then centrifuged at 20,000 × g for 20 min (Pylkas et al., 2005; Farooq et al., 2013). The protein-free supernatants were filtered using 0.45-µm syringe filters and used for GC and cell culture experiments.

#### Measurements of Acidification Kinetics

The pH value of the yogurt was estimated during fermentation every 30 min using a pH meter (Mettler Toledo). The acidification kinetic parameters of yogurt samples were measured as previously described (Jeong et al., 2018). The maximum acidification rate (*V*<sub>max</sub>), time taken to reach *V*<sub>max</sub> (*T*<sub>max</sub>), time required to reach pH 5.0 (*T*<sub>pH 5.0</sub>), and time required to complete fermentation (*T*<sub>f</sub>) were calculated. The acidification rate was calculated as pH time variation (dpH/dt) and expressed as 10⁻³ pH units/min.

#### Measurements of Physicochemical Characteristics

Titratable acidity was measured by titrating 10 g of the yogurt blended with 10 mL of distilled water using 0.1 N NaOH solution until pH 8.3. Titratable acidity was calculated as percent lactate (Alhejaili et al., 2019). Titratable acidity was calculated as below:

\[
\text{Titratable acidity (\%)} = \frac{\text{used NaOH (mL)} \times 0.009}{\text{weight of yogurt (g)} \times 100},
\]

where 0.009 is the conversion factor for lactic acid.

The viscosity of the yogurt was measured using a DV-E Viscometer with 63 spindle (Brookfield). The spindle was immersed in the yogurt and viscosity was measured at 1-min intervals from 5 to 8 min (Kwon et al., 2019).

Water-holding capacity (WHC) was determined as previously described (Zhang et al., 2019). Yogurt samples (10 g) were centrifuged at 2,000 × g for 10 min at 4°C, and the supernatants were weighed. The WHC was calculated as below:

\[
\text{WHC (\%)} = \left(1 - \frac{W_2}{W_1}\right) \times 100,
\]
where \(W_1\) = weight of yogurt and \(W_2\) = weight of supernatant after centrifugation.

**Gas Chromatography Analysis**

The compositions of SCFA and lactate in yogurt supernatants were analyzed using a 6890 gas chromatography system (Agilent Technologies) equipped with a flame-ionization detector and DB FFAP column (122–3232, 30.0 m length, 250 µm film, 0.25 µm internal diameter; Agilent Technologies; Playne, 1985; Zened et al., 2012; Trigueros and Sendra, 2015). Yogurt supernatants (1 mL) and 50 µL of 2% pivalic acid (wt/vol) were added to a vial. Pivalic acid was used as an internal standard and 1 µL of the sample was injected into the gas chromatograph system (split 1:50). A standard solution (100 mL) was prepared by mixing acetate, propionate, butyrate, lactate, and pivalic acid in deionized water. The flow rate of the carrier gas (helium) was 1.6 mL/min. The temperature program started at 100°C for 1 min, and the temperature was increased by 20°C/min to 190°C, held at 190°C for 5 min 18 s, and finally increased to 200°C and held for 1 min at 200°C.

**Cell Culture**

Mucus-secreting human colon epithelial cells, HT29-MTX, were used to evaluate the expression and secretion of mucin as previously described (Mahler et al., 2009; Volstatova et al., 2019). The HT29-MTX cells were cultured in high glucose Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin in a CO2 incubator at 37°C. The HT29-MTX cells were cultured until 100% confluency and then treated with protein-free yogurt supernatants.

**Real-Time PCR Analysis**

To examine the expression of MUC2, CDX2, and TFF3, HT29-MTX cells were cultivated on 12-well plates (Adamczak et al., 2016; Wu et al., 2019) and treated with yogurt supernatants at a final concentration of 5% for 6 h. The mRNA expression was determined by real-time PCR (Thermo Scientific). Relative quantification of mRNA expression was performed by the \(\Delta\Delta Cq\) method using GAPDH as the reference control gene. The primer sequences used were MUC2 (forward 5’-ACAACCCCAACTTACGCTGA-3’ and reverse 5’-GCTGTCATCATCTCAATGGCAGTGG-3’), CDX2 (forward 5’-GGACACCTTGCGAGTGG-3’ and reverse 5’-TGTCACCTGTAGTGGAACACTCC-3’), and GAPDH (forward 5’-GACCCCTTCATTGACCTCAACTAC-3’ and reverse 5’-ATGACAAAGCTTCCGTTTCAG-3’).

**Alcian Blue Staining for Detection of Mucin Glycoproteins**

To determine the secretion of mucin glycoproteins, Alcian blue staining (Sigma-Aldrich) was performed (Araújo and Sarmento, 2013; Schimpel et al., 2014). The HT29-MTX cells were treated with yogurt supernatants at final concentrations of 5% and 10% for 24 h. Following treatment, cells were washed twice with PBS, fixed with 4% paraformaldehyde for 30 min, and then washed again with PBS. To stain mucin glycoproteins, a 1% Alcian blue solution in 3% acetic acid was added to each well and the cells were incubated for 30 min. Alcian blue solution was removed, and the cells were washed with PBS. Mucin production was examined using an Olympus IX71 microscope at a magnification of 200×, and the images were digitally captured using an Olympus DP71 camera and DP controller software (Olympus Optical Co. Ltd.). Quantification of the staining area was performed using ImageJ software (Sato et al., 2014).

**Determination of Probiotics Adhesion Onto HT29-MTX Cells**

To evaluate the effects of yogurt supernatants on the adhesion of probiotics onto HT29-MTX cells, Lactobacillus plantarum ATCC 8014 and L. rhamnosus ATCC 7469 served as test organisms. The adhesion properties were evaluated as previously described (Jayashree et al., 2018; Cheng et al., 2020). In brief, L. plantarum and L. rhamnosus were activated in MRS broth. The HT29-MTX cells cultured on 12-well plates were treated with yogurt supernatants at a final concentration of 5% for 24 h, washed with PBS, and exposed L. plantarum and L. rhamnosus resuspended in antibiotic-free DMEM at a multiplicity of infection of 10:1 for 1 h. After incubation, HT29-MTX cells were washed with PBS and treated with 500 µL of 0.1% Triton X-100 for 10 min. Serial dilutions of cell lysates containing adhered bacteria with PBS were spread on MRS agar and incubated at 37°C for 48 h, followed by enumeration of viable colony count.

**Statistical Analysis**

All data are expressed as mean ± standard error of the mean. Statistical significance was determined by an independent 2-sample 𝑡-test and the Tukey’s post-hoc
RESULTS AND DISCUSSION

Changes in pH and Acidification Kinetics of Yogurts

The changes in pH and acidification kinetic parameters of yogurt were measured to determine whether the different combinations of probiotics affect these properties of yogurt. The pH of C-Bb, C-La, and C-Lg yogurts decreased faster than that of C yogurt during fermentation (Figure 1). It has been reported that yogurt is fermented by mixing conventional starter culture bacteria with probiotics to reduce fermentation time (Damin et al., 2008). In fact, C-Bb, C-La, and C-Lg yogurts had higher Vmax values than C yogurt, and their fermentation time decreased (Table 1). The tmax of C-Bb yogurt was lower than that of C yogurt, whereas C-La and C-Lg yogurts showed higher tmax values than C yogurt. Both tph 5.0 and tf values of C-Bb, C-La, and C-Lg yogurts were lower than those of C yogurt.

Physicochemical Characteristics of Yogurts

The titratable acidity of C-Bb, C-La, and C-Lg yogurts was slightly higher than that of C yogurt, but the difference was not significant (Table 2). The viscosity of C-Bb, C-La, and C-Lg yogurts was higher than that of C yogurt. The C-Lg group showed markedly higher viscosity, and this starter combination could produce substances that enhance viscosity. Some probiotics are known to produce exopolysaccharides that improve the texture of the yogurt (Han et al., 2016). In fact, exopolysaccharides can stabilize the structure of yogurt (Fazilah et al., 2018). The WHC was similar between different yogurt groups but was the highest for C yogurt (Table 2). WHC reflects the coagulation stability of yogurt (Mousavi et al., 2019). The difference in WHC among yogurt groups was not remarkable except for C and C-La yogurts. Taken together, the combination of each probiotic (B. bifidum, L. acidophilus, and L. gasseri) with starter bacteria (S. thermophilus and L. bulgaricus) produced quality characteristics similar to those of the control yogurt fermented with S. thermophilus and L. bulgaricus.
Concentrations of SCFA in Yogurts

The levels of SCFA (acetate, propionate, and butyrate) and lactate in yogurt were measured using GC (Figure 2). The concentration of acetate in yogurt ranged from 0.874 to 1.267 mmol/L and was significantly higher in C-Bb, C-La, and C-Lg yogurts than in C yogurt (Figure 2A). Heterofermentative lactic acid bacteria such as Lactobacillus strains can produce acetate and lactate via the phosphoketolase and Wood-Ljungdahl pathways (Zaunmüller et al., 2006; Koh et al., 2016). Bifidobacterium species such as Bifidobacterium adolescentis yield acetate and lactate via the bifid shunt route (Flint et al., 2015). In our study, we inoculated each group with the same (3 × 10⁷ cfu/mL) density of bacteria. The combination of S. thermophilus and L. bulgaricus (C) seems to produce acetate, whereas the additional supplementation with B. bifidum, L. acidophilus, or L. gasseri would result in the production of higher levels of acetate during fermentation. In a previous study, the level of acetate in the yogurt inoculated with S. thermophilus and B. animalis ssp. lactis (1.2 ± 0.0 g/L) was higher than that in the yogurt inoculated with S. thermophilus (de Souza Oliveira et al., 2012). In contrast, we found that the concentrations of propionate (0.118–0.157 mmol/L), butyrate (0.091–0.143 mmol/L), and lactate (0.262–0.274 mmol/L) were not significantly different between different yogurt groups (Figure 2B–2D). Similar results were reported for L. rhamnosus, L. acidophilus, Bifidobacterium longum, and Bifidobacterium breve, which produced higher amounts of acetate than propionate and butyrate (Fernando et al., 2010); the amount of acetate produced was 1,000 times higher than that of propionate (LeBlanc et al., 2017). The pathway underlying propionate and butyrate production by Lactobacillus and Bifidobacterium is yet unknown. Probiotics are known to produce lactate through the pathways used for producing acetate, but there was no difference in lactate levels among different yogurt groups. Similar data have been reported in the past studies where no significant difference in lactate contents was found in yogurts by the variety of inoculated lactic acid bacteria (Prasanna et al., 2013; Ozturkoglu-Budak et al., 2019).

Expression of Mucin mRNA in HT29-MTX Cells Treated with Yogurt Supernatants

Mucin, a substance produced by the mucus layer on the intestinal epithelium, enhances the gut immunity by protecting the epithelial barrier from pathogens or toxins (Boegh and Nielsen, 2015). The HT29-MTX cells are human colon epithelium-derived goblet cells that produce mucin and are widely used to observe mucin expression (Smirnova et al., 2003; Volstato et al., 2019). Several studies have demonstrated the increase in the expression of mucin-encoding genes mediated by SCFA such as acetate in human colorectal epithelial cells (LS174T; Willemsen et al., 2003; Burger-van Paassen et al., 2009). We evaluated the effects of yogurt supernatants at 5% or 10% concentration on the secretion of mucin by HT29-MTX cells. The supernatant from C yogurt supplemented with 2 mmol/L acetate was used as a positive control. The expression of MUC2, CDX2, and TFF3 was evaluated by treating cells with 5% yogurt supernatants. MUC2 is a major mucin protein in the mucus layer, whereas CDX2 regulates the expression of MUC2 (Yamamoto et al., 2003). The expression of MUC2 and CDX2 was significantly higher in HT29-MTX cells treated with C-La (P < 0.005) as well as C-Bb and C-Lg (P < 0.05) yogurts than in those treated with C yogurt (Figure 3A and B). The mRNA levels of MUC2 and CDX2 in untreated cells were lower than those detected in cells treated with C yogurt (P < 0.05), whereas the positive control showed the highest expression of MUC2 and CDX2. Our data suggest that acetate contributed to the expression of these genes because the positive control, C-Bb, C-La, and C-Lg yogurts contained higher levels of acetate than C yogurt. In previous studies,
SCFA such as acetate, propionate, and butyrate were shown to stimulate the expression of mucin in T84 and LS174T cells (Willemsen et al., 2003). Further, higher concentrations of acetate (1–15 mmol/L) induced MUC2 expression in LS174T cells (Burger-van Paassen et al., 2009). On the contrary, the expression level of TFF3 was not significantly different between the various treatment groups (Figure 3C). The TFF3 protein also plays a role in protecting the intestinal mucin environment by increasing the viscosity of mucin and repairing the damaged mucus layer (Matsuda et al., 2008, Petrou and Crouzier, 2018). However, studies have shown that SCFA reduce the expression of TFF3 in colon cancer cells (Tran et al., 1998) or have no effect on the expression of TFF3 in mice (Nakamura et al., 2017). In our experimental setting, we assumed that SCFA or lactate in yogurt supernatants failed to influence the expression of TFF3 in HT29-MTX cells.

Figure 2. The concentrations of acetate (A), propionate (B), butyrate (C), and lactate (D) in yogurt, as determined by gas chromatography. C = yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*; C-Bb = yogurt fermented with *S. thermophilus*, *L. bulgaricus*, and *Bifidobacterium bifidum*; C-La = yogurt fermented with *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus acidophilus*; C-Lg = yogurt fermented with *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus gasseri*. Results represent means ± SEM (n = 3). Means with different letters (a,b) indicate significant differences (P < 0.05).
Secretion of Mucin Glycoproteins in HT29-MTX Cells Treated with Yogurt Supernatants

Alcian blue staining was performed to evaluate the secretion of mucin glycoproteins. Results showed that HT29-MTX cells treated with C-Bb, C-La, and C-Lg yogurt supernatants had higher staining intensity than those treated with C yogurt supernatant (Figure 4A and B). Treatment of cells with 10% yogurt supernatants resulted in higher staining intensity than the treatment with 5% yogurt supernatant. Faint staining was observed for control cells without any yogurt supernatant treatment, whereas the positive control treated with 2 mmol/L acetate showed the highest staining intensity. Our data provide evidence that acetate activates the secretion of mucin glycoproteins by increasing the expression of $MUC2$ and $CDX2$ and that may contribute to defense against invasion of pathogens. Like our data, SCFA increased intestinal integrity in the intestinal epithelial cells. For example, SCFA mixtures (i.e., acetate, propionate, and butyrate) improved intestinal barrier function by increasing trans-epithelial electrical resistance in Caco-2 cells (Chen et al., 2017).

Adhesion Ability of Probiotics Onto HT29-MTX Cells

To evaluate the positive role of mucin produced by yogurt supernatants in HT29-MTX cells, we examined the adhesion ability of $L.\ plantarum$ and $L.\ rhamnosus$ onto HT29-MTX cells. The adhesion of $L.\ plantarum$ onto HT29-MTX cells tended to increase following cell treatment with C-Bb, C-La, and C-Lg yogurts (Figure 5A). The adhesion ability of $L.\ rhamnosus$ also slightly increased after the treatment of cells with C-Bb, C-La, and C-Lg yogurts (Figure 5B). Further, the positive control containing 2 mmol/L acetate enhanced the adhesion abilities of these probiotics onto HT29-MTX cells (Figure 5A and B). Probiotics colonize the intestinal epithelium, compete with pathogens that inhibit the adhesion and invasion of pathogens, and protect the epithelium (Kim and Ho, 2010). It has been reported that $Escherichia\ coli$ was reduced by acetate produced by $B.\ bifidum$ (Fukuda et al., 2011) and butyrate stimulated mucin production and increased the adhesion of $L.\ acidophilus$ ATCC 4356 and $B.\ longum$ ATCC 15707 to human colon epithelial cells, thereby inhibiting the adhesion of $Escherichia\ coli$ ATCC 43896 (Jung et al., 2015). Similarly, our data also demonstrated the increase in the adhesion of probiotics onto intestinal epithelial cells following treatment with yogurt supernatants, particularly through the mucin produced by acetate. Previously published data also demonstrated that SCFA including acetate plays an important role in the intestinal epithelium. Vinegar, which is rich in acetate, increased tight junction mRNAs (i.e., ZO-1, occludin, and claudin-1; Xia et al., 2020a), and regulated gut microbiota compositions by increasing $Lactobacillus$ and $Bacteroidetes$ in alcohol-ingested mice (Xia et al., 2020b). Therefore, we assumed that intake of foods containing high acetate, such as yogurt and vinegar, can improve intestinal health through producing mucin proteins and modulating gut microbiota.
Figure 4. The staining of acidic mucin glycoproteins (A) and quantification of relative staining area (B) were performed using Alcian blue staining method. For staining, HT29-MTX cells were treated with 5% or 10% yogurt supernatants in Dulbecco’s modified Eagle medium and incubated for 24 h. C = yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*; C-Bb = yogurt fermented with *S. thermophilus*, *L. bulgaricus*, and *Bifidobacterium bifidum*; C-La = yogurt fermented with *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus acidophilus*; C-Lg = yogurt fermented with *S. thermophilus*, *L. bulgaricus*, and *Lactobacillus gasseri*. Values represent means ± SEM (n = 3). Different letters (a–f) indicate significant differences (P < 0.05).
CONCLUSIONS

We demonstrated that the yogurt fermented with probiotics (B. bifidum, L. acidophilus, and L. gasseri) contain high SCFA, particularly acetate, which may exert beneficial effects on the production of mucin by HT29-MTX cells. Fermentation, acidification kinetic parameters, and physicochemical characteristics of the yogurts were not significantly different following supplementation with additional probiotics. The expression levels of MUC2 and CDX2 in HT29-MTX cells treated with yogurt supernatants were consistent with the acetate content of the yogurt. In addition, mucin glycoprotein production and the adhesion of L. plantarum and L. rhamnosus to HT29-MTX cells increased following treatment with yogurt supernatants containing high amounts of acetate. This study suggests that consumption of yogurt containing high amounts of SCFA, such as acetate from the fermentation of probiotics, may contribute to the protection of the intestinal barrier through epithelial mucin production.

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