Journal of Dental Sciences (2020) 15, 403–410

Available online at www.sciencedirect.com

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Original Article

Fermented milk containing a potential probiotic *Lactobacillus rhamnosus* SD11 with maltitol reduces *Streptococcus mutans*: A double-blind, randomized, controlled study

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Received 24 December 2019; Final revision received 3 March 2020
Available online 6 May 2020

KEYWORDS
Probiotic;
Lactobacillus rhamnosus SD11;
Maltitol;
Streptococcus mutans

Abstract *Background/purpose:* Sucrose has been considered as a cariogenic substrate due to large amounts of acid production after fermentation by certain oral bacteria, thus sugar alcohols are often used to replace sucrose. The aims of this study were to investigate the effect of maltitol on the growth and acid production of *Streptococcus mutans* and *Lactobacillus rhamnosus*-SD11 compared to various sugars, and to examine whether the fermented milk containing a potential probiotic *L. rhamnosus*-SD11 with maltitol could reduce *S. mutans*.
Materials and methods: The acid production of tested sugars by cariogenic *S. mutans* was measured using pH meter. In a clinical trial, 123 children were recruited and randomly assigned to either the probiotic- or control-fermented milk, once daily for 4 weeks. The target bacteria levels in the saliva were examined using a real-time PCR at baseline, 4 and 8 weeks. The oral examination was recorded at the baseline and 8 weeks.
Results: The results showed that maltitol exhibited less acid production than simple sugars. In the clinical trial, a significant reduction of salivary total streptococci and *S. mutans* occurred, while the levels of salivary lactobacilli significantly increased in the probiotic group compared to the control group after receiving the probiotic fermented milk.

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https://doi.org/10.1016/j.jds.2020.03.003

Conclusion: The daily consumption of the fermented milk containing *L. rhamnosus*-SD11 with maltitol had beneficial effects on oral health by reducing salivary *S. mutans*. Thus, the substitution of simple sugars by maltitol in dairy products containing *L. rhamnosus*-SD11 may be an alternative way to prevent the risk of caries.

Introduction

Probiotics were first known in forms of yoghurt and fermented milk, and have been used for decades to promote human health benefits.[1–3] Systemic reviews and meta-analysis suggest that some probiotics could decrease the cariogenic mutans streptococci (MS) levels, and thus the consumption of these strains may have a positive effect on the prevention of caries.[4,5] Our previous study examined *Lactobacillus* strains isolated from the saliva of children, and demonstrated that some of those strains could inhibit the growth of oral pathogens by producing antimicrobial substances.[6,7] Among those strains, *Lactobacillus rhamnosus* SD11 has the potential to be a probiotic strain for caries prevention due to its ability to produce a bacteriocin against cariogenic mutans streptococci (MS) with molecular weights of 33,000 Da.[7] Subsequently, further clinical trials demonstrated that daily consumption of fermented milk containing *L. rhamnosus* SD11, without adding any sugars, showed a beneficial effect on oral health by reducing cariogenic MS levels in saliva.[8,9] However, it was suggested that adding the sugar to improve the taste of fermented milk was recommended.

Among commonly used sugars, sucrose has been considered as a highly cariogenic substrate due to large amounts of acid production after fermentation by oral bacteria, especially MS and lactobacilli. Frequent exposure to sucrose creates a condition for the onset of caries by promoting demineralization.[10] Increasingly, consumers are becoming concerned about sugar and its cariogenicity in food products, therefore, sugar substitutes, especially sorbitol and xylitol, are often used to replace sucrose, glucose and fructose. However, the ability to ferment sugar alcohols, especially sorbitol, has been reported in some probiotic *Lactobacillus* strains (*L. johnsonii* LA1, *L. plantarum* 299V, *L. rhamnosus* GG and *L. rhamnosus* LC 705) leading to a pH lower than 5.5 which is considered as the critical level for enamel demineralization.[11] It was also shown that 8 clinical *L. rhamnosus* strains could ferment sugar alcohols, xylitol and sorbitol to a pH lower than 5.5.[12] However, maltitol has not been included in those studies.

Maltitol, one of the sugar alcohols, was not fermented by *Streptococcus mutans*[13]; however, the fermentation ability of *L. rhamnosus* strains on maltitol has not yet been studied. Clinical trials and a systematic review have shown that sugar free chewing gum contained xylitol could reduce cariogenic mutans streptococci.[14,15] One clinical study reported that maltitol could improve general oral health in a similar way to xylitol.[16]

Considering the information relates to acid production of maltitol by *L. rhamnosus* SD11 has not been carried out, and its application in fermented milk with maltitol for preventing dental caries in a clinical trial has not been reported. Our hypothesis was that the potential probiotic *L. rhamnosus* SD11 with maltitol could reduce cariogenic bacterium, *S. mutans*, in a clinical trial. The objectives of this study were to investigate the effect of maltitol on the growth and acid production of *S. mutans* and *L. rhamnosus* SD11 compared to various sugars, and to examine whether the fermented milk containing a potential probiotic *L. rhamnosus* SD11 with maltitol could reduce *S. mutans*.

Materials and methods

Bacterial strains and culture conditions

L. rhamnosus SD11 and cariogenic *S. mutans* ATCC 25175 used in this study were both strains retrieved from culture collection (kept in −80 °C) at the Department of Stomatology, Faculty of Dentistry, Prince of Songkla University, Thailand.

L. rhamnosus SD11, a strain selected from caries-free subjects, was previously identified using restriction fragment length polymorphism analysis (PCR-RFLP) of 16S-rRNA gene profiles and protein profiles of sodium dodecyl sulfate polyacrylamide gel electrophoresis.[17] The further identity of the strain was confirmed as *L. rhamnosus* due to its 16S-rRNA gene sequences and presence of a specific band at the same level of *L. rhamnosus* GG using the denaturing gradient gel electrophoresis with primers of CARP according to Piwat and Teanpaisan.[18]

L. rhamnosus SD11 was cultured on De Man, Rogosa and Sharpe agar (MRS, Difco™, Sparks, MD, USA), while *S. mutans* was cultured on 5% blood agar (Difco™). The plates were anaerobically incubated at 37 °C for 24—48 h before usage.

Acid production of *L. rhamnosus* SD11 and *S. mutans* after fermenting maltitol and various sugars

In this study were tested simple sugars (sucrose, glucose and lactose), sugar alcohols (mannitol, maltitol, sorbitol, and xylitol) and a plant sweetener (stevia). All individual sugars were prepared at 5 different concentrations at 10, 5, 2.5, 1, and 0.5% (w/v).

The basal growth media (BHI for *S. mutans* and MRS for *L. rhamnosus* SD11) were prepared without any sugars and

were adjusted to pH 7.0. Then, filtered sterilized sugars at different concentrations were added in the basal growth media. Individual basal growth medium without any sugar was used as a control.

A single colony of *S. mutans* and *L. rhamnosus* SD11 was inoculated in 10 ml of brain heart infusion broth (BHI, Difco™) and MRS broth (Difco™), respectively. After incubation at 37 °C for 24 h, bacterial cells were centrifuged at 3000 *g* for 5 min and washed with 0.85% NaCl (RCI Labscan Limited, Bangkok, Thailand). Each bacterial suspension was adjusted to 108 CFU/ml and 1% of starter was inoculated into the prepared BHI (for *S. mutans*) and MRS (for *L. rhamnosus* SD11). The growth of each strain was monitored by measuring the absorbance at a wavelength of 600 nm, and acid production was detected by using a pH meter (Mettler Toledo, Zürich, Switzerland) after incubation at 37 °C for 0, 6, 12, 24, 48, and 72 h.

Effect of fermented milk containing the *L. rhamnosus* SD11 and maltitol on *S. mutans*

Fermented milk preparation

The fermented milks (control and tested) were prepared by the Dairy Home Industries Company from Nakhon Ratchasima, Thailand. Commercial fermented milk was used as the control milk, which was prepared from a thermophilic lactic acid starter culture (FD-DVS ABY-3 Probio-Tec, Chr. Hansen, Horsholm, Denmark) containing *Bifidobacterium* species, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophiles* according to the manufacturer's instructions.

For the tested probiotic milk, the details to prepare the strain can be found in the study of Rungsri et al.[8] Briefly, *L. rhamnosus* SD11 was inoculated into 50 ml of MRS broth (Difco™) at 37 °C overnight, and the culture was then added to 450 ml MRS broth (Difco™) at 37 °C for 48 h. Cells were harvested from the MRS broth (Difco™) by centrifugation at 3000 *g* for 5 min and washed 3 times with 0.85% NaCl (RCI Labscan Limited) before being used.

One ml of *L. rhamnosus* SD11 (5×10^{10} CFU/ml) was inoculated in 1 L of fresh milk and then incubated at 45 °C for 6 h. Afterwards, fermented milk with *L. rhamnosus* SD11 was thoroughly mixed with the commercial fermented milk in a proportion of 1:4 (v/v) giving the final *L. rhamnosus* SD11 as 10^8 CFU/ml. The number of *L. rhamnosus* SD11 was counted by observing its unique colonial morphology under a stereomicroscope after incubating on MRS agar (Difco™) at 37 °C for 48 h, showing as large, and white and creamy in appearance, while other bacteria were small and semi-transparent. The total lactobacilli count of both probiotic- and control-fermented milk was approximately 10^{10} CFU/ml. Both fermented milk preparations (control- and probiotic-fermented milk) were supplemented to a final concentration of 5% maltitol.

Study design, subjects and intervention

The study was designed as a prospective double-blind, randomized, placebo-controlled trial with an experimental period of 8 weeks. The protocol was approved by the Faculty of Dentistry Ethics Committee at Prince of Songkla University, Thailand (EC6011-36-L-HR) and the project was registered at http://www.clinicaltrials.in.th/, being one of the WHO Registry Networks, Clinical Trials identifier (TCTR20171221001).

The sample size calculation in this study was based on our pilot study in a small group of school children with the same age, which was carried out in a separate school prior to the main study. A reduction in the primary outcome, *S. mutans* levels, of this study would be an estimated 90% power at the 0.01 level of significance ($\alpha = 0.05$, $\beta = 0.10$). Therefore, 46 participants per group were needed to allow for a 30% dropout. At least 100 participants of the total sample size (50 subjects/group) were judged as necessary for this study. The project was thoroughly explained to the children and their parents in a meeting organized at the school. A total of 123 children (67 females, 56 males) aged 13–14 years old (mean 13.1 ± 0.5 years) participated in this study. A flowchart of the study is outlined in Fig. 1.

Eligible participants included those with good oral health who had to have $\geq$20 permanent teeth, have caries in $\leq$ two teeth, have an absence of periodontal disease, be a non-smoker, and have daily tooth brushing habits using a fluoride containing toothpaste. The exclusion criteria were (a) having habitual consumption of probiotics or xylitol, (b) having systemic antibiotic medication taken within the past 6 weeks, (c) having an allergy to cows' milk, lactose intolerance, and any severe food allergy, (d) having systemic or severe chronic diseases, and (e) undergoing orthodontic treatment. Dental caries status (DMFT) of all children were examined according to WHO criteria.[19]

Children were randomly assigned to the probiotic or control group using a random number table, whereupon they received 100 ml of fermented milk with or without *L. rhamnosus* SD11, respectively, once daily for 4 weeks under observation by a teacher. The content of the fermented milk was unknown to the children, teacher and clinician responsible for the sampling. The study was blinded until the time of statistical calculations. All children were asked to immediately report any adverse side effects and to fill in the questionnaire form after 4 weeks of milk consumption regarding ailments such as vomiting, diarrhea, fever, rash, or feeling uncomfortable etc. after receiving the fermented milk. The children were also asked about their satisfaction of the products concerning the taste, texture, smell, and color.

Microbial evaluation using a real-time PCR

The unstimulated whole salivary samples (2–3 ml) were collected from individual subjects at baseline, 4 and 8 weeks by having them spit into a sterile plastic ware. The salivary samples (1 ml) were extracted for DNA using a PureDirex Genomic DNA Isolation kit (Bio-Helix Co., LTD., Keelung City, Taiwan) following the manufacturer's protocol for salivary bacteria, and bacterial DNA were kept at −20 °C until used.

The quantities of targeted bacteria in the saliva at baseline (T0), 4 weeks (T4), and 8 weeks (T8) were evaluated using a real-time PCR. Total bacterial DNA (5 μl) was added in to a Sensi-FAST™ SYBR kit (Bioline Reagent Ltd., California, USA). The sequences of primer used were as follows: total bacteria (5′-TCCTACGGGAGGCAGCAGT-3′ and 5′-GGACTACCAGGGTATCTAATCCTGTT-3′),[20] total lactobacilli (5′-CATTTGGAAACAGATGCTAATACC-3′ and 5′-

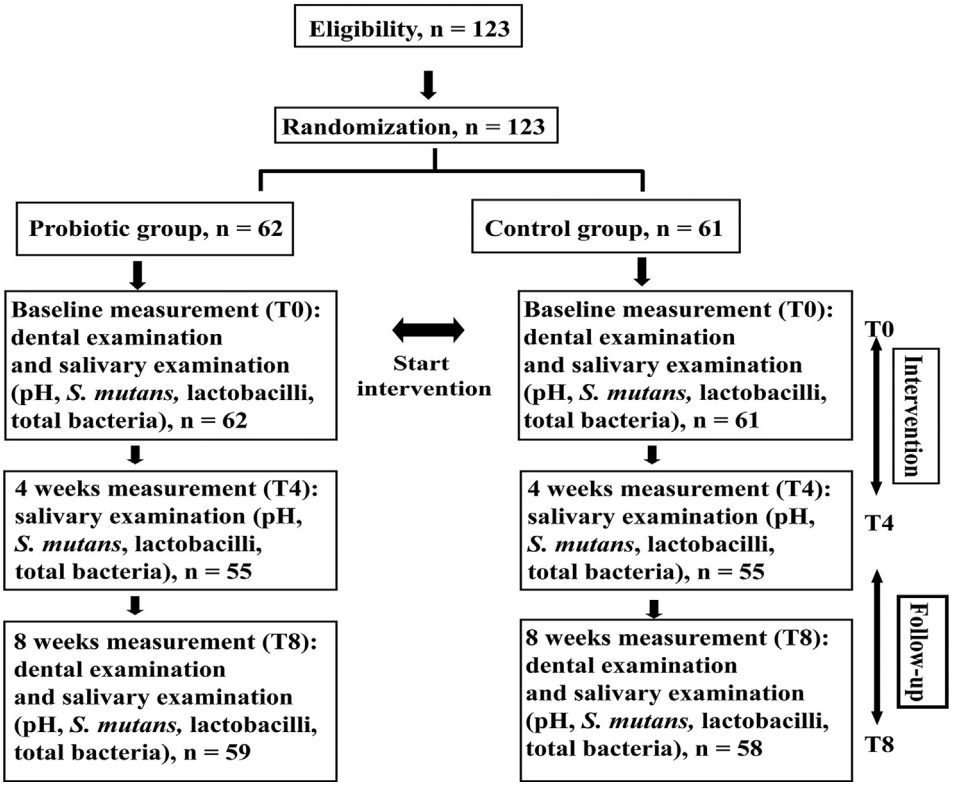

Figure 1 Flowchart showing the progress of children participating at each time period of the 8 week study.

GTCCATTGTGGAAGATTCCC-3′),[21] total streptococci (5′-CCGGTGACGGCAAGCTAA-3′ and 5′-TCATGGAGGCGAGTTGCA-3′),[22] and *S. mutans* (5′-ACTACACTTTCGGGTGGCTTGG-3′ and 5′-CAGTATAAGCGCCAGTTTCATC-3′).[23] The PCR thermal profile consisted of an initial DNA step of 10 min at 95 °C followed by 2 min at 50 °C and 40 cycles at 95 °C for 20 s with different annealing temperatures including 60 °C for total bacteria and *S. mutans*, 58 °C for total lactobacilli, and 56 °C for total streptococci for 20 s. The polymerizing temperature was set at 72 °C for 25 s. Amplification, detection and data analysis were performed with the CFX96™ Real time system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each sample was run in duplicate and the number of targeted bacteria in the saliva samples was quantified using a standard curve.

The standard curve of each targeted bacteria was previously described.[21] The bacterial pellets of targeted bacteria were collected after centrifugation at 3000 *g* for 5 min, washed twice with phosphate-buffered saline (PBS, pH 7.0) and adjusted to $OD_{600\,nm} = 2.0$ using a UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, UK). The bacterial cell suspensions were two-fold dilutions which were then divided into two aliquots. To determine the bacterial number as CFU/ml, the first aliquot was counted using the cultivation method. The second aliquot was used for DNA extraction as aforementioned to determine the Cq of real-time PCR using the CFX96 Touch™ Real-Time PCR detection system (Bio-Rad Laboratories). A linear standard curve was plotted for each bacterial species from log CFU/ml against the corresponding Cq, showing a high correlation coefficient ($R^2 > 0.99$).

Data analysis

All data were presented as means ± standard deviations. The general characteristics of the children (gender, age, pH of saliva, and DMFT) between the control and probiotic groups were analyzed using the chi-square test for categorized/dichotomized variables and the Mann–Whitney U test for interval variables. The bacterial levels were presented as log CFU/ml. The changes in bacteria levels from baseline to the intervention period were analyzed using one-way ANOVA followed by the post hoc test Bonferroni. The levels of total bacteria, total lactobacilli, total streptococci, and *S. mutans* between the two groups were analyzed using the independent T-test. The differences were considered significant when $p < 0.05$.

Results

Acid production and growth of *L. rhamnosus* SD11 and *S. mutans*

The growth (increase in $OD_{600\,nm}$) and acid production (pH values) of *L. rhamnosus* SD11 and *S. mutans* varied between the sugars and their different concentrations tested. A lower pH correlated to an increase in each bacterial growth. No significant differences in growth and pH within the same strain upon the different concentrations (10, 5, 2.5, 1, and 0.5%) of sugars tested were observed. After 24 h incubation, the growth of both strains (*L. rhamnosus* SD11

and *S. mutans*) in all sugars reached maximum growth (Figs. 2A and 3A).

After 24 h incubation of *L. rhamnosus* SD11 with 5% sugars; glucose, sucrose, lactose and mannitol showed the maximum growth of approximately $OD_{600\,nm} = 1.5$, followed by sorbitol ($OD_{600\,nm} = 1.0$), maltitol ($OD_{600\,nm} = 0.6$), and stevia and xylitol ($OD_{600\,nm} = 0.2$), respectively (Fig. 2A). The pH values corresponded with the growth in glucose, sucrose, lactose and mannitol, showing pH values below 5.0 after 24 h incubation and after 48 h incubation for sorbitol (Fig. 2B). In contrast, the pH values of maltitol, xylitol and stevia slightly decreased, and that remained above pH 6.2 throughout the time of the study. There was no significant difference of the pH values among maltitol, xylitol and stevia (Fig. 2B).

A similar pattern for sugar fermentations of *S. mutans* was observed for 5% sucrose, glucose, lactose and mannitol showing the maximum growth of approximately $OD_{600\,nm} = 1.1$, followed by sorbitol ($OD_{600\,nm} = 0.6$), maltitol ($OD_{600\,nm} = 0.3$), stevia and xylitol ($OD_{600\,nm} = 0.2$), respectively (Fig. 3A). The pH values also corresponded with their (glucose, sucrose, lactose and mannitol) growth, showing pH values below 5.0 after 24 h, followed by sorbitol (Fig. 3B). The pH values of maltitol, xylitol and stevia remained the same (pH 7.0) as at the beginning (Fig. 3B).

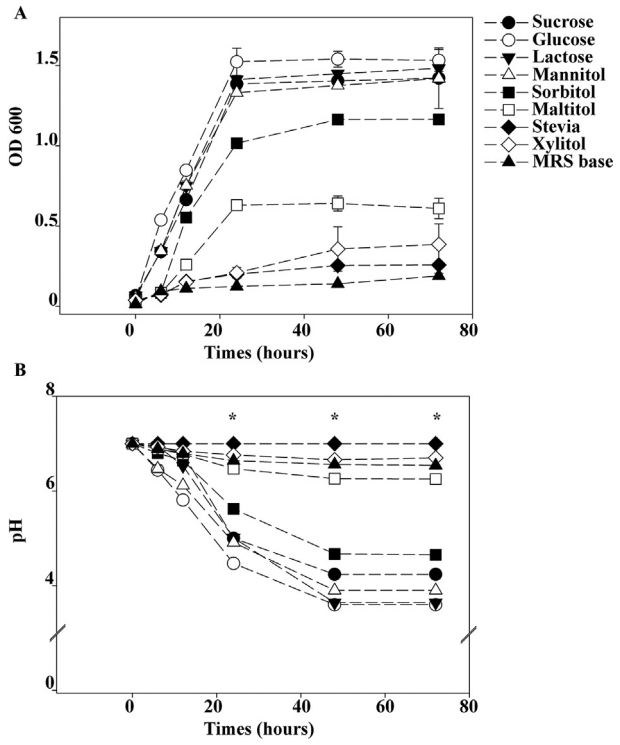

Figure 2 The effect of 5% various sugars on growth-(A) and acid production-(B) of *Lactobacillus rhamnosus* SD11. *Significant lowering in the pH of *L. rhamnosus* SD11 cultured in sucrose, glucose, lactose, mannitol, and sorbitol compared to maltitol, stevia, and xylitol ($p < 0.05$).

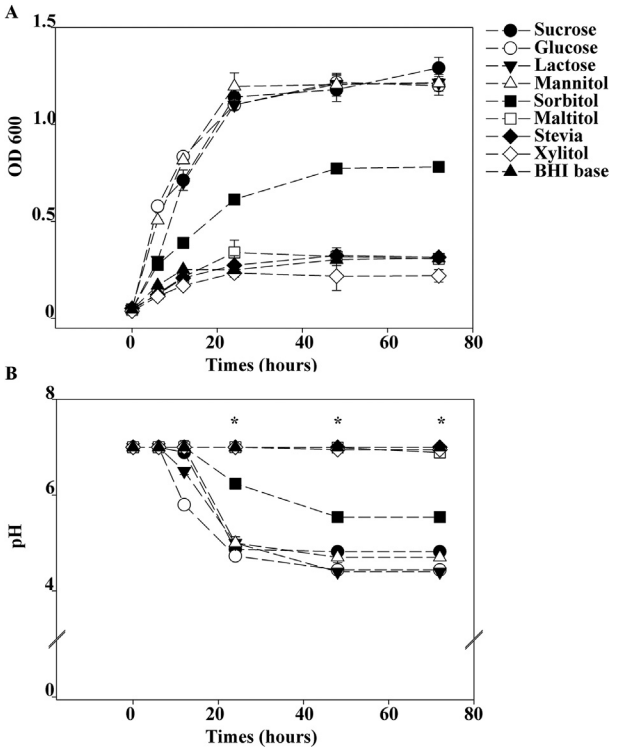

Figure 3 The effect of 5% various sugars on growth-(A) and acid production-(B) of *Streptococcus mutans*. *Significant lowering in the pH of *S. mutans* cultured in sucrose, glucose, lactose, mannitol, and sorbitol compared to maltitol, stevia, and xylitol ($p < 0.05$).

Effect of the fermented milk with *L. rhamnosus* SD11 on *S. mutans* in the clinical trial

For the clinical study, details of baseline characteristics for age and DMFT between the control and probiotic groups generally did not differ, except that the number of females and salivary pH was significantly higher in the probiotic group compared with the control group ($p < 0.05$) (Table 1).

This study monitored the effect of fermented milk on cariogenic *S. mutans* levels. In the probiotic group, it was demonstrated that *S. mutans* together with total streptococci levels significantly decreased after receiving the fermented milk containing *L. rhamnosus* SD11 (Fig. 4A and B). Means of *S. mutans* and total streptococci levels were 5.7 ± 0.2 and 7.4 ± 0.2 log CFU/ml at the baseline, however, it reduced to 4.7 ± 0.2 and 5.8 ± 0.2 log CFU/ml at 4 weeks and 4.8 ± 0.2 and 6.1 ± 0.2 log CFU/ml at 8 weeks, respectively.

Lactobacilli levels in both groups were not significantly different at the baseline with means of 7.2 ± 0.4 (probiotic group) and 7.2 ± 0.3 log CFU/ml (control group). A significant increase of lactobacilli was found in the probiotic group (mean 8.1 ± 0.2 log CFU/ml at 4 weeks and 8.0 ± 0.3 log CFU/ml at 8 weeks) after receiving the probiotic fermented milk. Nevertheless, the lactobacilli level was not a significant difference in the control group (means 7.5 ± 0.2

Table 1 Characteristics of the study population.

Characteristics	Probiotic group $n = 62$	Control group $n = 61$
Sex*		
Male	21	35
Female	41	26
Age (years old)	13.1 ± 0.4	13.1 ± 0.5
Decayed, missing and filled teeth at baseline	1.7 ± 0.6	1.5 ± 0.5
Decayed, missing and filled teeth at week 8	1.7 ± 0.6	1.5 ± 0.5
pH of saliva at:		
baseline (T0)*	6.8 ± 0.4	6.6 ± 0.4
4 weeks (T4)	6.8 ± 0.4	6.7 ± 0.4
8 weeks (T8)*	6.8 ± 0.4	6.4 ± 1.0

*Significant difference between the probiotic and control group.

log CFU/ml at 4 weeks and 7.2 ± 0.1 log CFU/ml at 8 weeks) (Fig. 5A). Total bacteria levels were not significantly different throughout the study in both groups ($p > 0.05$, Fig. 5B).

No adverse effects were recorded from any child in either group.

Discussion

The principle cause of dental caries is well understood: the consumption of sugars stimulates the growth of oral microbes, most notably *S. mutans*. It can rapidly colonize and grow on teeth, and organic acids, the products from some sugars fermentation, can lead to reducing the pH in the environment.[24] A pH below 5.5 (critical pH) can initiate the demineralization of the tooth surface. Although sugar or sweetness is one of the major factors for dental caries, sugar is the fundamental component for taste. It can be divided into 2 major groups: simple sugars such as glucose, sucrose, fructose, and lactose, and sugar alcohols such as xylitol, sorbitol and maltitol. In Thailand, most commercial

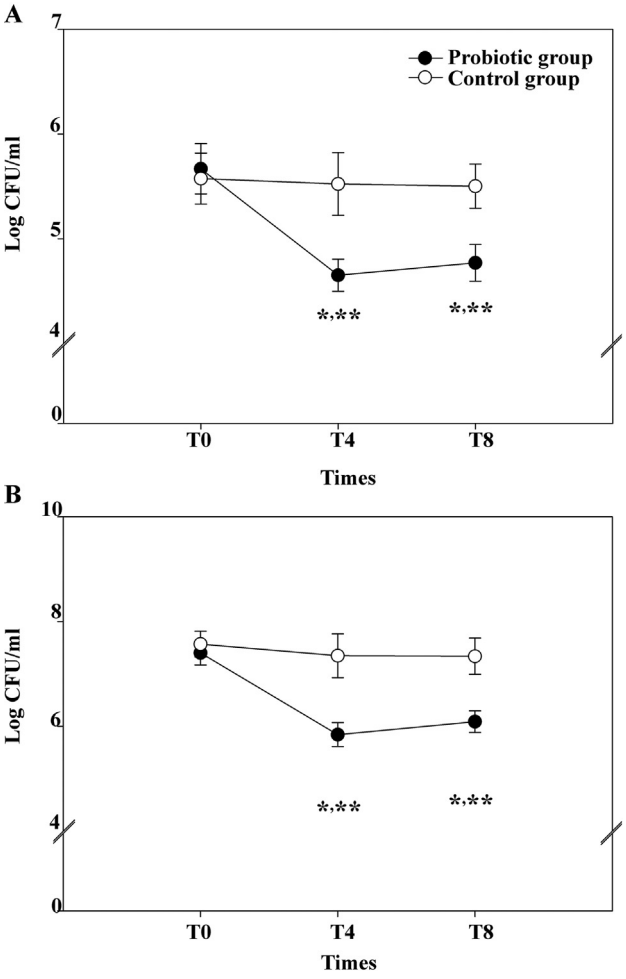

Figure 4 The number of salivary *Streptococcus mutans*-(A) and total streptococci-(B) measured at the baseline (T0), 4 weeks (T4) and 8 weeks of study (T8). *Significant difference in the probiotic group compared to the control group ($p < 0.05$), **Significance difference versus baseline ($p < 0.05$).

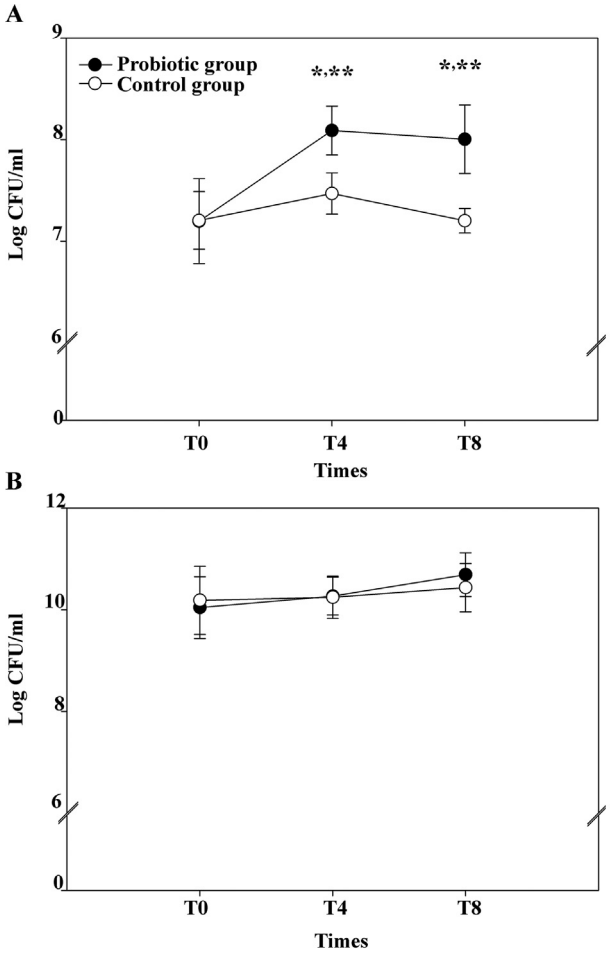

Figure 5 The number of salivary total lactobacilli-(A) and total bacteria-(B) measured at the baseline (T0), 4 weeks (T4) and 8 weeks of study (T8). *Significant difference in the probiotic group compared to the control group ($p < 0.05$), **Significance difference versus baseline ($p < 0.05$).

fermented milk in the market generally contains 10—20% of sugars. This may promote caries development resulting in high caries prevalence among Thai children.[25] The World Health Organization recommends a reduction in simple sugar intake between meals to decrease caries occurrence. It also suggests that sugar substitutes could be an alternative way to eliminate the caries risk, as polyols are not fermented by oral bacteria.[26—28] The results in the present study demonstrated that maltitol yielded less acid production compared to all other simple sugars when fermented by *L. rhamnosus* SD11. *S. mutans* could not grow well in maltitol culture and so the pH remained 7.0. It indicates that maltitol may be a good choice for a sugar substitute. In addition, one study has demonstrated that sugar-free chewing gum with either maltitol or xylitol could give similar results in caries prevention by inducing a higher salivation and salivary pH, significantly decreasing in the activity of glucan sucrase and in the concentration of free sialic acid and inducing a better resistance of the oral ecosystem to sucrose challenge.[29] Therefore, 5% (w/v) maltitol was chosen for adding into both the control and probiotic fermented milk for a continued study in the present clinical trial.

The results in the clinical trial have shown that children who received the fermented milk containing *L. rhamnosus* SD11 had significantly lower total streptococci and *S. mutans* levels compared to their baseline. Such levels were also significantly lower compared to the children who received the control fermented milk containing 4 commercial strains. This finding confirmed our previous study showing that the fermented milk containing *L. rhamnosus* SD11 could reduce mutans streptococci compared to fermented milk containing *L. bulgaricus*.[8,9] The review articles have shown that probiotic strains could reduce the number of cariogenic mutans streptococci,[4,5] however, some reports could not find the reducing effect of probiotics on mutans streptococci.[30,31] This may depend on the different probiotic strains used, which could give different results. In the present study, the reason for the ability of *L. rhamnosus* SD11 to reduce cariogenic *S. mutans* may be explained by its combination abilities; producing bacteriocin,[7] adhering to human oral mucosa[32] and producing anti-oxidative substances.[33] Therefore, it is important to recognize that the health effects of probiotics can be strain-specific, thus different results may occur from the different strains used.

After receiving the fermented milk, the salivary pH of children in the control fermented milk significantly decreased compared to the children who received the probiotic fermented milk containing *L. rhamnosus* SD11 with maltitol. This may explain by that the *S. mutans* level in the control group was higher than in the probiotic group as one study showed that a high salivary *S. mutans* level correlated to a low salivary pH.[34]

The addition of 5% maltitol in the fermented milk product did not change any effect of *L. rhamnosus* SD11 in reducing cariogenic *S. mutans* in volunteers. Moreover, it was observed that 5% of maltitol could enhance the growth of *L. rhamnosus* SD11 without decreasing the pH value below the critical level of pH 5.5. This concurred with the finding that total lactobacilli levels were found to increase in the probiotic group compared to the control group in this study. The composition of fermented milks used for the control- and probiotic-group was the same, except *L. rhamnosus* SD11 was added for the probiotic group. Thus, it was assumed the increase of lactobacilli in the probiotic group may result from the growth of *L. rhamnosus* SD11. This suggests that maltitol may be an alternative prebiotic for promoting the growth of *L. rhamnosus* SD11. Our concern regarding the 5% maltitol was that it may have any effect on the oral microbiota. A study analyzing the effect of maltitol-containing chewing gum on the composition of dental plaque microbiota in subjects with active dental caries by pyrosequencing of 16S rRNA genes, reported that chewing gum containing maltitol had minor effects on the composition of the plaque microbiome.[35] In this study, it was found that the total bacteria levels in both groups did not change throughout the study, however, it was not assured that oral microbiota was not affected by 5% maltitol. Thus, a study of microbiome has been planned in the future.

In addition, the children were satisfied with the taste of the fermented milk analyzed from the questionnaire form, which was an improvement in the taste of products used in our previous study.[8] No side or adverse effects were displayed during the trial, indicates the safety of the strain used. A limitation of this study was that the change of DMFT could not be evaluated due to the study length of time being too short. Further study should include an extended longer study time to monitor for caries progression.

In conclusion, the maltitol yielded less acid production compared to the simple sugars after fermenting by *L. rhamnosus* SD11 and *S. mutans*. Maltitol could enhance the growth of *L. rhamnosus* SD11, this suggests that maltitol may be an alternative prebiotic for promoting the growth of *L. rhamnosus* SD11. The daily consumption of fermented milk containing a potential probiotic *L. rhamnosus* SD11 with 5% (w/v) maltitol showed a beneficial effect on oral health by reducing salivary cariogenic *S. mutans*, and it also did not have any adverse effect on volunteers. The results demonstrated that maltitol at 5% (w/v) is a good choice for substituting sucrose. Therefore, the fermented milk containing *L. rhamnosus* SD11 with 5% maltitol might be an alternative way to prevent caries risk.

Declaration of competing interest

The authors declare no potential conflict of interest with respect to the authorship and/or publication of this article.

Acknowledgments

This study was supported by the National Innovation Agency (NIA) (Bangkok, Thailand), the National Research Council of Thailand, Super clusters (Bangkok, Thailand; DEN600729S), and the government budget of Prince of Songkla University (DEN6201051S). The experimental products were kindly provided by the Dairy Home Industries Company, Thailand. The suppliers had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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