Low-sugar yogurt making by the co-cultivation of *Lactobacillus plantarum* WCFS1 with yogurt starter cultures

S. S. Zhang,¹ Z. S. Xu,² L. H. Qin,¹ and J. Kong^{1*}

¹State Key Laboratory of Microbial Technology, Shandong University, Qingdao, 266237, P. R. China

²State Key Laboratory of Biobased Material and Green Papermaking, Qilu University of Technology, Shandong Academy of Science, Jinan, 250353, P. R. China

ABSTRACT

Yogurt making traditionally relies on the simultaneous utilization of 2 starters: *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, but these 2 strains normally metabolize the glucose portion of lactose and release galactose into extracellular medium, resulting in high levels of residual galactose and unfermented lactose in yogurt, which leads to several industrial and health concerns. In this study, we found that *Lactobacillus plantarum* could effectively metabolize both lactose and galactose. Comparative genomic analysis demonstrated the constant presence of a chromosome-encoded Leloir pathway for galactose metabolism in *Lb. plantarum* species, and the *gal* operon was driven by a strong constitutive promoter in *Lb. plantarum* WCFS1, displaying great potential in low-sugar yogurt making. To test this hypothesis, *Lb. plantarum* WCFS1 was co-cultured with *S. thermophilus* or *Lb. bulgaricus* in lactose-based medium. Results showed that lactose was consumed completely and galactose was metabolized efficiently. For yogurt making, co-cultivation of *Lb. plantarum* WCFS1 with yogurt starter cultures produced a higher reduction of total sugar content compared with the traditional fermentation processes. In addition, the sensory analysis indicated that the yogurt fermented with yogurt starter cultures and *Lb. plantarum* WCFS1 was acceptable to consumers in appearance, texture, and flavor. Therefore, this study emphasized the potential to manufacture low-sugar yogurt by the co-cultivation of *Lb. plantarum* with yogurt starter cultures.

Key words: *Lactobacillus plantarum*, low-sugar yogurt, traditional starter, Leloir pathway

INTRODUCTION

Yogurt is one of the most popular fermented dairy products and has high consumer acceptability world-

wide for its high nutritional value, unique flavor, and health benefits (Benozzi et al., 2015; Aryana and Olson, 2017). Yogurt is traditionally produced by the cultivation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Horiuchi et al., 2009). However, most strains of *S. thermophilus* and *Lb. bulgaricus* only metabolize the glucose moiety of lactose and release the galactose into the medium, resulting in significant amounts of galactose as well as high levels of residual unfermented lactose in yogurt (Turner and Martley, 1983; Hols et al., 2005). Lactose and galactose are not recommended for individuals with symptoms of lactose intolerance and galactosemia (Van Calcar et al., 2014; Silanikove et al., 2015). In addition, residual galactose in fermented dairy products was associated with several industrial problems such as reduced quality of fermented products, browning in pizza, and textural defects in cheese (Neves et al., 2010; Wu et al., 2015). Therefore, efforts to remove or reduce lactose and galactose are of great importance to human health and industrial processes (Chollet et al., 2013).

Lactase is commonly used to hydrolyze lactose into galactose and glucose to produce lactose-free milk (Jelen and Tossavainen, 2003; Harju et al., 2012). This method is expensive and cannot eliminate the galactose from milk. The obtained dairy foods are only suitable for individuals with lactose intolerance and not for the patients with galactosemia. In this case, various chromatographic methods for the separation of lactose from other milk constituents were carried out, which can eventually produce lactose-free and galactose-free milk products (Harju et al., 2012). However, these commercial lactose-free dairy products are sold in unfermented status. Considering the nutrition and health-promoting benefits of yogurt compared with nonfermented dairy products, research has been done on isolating or constructing wild-type or modified *S. thermophilus* strains with galactose-positive (**Gal**⁺) phenotype, and then coculturing with *Lb. bulgaricus* for the preparation of low-galactose yogurt (Robitaille et al., 2007; Anbukarasi et al., 2014a). However, the modified strain only metabolized galactose when the amount of lactose was

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*Corresponding author: kongjian@sdu.edu.cn

less than 0.1% (wt/vol), and the Gal⁺ phenotype was not expressed during milk fermentation and mozzarella cheese production (Robitaille et al., 2007). Moreover, there is a ceiling on lactose hydrolysis when *S. thermophilus* and *Lb. bulgaricus* grow in milk. Therefore, an alternative method is needed to manufacture lactose-free or galactose-free yogurt.

Lactobacillus plantarum is a versatile facultative hetero-fermentative lactic acid bacterium present in plant-based fermented foods as well as meat, fish, and fermented dairy products (Marco et al., 2017). Its dual role as an indigenous human gut inhabitant and a safe starter culture in food fermentation makes it a preferred candidate for the development of novel functional products (Capozzi et al., 2017). Several studies have confirmed that consuming *Lb. plantarum*-containing fermented milk brought in vivo functionality to host health (Chiu et al., 2006; Valente et al., 2019). However, the effects of *Lb. plantarum* on residual lactose or galactose in fermented dairy foods have not been reported yet. In our previous studies, 2 functional β -galactosidase genes responsible for lactose hydrolysis were identified in the genome of *Lb. plantarum* WCFS1. Here, we investigated the genetic organization of the lactose hydrolysis and galactose metabolism in *Lb. plantarum* by comparative genome analysis, and determined the residual sugar content in the medium when *Lb. plantarum* WCFS1 was inoculated alone or co-cultured with traditional yogurt starters. The objective of this study was to determine whether the content of lactose or galactose could be decreased by the addition of *Lb. plantarum*, as well as its effect on the properties of dairy products.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Cultivation Conditions

Bacterial strains and plasmids used in this study are listed in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-17347>). As the host for plasmid construction, *Lactococcus lactis* MG1363 was grown statically in M17 broth (Oxoid, Basingstoke, UK) supplemented with 0.5% (wt/vol) glucose (GM17) at 30°C. *Streptococcus thermophilus* CGMCC 7.179 was a Gal⁺ strain and SDMCC050215 was a Gal⁻ strain, and they were grown statically in M17 broth supplemented with 1% (wt/vol) lactose (LM17) at 42°C, and the strains of *Lb. plantarum* and *Lb. bulgaricus* were grown statically in de Man, Rogosa, and Sharpe (MRS) medium (Oxoid, Basingstoke, UK) at 37°C. The M17-galactose broth was prepared by supplemented with 1% (wt/

vol) galactose in M17 broth. The MRS-lactose or MRS-galactose broth was prepared by substitution of glucose with 1% (wt/vol) lactose or galactose in MRS medium, respectively. If necessary, chloramphenicol with a final concentration of 5 μ g/mL was added to the medium for *Lc. lactis* or *Lb. plantarum* recombinant strains.

Growth and Metabolism of *Lb. plantarum* WCFS1 and Traditional Starters in Lactose/Galactose-Based Medium

The MRS-lactose and MRS-galactose medium were used to test the ability of *Lb. plantarum* WCFS1 and *Lb. bulgaricus* to use lactose and galactose. The LM17 and M17-galactose medium were used to test the ability of *S. thermophilus* to use lactose and galactose. After overnight static incubation, *Lb. plantarum* WCFS1 and *Lb. bulgaricus* (ATCC11842 and SDMCC050201) were inoculated (2% vol/vol) into the MRS-lactose and MRS-galactose medium at 37°C, *S. thermophilus* (CGMCC 7.179 and SDMCC050215) were inoculated (2% vol/vol) into the LM17 and M17-galactose medium at 42°C. Samples of the cultures were collected at interval of 2 h for analysis during a period of 24 h. The growth was measured by optical density at 600 nm (OD₆₀₀) with Biophotometer. The pH was measured with a glass electrode pH meter (Mettler Toledo, Zurich, Switzerland). The content of residual sugars and metabolites in the cultures were determined by HPLC as described previously (Xu et al., 2017).

Molecular Manipulation

All the molecular manipulations in this study were performed as described previously (Xu et al., 2018). Ex Taq polymerase, restriction enzymes, and T₄ DNA ligase were used according to manual protocols (TaKaRa, Tokyo, Japan). Genomic DNA of *Lb. plantarum* WCFS1 was extracted using the Bacterial Genomic Extraction Kit (Tiangen, Beijing, China). Primers Pgal-F (5'-CG-CAGATCTGAGGAAGCTCCTTTCAAAGT-3') and Pgal-R (5'-CGCGAATTCCATGAAAAATCCCTC-CGTAAATAG-3') were designed for amplification of the *gal* operon promoter from the genomic DNA of *Lb. plantarum* WCFS1. The PCR product was digested with *Bgl*II and *Eco*RI and cloned into the corresponding sites of plasmid pSec:Leiss:Nuc, resulting in recombinant plasmid pGal. Primers GFP-F (5'-CGCGAATTCAG-CAAAGGAGAAGAAGCTTTT-3') and GFP-R (5'-GCGGATATCTTAGTATAGCTCATCCATGC-3') were used for amplification of the reporter gene *gfp* using pCD4033-gfp as the template (Sun et al., 2010). The PCR product was digested with *Eco*RI and *Eco*RV and

ligated into the corresponding sites of plasmid pGal, generating the recombinant plasmid pGal-GFP.

Fluorescence Analysis

Plasmid pGal-GFP was transformed into *Lb. plantarum* WCFS1, generating recombinant *Lb. plantarum* WCFS1/gal-GFP. This strain was grown statically in the MRS-glucose, MRS-lactose, and MRS-galactose media at 37°C for 12 h. Cells were harvested by centrifugation at $6,000 \times g$ for 5 min at 4°C, washed twice, and resuspended in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 2 mM KH₂PO₄, pH 7.4). For measurement of fluorescence intensity, 200 µL of bacterial suspension was transferred into a 96-well plate, and the Multi-Detection Microplate Reader (BioTek, Winooski, VT) was used by excitation at 485 nm and emission at 535 nm. The empty vector-containing strain was used as control in each respective medium.

Coculture of *Lb. plantarum* with Traditional Starters in Lactose-Based Medium

Streptococcus thermophilus strains (CGMCC 7.179 and SDMCC050215) and *Lb. bulgaricus* strains (ATCC11842 and SDMCC050201) were used to test the utilization of lactose and galactose with or without *Lb. plantarum* WCFS1. The cultures of *S. thermophilus* were inoculated (2% vol/vol) to the LM17 broth at 42°C and *Lb. bulgaricus* were inoculated (2% vol/vol) to the MRS-lactose broth at 37°C. When co-cultured, 2% (vol/vol) *Lb. plantarum* WCFS1 was added. Samples of the cultures were collected at interval of 2 h for analysis during a period of 24 h. The growth was measured by OD₆₀₀ and viable cell counts. The pH was measured with a glass electrode pH meter. The sugars and lactic acid were measured by HPLC as described above.

Fermented Milk

Reconstituted skim milk (RSM) medium was prepared by reconstituting skim milk powder (10% wt/vol) with distilled water and sterilized at 121°C for 15 min. Three strains (*S. thermophilus*, *Lb. bulgaricus*, and *Lb. plantarum* WCFS1) were co-cultured in the ratio of 1:1:1 in 100 mL of RSM medium at 42°C for 12 h, and the fermentation without *Lb. plantarum* WCFS1 was used as control. The curd time was observed visually, which indicated the time when milk changed from a flowing liquid to an immobile semi-solid state. At the end of the fermentation, the samples were cooled in an ice-water bath and stored at 4°C for 14 d. The determi-

nations of lactose and galactose content, pH, titratable acidity, and syneresis were carried out at the end of fermentation, and the viable cell counts were detected at 0, 7, and 14 d as described by da Costa et al. (2016) and Li et al. (2017).

Sensory Analysis

Thirty panelists were selected and invited to take part in the sensory analysis. The yogurt samples were served in 20-mL plastic cups coded with random digit numbers at approximately 10°C. Water was provided between evaluations of samples for mouth rinsing. Sample acceptance including the appearance, texture, and flavor was rated using a 10-point hybrid hedonic scale, where 1 = disliked extremely and 10 = liked extremely. Overall mean score was calculated for each sample by averaging the score of all participants.

Statistical Analysis

Each experiment was carried out in triplicate, and the data were presented as mean $\pm$ standard deviation. Statistical significance between yogurts fermented by traditional starters with or without *Lb. plantarum* WCFS1 was performed using the Statistical Packages for the Social Sciences (SPSS version 19.0, IBM Corp., Armonk, NY). Probability values of <0.05 were considered as statistically significant.

RESULTS

Utilization of Lactose and Galactose by *Lb. plantarum* WCFS1

Lactobacillus plantarum WCFS1 was cultivated for 24 h in the medium using lactose as the sole carbon source. Results showed that 99% lactose was consumed, galactose was almost undetectable, and a high level of lactic acid was produced, which indicated that *Lb. plantarum* WCFS1 could metabolize lactose effectively and consume galactose at the same time (Figure 1A). To further confirm the ability of *Lb. plantarum* WCFS1 to use galactose, it was cultivated in the MRS-galactose medium. The results indicated that *Lb. plantarum* WCFS1 could consume 94% galactose after 24 h of incubation with high production of biomass, and a diminution of pH until 4.2 (Figure 1B and Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-17347>). As for traditional yogurt starters, the *Lb. bulgaricus* strains tested were unable to survive in the medium with galactose as the sole carbon source, whereas the *S. thermophilus* results were strain dependent in the galac-

Figure 1. Residual sugars and lactic acid content in de Man, Rogosa, and Sharpe (MRS)-lactose (A) and MRS-galactose (B) inoculated with *Lactobacillus plantarum* WCFS1. The contents of lactose, galactose, and lactic acid are represented by solid squares, solid circles, and solid triangles, respectively. Error bars represent SE from 3 replicate experiments.

tose utilization. The optical density at 600 nm (OD_{600}) of *S. thermophilus* CGMCC 7.179 and SDMCC050215 reached values of 1.3 and 0.5, and the pH reached values of 5.3 and 5.7, respectively (Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-17347>). These results indicated that *Lb. plantarum* WCFS1 was more capable to use lactose and galactose than *S. thermophilus* and *Lb. bulgaricus* strains.

Genetic Organization for Lactose/Galactose Metabolism in *Lb. plantarum* WCFS1

To investigate the lactose or galactose metabolic flux, we predicted the metabolic pathway by Kyoto Encyclopedia of Genes and Genomes (KEGG database) in *Lb. plantarum* WCFS1. As shown in Figure 2A, lactose or galactose was imported through the PTS regulated carbohydrate transporter LacS and RafP. Subsequently, lactose was hydrolyzed by cytosolic β -galactosidase into glucose and galactose, and these 2 monosaccharides were metabolized by the Embden-Meyerhof-Parnas pathway and Leloir pathway, respectively. In our previous studies, 2 functional β -galactosidase genes (*lacA* and *lacLM*) responsible for lactose hydrolysis were identified in the genome of *Lb. plantarum* WCFS1 (Zhang et al., 2019). In this study, we found that the genes involved in galactose metabolism were located in the *gal* operon in the order of *galKE₂T* in *Lb. plantarum* WCFS1. Among them, *galK* gene encoded the galactokinase, which is responsible for ATP-dependent phosphorylation of α -D-galactose to galactose 1-phosphate. The *galT* gene encoded the galactose-1-phosphate uridylyltransferase, catalyzing the transfer of a UMP (uridine monophosphate) group from UDP (uridine diphosphate)-glucose to galactose 1-phosphate, thereby generating glucose

1-phosphate and UDP-galactose. The *galE* gene encoded the UDP-glucose 4-epimerase, responsible for converting UDP-galactose to UDP-glucose to complete the Leloir pathway. In addition, a regulator gene *galR* was located downstream of the *gal* operon and was transcribed in the same direction (Figure 2B).

To test the activity of the Leloir pathway in *Lb. plantarum* WCFS1, the promoter P_{gal} of *gal* operon was cloned and ligated with the GFP reporter gene, generating the recombinant plasmid pGal-gfp to explore the characteristics of this promoter. As results, the GFP under *gal* promoter in *Lb. plantarum* WCFS1 could express efficiently in the medium supplemented with glucose, lactose, or galactose as the sole carbon source with fluorescence intensity of 455,651, 476,936, and 369,153, respectively (Figure 3). In addition, compared with the promoter P_{ldh} of the lactate dehydrogenase gene (*ldh*) from *Lb. delbrueckii* 11842, which has been proven to be a constitutive promoter for the intracellular production of heterologous proteins in lactobacilli (Sun et al., 2010), the strength of promoter P_{gal} was 7- to 9-fold higher than that of promoter P_{ldh} (data not shown). These results suggested that the *gal* operon promoter P_{gal} in *Lb. plantarum* WCFS1 was a strong constitutive promoter. It was accordance with the above galactose metabolism data of *Lb. plantarum* WCFS1, indicating that the *gal* operon was active in *Lb. plantarum* WCFS1.

Effects of *Lb. plantarum* on the Growth and Metabolites of Traditional Starters

The traditional yogurt starters *S. thermophilus* (CGMCC 7.179 and SDMCC050215) and *Lb. bulgaricus* (ATCC11842 and SDMCC050201) were adopted to

Figure 2. Diagram for lactose and galactose metabolism (A) and genetic organization of *lac* and *gal* operon (B) in *Lactobacillus plantarum* WCFS1. EPS = exopolysaccharides; UDP = uridine diphosphate.

test the growth and metabolism in lactose-based medium with or without *Lb. plantarum* WCFS1. When *S. thermophilus* CGMCC 7.179 and SDMCC050215 were cultured alone, the OD₆₀₀ reached values of 4.2 and 3.8, and the pH reached values of 5.3 and 4.9, respectively.

Figure 3. Effects of different carbon sources on the fluorescence intensity of *Lactobacillus plantarum* WCFS1 (black bar) and WCFS1/gal-GFP (gray bar). Error bars represent SE from 3 replicate experiments.

When co-cultured with *Lb. plantarum* WCFS1, the OD₆₀₀ increased to 5.0 and 6.0, and pH decreased to 4.5 and 4.0, respectively. The same phenomenon was also observed when the strains *Lb. bulgaricus* ATCC11842 and SDMCC050201 were cultured alone or co-cultured with *Lb. plantarum* WCFS1, the OD₆₀₀ increased from 3.0 and 4.0 to 7.8, and pH decreased from 4.8 and 4.5 to 4.2, respectively (Figure 4). These results indicate that co-cultivation of *Lb. plantarum* WCFS1 with traditional starters could increase the total biomass and reduce pH in lactose-based medium.

The results of viable cell count for each strain are shown in Supplemental Figure S2 (<https://doi.org/10.3168/jds.2019-17347>). The survival of *S. thermophilus* and *Lb. bulgaricus* was not damaged in the co-cultivation with *Lb. plantarum* WCFS1. In addition, *Lb. plantarum* WCFS1 remained at high levels around 9 log cfu/mL in the mixed culture, whereas the viable cells counts of *S. thermophilus* and *Lb. bulgaricus* were lower compared with *Lb. plantarum* WCFS1. In effect, *S. thermophilus* CGMCC 7.179 reached 8 log cfu/mL, whereas *S. thermophilus* SDMCC050215, *Lb. bulgaricus* ATCC11842 and *Lb. bulgaricus* SDMCC050201 only reached 7 log cfu/mL.

The determination of residual sugars and lactic acid content in the lactose-based medium showed that

Figure 4. Growth curves and pH changes of *Streptococcus thermophilus* CGMCC 7.179 (A), *S. thermophilus* SDMCC050215 (B), *Lactobacillus bulgaricus* ATCC11842 (C) and *Lb. bulgaricus* SDMCC050201 (D) with or without *Lb. plantarum* WCFS1 in lactose-based medium. Absorbance (optical density at 600 nm; OD₆₀₀) and pH are represented by solid lines and dotted lines, respectively. The culture of *S. thermophilus* or *Lb. bulgaricus* alone is represented by solid squares or triangles, respectively. The coculture of *Lb. plantarum* WCFS1 with *S. thermophilus* or *Lb. bulgaricus* is represented by solid circles or inverted triangles, respectively.

S. thermophilus was more efficient in lactose utilization than *Lb. bulgaricus*. *Streptococcus thermophilus* CGMCC 7.179 consumed up 95% lactose after 6 h of cultivation, *S. thermophilus* SDMCC050215 consumed 90% lactose in the stationary phase, whereas *Lb. bulgaricus* ATCC11842 and SDMCC050201 only consumed 77 and 79% lactose, respectively. In addition, *S. thermophilus* and *Lb. bulgaricus* accumulated 4 g/L to 5 g/L galactose in lactose-based medium. Although *S. thermophilus* CGMCC 7.179 was a galactose-positive strain, a large amount of galactose was left in the medium. When they were co-cultured with *Lb. plantarum* WCFS1, lactose was consumed more efficiently and galactose also decreased significantly until it was undetectable in the stationary phase. Meanwhile, the content of lactic acid was increased with the decrease of the total sugar content (Figure 5).

Characteristics of Yogurt Fermented by Co-cultivation with *Lb. plantarum* WCFS1

To investigate the ability to use lactose or galactose in milk, *Lb. plantarum* WCFS1 was co-cultured with *S. thermophilus* and *Lb. bulgaricus* in RSM for 12 h. The residual sugar content of yogurt is shown in Table 1. The initial lactose content in the RSM was 138.77 ± 3.61 mmol/kg, and the level of lactose decreased in varying degrees at the end of fermentation. Compared with the yogurt fermented by traditional starters, the cultivation with *Lb. plantarum* WCFS1 significantly ($P < 0.05$ for all groups) reduced the lactose content. As for galactose content, no significant difference ($P > 0.05$) was observed for SDMCC050201 + SDMCC050215 alone or with WCFS1. In the other 3 combinations, the galactose content increased due the

Figure 5. Sugars and lactic acid content in lactose-based medium fermented by *Streptococcus thermophilus* CGMCC 7.179 (A), *S. thermophilus* SDMCC050215 (B), *Lactobacillus bulgaricus* ATCC11842 (C), and *Lb. bulgaricus* SDMCC050201 (D) with or without *Lb. plantarum* WCFS1. The results for *S. thermophilus* or *Lb. bulgaricus* alone are represented by solid symbols, and the results for the coculture of *Lb. plantarum* WCFS1 with *S. thermophilus* or *Lb. bulgaricus* are represented by open symbols. The contents of lactose, galactose, and lactic acid are represented by squares, circles, and triangles, respectively.

cultivation with *Lb. plantarum* WCFS1. The percentages of total sugar reduction, calculated for the molar weight of monosaccharides, are shown in Table 1. Compared with RSM medium, the maximum percentages of total sugar reduction of yogurt fermented by traditional starters and co-cultured with *Lb. plantarum* WCFS1 were 18.40% (ATCC11842 + SDMCC050215) and 26.00% (ATCC11842 + SDMCC050215 + WCFS1), respectively.

The physicochemical properties demonstrated that the cultivation of *Lb. plantarum* with traditional yogurt starters could give new characteristics to yogurt (Supplemental Table S2; <https://doi.org/10.3168/jds.2019-17347>). Compared with the traditional yogurt

starters, the co-cultivation with *Lb. plantarum* WCFS1 could shorten the curd time significantly ($P < 0.05$ for all groups), and the pH of yogurt decreased and the titratable acidity increased, indicating that the co-cultivation of *Lb. plantarum* WCFS1 with traditional starters might improve the yield of lactic acid. In addition, the water-holding capacity was improved for the yogurt fermented by the co-cultivation of *Lb. plantarum* WCFS1 with traditional starters. The viable cell counts of *Lb. bulgaricus* and *S. thermophilus* in yogurt reached 8 log cfu/mL, and they were not impaired when co-cultured with *Lb. plantarum* WCFS1 during fermentation and storage. In addition, *Lb. plantarum* WCFS1 also remained at high levels around 8 log cfu/

Table 1. Residual sugar content in fermented milks¹

Strain	Lactose (mmol/kg)	Galactose (mmol/kg)	Total sugar reduction (%)
RSM medium	138.77 ± 3.61	—	—
ATCC11842 + CGMCC 7.179	116.57 ± 4.44 ^a	24.42 ± 1.11 ^b	7.20
ATCC11842 + CGMCC 7.179 + WCFS1	94.09 ± 1.67 ^b	29.97 ± 1.67 ^a	21.40
ATCC11842 + SDMCC050215	89.37 ± 0.56 ^a	47.74 ± 1.11 ^b	18.40
ATCC11842 + SDMCC050215 + WCFS1	73.00 ± 0.56 ^b	59.39 ± 0.56 ^a	26.00
SDMCC050201 + CGMCC 7.179	112.13 ± 1.94 ^a	22.76 ± 0.56 ^b	11.00
SDMCC050201 + CGMCC 7.179 + WCFS1	102.41 ± 4.16 ^b	27.20 ± 1.11 ^a	16.40
SDMCC050201 + SDMCC050215	93.81 ± 4.16 ^a	45.52 ± 2.22 ^a	16.00
SDMCC050201 + SDMCC050215 + WCFS1	81.60 ± 2.22 ^b	47.74 ± 1.67 ^a	24.00

^{a,b}Different lowercase superscript letters indicate significant differences ($P < 0.05$) between yogurt fermented by traditional starters with or without *Lactobacillus plantarum* WCFS1.

¹Values are presented as mean ± SD of 3 individual experiments ($n = 3$). — indicates data not shown. The percentages of total sugar reduction were calculated by the molar weight of monosaccharide. RSM = reconstituted skim milk.

mL (Supplemental Table S3; <https://doi.org/10.3168/jds.2019-17347>).

Sensory Analysis

Results of the sensory analysis are presented in Supplemental Table S4 (<https://doi.org/10.3168/jds.2019-17347>). The results showed that co-cultivation of *Lb. plantarum* WCFS1 with traditional starters did not have a negative effect on the scores of appearance and texture. In contrary, the consumer acceptance of appearance and texture improved significantly ($P < 0.05$) by the cultivation with *Lb. plantarum* WCFS1, for the traditional starters ATCC11842 + CGMCC 7.179 and SDMCC050201 + CGMCC 7.179. The flavor scores showed that the effect of co-cultivation with *Lb. plantarum* WCFS1 was variable according to the starter used. However, the scores were higher than 8.00 for all groups.

DISCUSSION

Yogurt making is traditionally performed by the combined actions of *S. thermophilus* and *Lb. bulgaricus* in milk (Agostoni et al., 2010). During milk fermentation, lactose was efficiently catabolized into glucose and galactose by the β -galactosidase of *S. thermophilus* and *Lb. bulgaricus*, among which the glucose portion was further metabolized by glycolysis. For the galactose portion, the *Lb. bulgaricus* strains has not got the genes for their metabolism, and most *S. thermophilus* strains are unable to ferment galactose despite having intact *galKTEM* genes encoding the required enzymes for the Leloir pathway (Vaughan et al., 2001; de Vin et al., 2005). The probable reason for this phenotype is due to the defective promoter of *gal* operon (Vaughan et al., 2001). The shortcoming in the metabolic pathway

of *S. thermophilus* and *Lb. bulgaricus* eventually lead to galactose accumulation as well as residual unfermented lactose in yogurt, which may cause a burden on human health, particularly in individuals with lactose intolerance and galactosemia. *Lactobacillus plantarum* is encountered as a natural inhabitant of the human gastrointestinal tract with identified candidate probiotic genes and potential health-associated properties (de Vries et al., 2006; Siezen et al., 2012). Several studies were conducted using *Lb. plantarum* as a starter for milk fermentation, but most of them focus on its effects on flavor profiles and beneficial effects on intestinal health (Arena et al., 2015; Li et al., 2017). Whether *Lb. plantarum* can reduce sugar content in yogurt has not been studied.

The ability of *Lb. plantarum* to use lactose and galactose was first detected in artificial media. The results indicated that *Lb. plantarum* had a strong ability to metabolize lactose and galactose, and it could catabolize lactose with limited galactose accumulation when lactose is present as the sole carbon. In our previous studies, 2 functional β -galactosidases responsible for lactose hydrolysis were identified in *Lb. plantarum* WCFS1 (Zhang et al., 2019). In this study, the Leloir pathway for galactose metabolism was found to be organized in an operon in this strain. Interestingly, our research also proved that the *gal* operon was expressed driven by a strong constitutive promoter in *Lb. plantarum* WCFS1. This property was different from that the sugar utilization is often induced by the corresponding substrate, such as the lactose metabolism in *Escherichia coli* and the xylose metabolism in *Bacillus subtilis* (Anbukkarasi et al., 2014b; Chen et al., 2016). Nevertheless, this characteristic of *Lb. plantarum* confer it great advantages to use together with traditional starters to reduce sugar content in fermented milk. Because lactose is the main sugar in milk, the MRS-lactose medium was used

to simulate the milk environment. The same behavior was also observed when *Lb. plantarum* WCFS1 was co-cultured with *S. thermophilus* or *Lb. bulgaricus*. Lactose was consumed more efficiently and galactose also declined significantly until it was undetectable. Meanwhile, the content of lactic acid was increased with the decrease of the total residual sugar content, resulting in a pH decline, obviously. These results indicated that the co-cultivation of *Lb. plantarum* with traditional yogurt starters could contribute to efficient lactose and galactose metabolism and rapid lactic acid production.

Therefore, the milk fermentation was further carried out by *Lb. plantarum* WCFS1 coupled with *S. thermophilus* and *Lb. bulgaricus*. The results showed that the co-cultivation of *Lb. plantarum* WCFS1 could significantly ($P < 0.05$ for all groups) reduce the lactose content, which is beneficial for the patients with lactose intolerance. However, the galactose content increased after co-cultivation with *Lb. plantarum* WCFS1, which was different from the results in artificial medium. The viable cell count in lactose-based medium showed that *Lb. plantarum* WCFS1 remained at high levels around 9 log cfu/mL, whereas *S. thermophilus* and *Lb. bulgaricus* only reached 7 to 8 log cfu/mL. On the contrary, the viable cell counts in fermented milks showed that all strains remained at levels around 8 log cfu/mL at the end of the fermentation. These results indicated that *Lb. plantarum* WCFS1 grew better in lactose-based medium, whereas *S. thermophilus* and *Lb. bulgaricus* grew better in milk. This could be one of the reasons why the residual galactose content was higher in RSM medium than in lactose-based medium. In addition, the high levels of lactose in milk could mean that the lactose/galactose transporters of *Lb. plantarum* WCFS1 prefer to transport lactose rather than galactose in the presence of both lactose and galactose. Therefore, the consumption of extracellular galactose secreted by *S. thermophilus* and *Lb. bulgaricus* was limited. Compared with RSM medium, the maximum sugar reductions for yogurts with and without *Lb. plantarum* were 18.40 and 26.00%, respectively. The molar quantity of the accumulated galactose was less than the molar quantity of the hydrolyzed lactose, indicating that a certain amount of galactose was metabolized by *Lb. plantarum*. Hence, co-cultivation of *Lb. plantarum* with traditional yogurt starters could reduce the total sugar content in yogurt.

The physicochemical characteristics demonstrated that co-cultivation of *Lb. plantarum* with traditional yogurt starters in yogurt making had several advantages. First, the curd time was shortened, which will increase production efficiency and promote the economic benefits of enterprises. Second, the syneresis was reduced significantly ($P < 0.05$ for all groups) compared with

traditional fermented milk, which may be related to the content of casein, whey protein, and exopolysaccharide (Hassan et al., 2003; Meletharayil et al., 2018). Considering syneresis (wheying off) is a major defect in the yogurt industry and is directly related to the extent of physical disturbance to the network of protein micelles, stabilizers such as pectin, gelatin, and starch are often used in yogurt making to prevent wheying off (Tasneem et al., 2014). Hence, the use of *Lb. plantarum* would reduce the cost of the product. Third, sensory analysis indicated that yogurt fermented by *Lb. plantarum* WCFS1 with traditional starters was acceptable to consumers in appearance, texture, and flavor. Above all, there is great development potential to prepare low-sugar yogurt by co-cultivation of *Lb. plantarum* with traditional yogurt starters.

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REFERENCES

- Agostoni, C., J. L. Bresson, S. Fairweather-Tait, A. Flynn, I. Golly, H. Korhonen, P. Lagiou, M. Løvik, R. Marchelli, A. Martin, B. Moseley, M. Neuhäuser-Berthold, H. Przyrembel, S. Salminen, Y. Sanz, S. Strain, S. Strobel, I. Tetens, D. Tomé, H. van Loveren, and H. Verhagen. 2010. Scientific opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion (ID 1143, 2976) pursuant to article 13(1) of regulation (EC) No 1924/2006. EFSA J. 8:1763. <https://doi.org/10.2903/j.efsa.2010.1763>.
- Anbukkarasi, K., D. K. Nanda, T. UmaMaheswari, T. Hemalatha, P. Singh, and R. Singh. 2014b. Assessment of expression of Leu-oir pathway genes in wild-type galactose-fermenting *Streptococcus thermophilus* by real-time PCR. Eur. Food Res. Technol. 239:895–903. <https://doi.org/10.1007/s00217-014-2286-9>.
- Anbukkarasi, K., T. UmaMaheswari, T. Hemalatha, D. K. Nanda, P. Singh, and R. Singh. 2014a. Preparation of low galactose yogurt using cultures of Gal(+) *Streptococcus thermophilus* in combination with *Lactobacillus delbrueckii* ssp. *bulgaricus*. J. Food Sci. Technol. 51:2183–2189. <https://doi.org/10.1007/s13197-014-1262-5>.
- Arena, M. P., G. Caggianiello, P. Russo, M. Albenzio, S. Massa, D. Fiocco, V. Capozzi, and G. Spano. 2015. Functional starters for functional yogurt. Foods 4:15–33. <https://doi.org/10.3390/foods4010015>.
- Aryana, K. J., and D. W. Olson. 2017. A 100-year review: Yogurt and other cultured dairy products. J. Dairy Sci. 100:9987–10013. <https://doi.org/10.3168/jds.2017-12981>.
- Benozzi, E., A. Romano, V. Capozzi, S. Makhoul, L. Cappellin, I. Khomenko, E. Aprea, M. Scampicchio, G. Spano, T. D. Märk, F. Gasperi, and F. Biasioli. 2015. Monitoring of lactic fermentation driven by different starter cultures via direct injection mass spectrometric analysis of flavour-related volatile compounds. Food Res. Int. 76:682–688. <https://doi.org/10.1016/j.foodres.2015.07.043>.

- Capozzi, V., M. Fragasso, R. Romaniello, C. Berbegal, P. Russo, and G. Spano. 2017. Spontaneous food fermentations and potential risks for human health. *Fermentation* 3:49. <https://doi.org/10.3390/fermentation3040049>.
- Chen, J., Y. Zhu, G. Fu, Y. Song, Z. Jin, Y. Sun, and D. Zhang. 2016. High-level intra- and extra-cellular production of D-psicose 3-epimerase via a modified xylose-inducible expression system in *Bacillus subtilis*. *J. Ind. Microbiol. Biotechnol.* 43:1577–1591. <https://doi.org/10.1007/s10295-016-1819-6>.
- Chiu, C. H., T. Y. Lu, Y. Y. Tseng, and T. M. Pan. 2006. The effects of *Lactobacillus*-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. *Appl. Microbiol. Biotechnol.* 71:238–245. <https://doi.org/10.1007/s00253-005-0145-0>.
- Chollet, M., D. Gille, A. Schmid, B. Walther, and P. Piccinali. 2013. Acceptance of sugar reduction in flavored yogurt. *J. Dairy Sci.* 96:5501–5511. <https://doi.org/10.3168/jds.2013-6610>.
- da Costa, M. P., S. Frasao Bda, B. R. Lima, B. L. Rodrigues, and C. A. Conte Junior. 2016. Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurts fermentation. *Talanta* 152:162–170. <https://doi.org/10.1016/j.talanta.2016.01.061>.
- de Vin, F., P. Rådström, L. Herman, and L. De Vuyst. 2005. Molecular and biochemical analysis of the galactose phenotype of dairy *Streptococcus thermophilus* strains reveals four different fermentation profiles. *Appl. Environ. Microbiol.* 71:3659–3667. <https://doi.org/10.1128/AEM.71.7.3659-3667.2005>.
- de Vries, M. C., E. E. Vaughan, M. Kleerebezem, and W. M. de Vos. 2006. *Lactobacillus plantarum*: Survival, functional and potential probiotic properties in the human intestinal tract. *Int. Dairy J.* 16:1018–1028. <https://doi.org/10.1016/j.idairyj.2005.09.003>.
- Harju, M., H. Kallioinen, and O. Tossavainen. 2012. Lactose hydrolysis and other conversions in dairy products: technological aspects. *Int. Dairy J.* 22:104–109. <https://doi.org/10.1016/j.idairyj.2011.09.011>.
- Hassan, A. N., R. Ipsen, T. Janzen, and K. B. Qvist. 2003. Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. *J. Dairy Sci.* 86:1632–1638. [https://doi.org/10.3168/jds.S0022-0302\(03\)73748-5](https://doi.org/10.3168/jds.S0022-0302(03)73748-5).
- Hols, P., F. Hancy, L. Fontaine, B. Grossiord, D. Prozzi, N. Leblond-Bourget, B. Decaris, A. Bolotin, C. Delorme, S. D. Ehrlich, E. Guédon, V. Monnet, P. Renault, and M. Kleerebezem. 2005. New insights in the molecular biology and physiology of *Streptococcus thermophilus* revealed by comparative genomics. *FEMS Microbiol. Rev.* 29:435–463. <https://doi.org/10.1016/j.femsre.2005.04.008>.
- Horiuchi, H., N. Inoue, E. Liu, M. Fukui, Y. Sasaki, and T. Sasaki. 2009. A method for manufacturing superior set yogurt under reduced oxygen conditions. *J. Dairy Sci.* 92:4112–4121. <https://doi.org/10.3168/jds.2008-1747>.
- Jelen, P., and O. Tossavainen. 2003. Low lactose and lactose-free milk and dairy products-prospects, technologies and applications. *Aust. J. Dairy Technol.* 58:161–165.
- Li, C., J. Song, L. Y. Kwok, J. Wang, Y. Dong, H. Yu, Q. Hou, H. Zhang, and Y. Chen. 2017. Influence of *Lactobacillus plantarum* on yogurt fermentation properties and subsequent changes during postfermentation storage. *J. Dairy Sci.* 100:2512–2525. <https://doi.org/10.3168/jds.2016-11864>.
- Marco, M. L., D. Heeney, S. Binda, C. J. Cifelli, P. D. Cotter, B. Folligné, M. Gänzle, R. Kort, G. Pasin, A. Pihlanto, E. J. Smid, and R. Hutkins. 2017. Health benefits of fermented foods: Microbiota and beyond. *Curr. Opin. Biotechnol.* 44:94–102. <https://doi.org/10.1016/j.copbio.2016.11.010>.
- Meletharayil, G. H., H. A. Patel, L. E. Metzger, C. Marella, and T. Huppertz. 2018. Influence of partially demineralized milk proteins on rheological properties and microstructure of acid gels. *J. Dairy Sci.* 101:1864–1871. <https://doi.org/10.3168/jds.2017-13670>.
- Neves, A. R., W. A. Pool, A. Solopova, J. Kok, H. Santos, and O. P. Kuipers. 2010. Towards enhanced galactose utilization by *Lactococcus lactis*. *Appl. Environ. Microbiol.* 76:7048–7060. <https://doi.org/10.1128/AEM.01195-10>.
- Robitaille, G., S. Moineau, D. St-Gelais, C. Vadeboncoeur, and M. Britten. 2007. Galactose metabolism and capsule formation in a recombinant strain of *Streptococcus thermophilus* with a galactose-fermenting phenotype. *J. Dairy Sci.* 90:4051–4057. <https://doi.org/10.3168/jds.2007-0140>.
- Siezen, R. J., C. Francke, B. Renckens, J. Boekhorst, M. Wels, M. Kleerebezem, and S. A. van Hijum. 2012. Complete resequencing and reannotation of the *Lactobacillus plantarum* WCFS1 genome. *J. Bacteriol.* 194:195–196. <https://doi.org/10.1128/JB.06275-11>.
- Silanikove, N., G. Leitner, and U. Merin. 2015. The interrelationships between lactose intolerance and the modern dairy industry: Global perspectives in evolutionary and historical backgrounds. *Nutrients* 7:7312–7331. <https://doi.org/10.3390/nu7095340>.
- Sun, Z., J. Kong, and W. Kong. 2010. Characterization of a cryptic plasmid pD403 from *Lactobacillus plantarum* and construction of shuttle vectors based on its replicon. *Mol. Biotechnol.* 45:24–33. <https://doi.org/10.1007/s12033-010-9242-0>.
- Tasneem, M., F. Siddique, A. Ahmad, and U. Farooq. 2014. Stabilizers: Indispensable substances in dairy products of high rheology. *Crit. Rev. Food Sci. Nutr.* 54:869–879. <https://doi.org/10.1080/10408398.2011.614702>.
- Turner, K. W., and F. G. Martley. 1983. Galactose fermentation and classification of *thermophilic lactobacilli*. *Appl. Environ. Microbiol.* 45:1932–1934. <https://doi.org/10.1128/AEM.45.6.1932-1934.1983>.
- Valente, G. L. C., L. B. Acurcio, L. P. V. Freitas, J. R. Nicoli, A. M. Silva, M. R. Souza, and C. F. A. M. Penna. 2019. Short communication: In vitro and in vivo probiotic potential of *Lactobacillus plantarum* B7 and *Lactobacillus rhamnosus* D1 isolated from Minas artisanal cheese. *J. Dairy Sci.* 102:5957–5961. <https://doi.org/10.3168/jds.2018-15938>.
- Van Calcar, S. C., L. E. Bernstein, F. J. Rohr, C. H. Scaman, S. Yannicelli, and G. T. Berry. 2014. A re-evaluation of life-long severe galactose restriction for the nutrition management of classic galactosemia. *Mol. Genet. Metab.* 112:191–197. <https://doi.org/10.1016/j.ymgme.2014.04.004>.
- Vaughan, E. E., P. T. van den Bogaard, P. Catzeddu, O. P. Kuipers, and W. M. de Vos. 2001. Activation of silent gal genes in the lactal regulon of *Streptococcus thermophilus*. *J. Bacteriol.* 183:1184–1194. <https://doi.org/10.1128/JB.183.4.1184-1194.2001>.
- Wu, Q., C. K. W. Cheung, and N. P. Shah. 2015. Towards galactose accumulation in dairy foods fermented by conventional starter cultures: Challenges and strategies. *Trends Food Sci. Technol.* 41:24–36. <https://doi.org/10.1016/j.tifs.2014.08.010>.
- Xu, Z., H. He, S. Zhang, and J. Kong. 2017. Effects of inoculants *Lactobacillus brevis* and *Lactobacillus parafarraginis* on the fermentation characteristics and microbial communities of corn stover silage. *Sci. Rep.* 7:13614. <https://doi.org/10.1038/s41598-017-14052-1>.
- Xu, Z., S. Zhang, Y. Mu, and J. Kong. 2018. *Paenibacillus panacisoli* enhances growth of *Lactobacillus* spp. by producing xylooligosaccharides in corn stover ensilages. *Carbohydr. Polym.* 184:435–444. <https://doi.org/10.1016/j.carbpol.2017.12.044>.
- Zhang, S., Z. Xu, L. Qin, and J. Kong. 2019. Development of strong lactose/galactose-inducible expression system for *Lactobacillus plantarum* by optimizing promoter. *Biochem. Eng. J.* 151:107316. <https://doi.org/10.1016/j.bej.2019.107316>.

ORCIDS

J. Kong <https://orcid.org/0000-0002-2872-2078>