ACTA ODONTOLOGICA SCANDINAVICA
2018, VOL. 76, NO. 5, 331–337
https://doi.org/10.1080/00016357.2018.1453083

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ORIGINAL ARTICLE

Reducing mutans streptococci and caries development by *Lactobacillus paracasei* SD1 in preschool children: a randomized placebo-controlled trial

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ABSTRACT

Objective: To examine a reducing effect of *Lactobacillus paracasei* SD1 on MS and caries in preschool children.

Materials and methods: A total of 124 children, aged 1.5–5 years old, participated and were randomly assigned to the probiotic or control group. The probiotic group received *L. paracasei* SD1-milk and the control group received standard-milk once daily for 3 months. MS/lactobacilli were enumerated and the caries score was examined. Association between probiotic consumption and bacterial level, or caries progression was assessed by a multivariate logistic regression. This study was registered at the Thai-Clinical-Trials-Registry (TCTR20140903001).

Results: Probiotic was found to be a factor associated with the MS level. Children in the probiotic group had a significantly lower risk of an increase in the MS level than in the control group after receiving the probiotic milk at 3- and 4-months with $p < .001$ and $p = .040$, respectively. Probiotic significantly reduced the risk for caries compared to the control group ($p = .016$). There were no adverse effects or non-compliance reported in either group.

Conclusions: Consumption of milk powder containing *L. paracasei* SD1 resulted in a reduction of both salivary MS and delayed new caries development, and the strain is safe for use in young children. Results suggest that *L. paracasei* SD1 may be an alternative way for caries prevention in young children.

ARTICLE HISTORY
Received 2 October 2017
Revised 31 January 2018
Accepted 11 March 2018

KEYWORDS
Lactobacillus paracasei SD1; mutans streptococci; probiotics; dental caries

Introduction

Early childhood caries (ECC) is considered as a serious public-health problem in developing countries, due to its impact on individuals and communities in terms of pain and suffering, impairment of function and reduced quality of life [1]. A number of studies including a systemic review have shown that the presence of mutans streptococci (MS) in saliva or plaque of 2- to 5-year-old children is correlated with an increased risk of dental caries [2–4]. However, other species clearly arise as main players in the microbial community including *Veillonella*, *Rothia*, and *Leptotrichia* in enamel caries and *Streptococcus sanguinis*, *Atopobium*, *Schlegelella*, *Pseudoramibacter* and *Lactobacillus* in dentine caries [5]. Although great efforts have been performed in oral health promotion, ECC remains a major oral health problem among Thai children. A high burden has been reported each year for treatment of the disease and its sequels, thus novel complementary strategies for caries prevention are required.

Previously, probiotics have been proven to have benefits for prevention or treatment of gastrointestinal diseases [6,7]. In recent years, an increased interest in probiotics for oral health has emerged, and the caries preventive effect of probiotics has been proposed [8,9]. Systemic review and meta-analysis suggest that probiotics decrease the MS counts, thus it could have a positive effect on the prevention of caries [10–12]. Among those studies, there are great variation in their set up including duration, the probiotics used, mode of application, and the number and age of the participants. Most studies were conducted in adults and young adults, while the study in young children is very few.

Our previous studies on screening to select potential probiotic strains of *Lactobacillus* strains derived from caries-free subjects demonstrated that *Lactobacillus paracase*i SD1 exhibits good properties e.g. inhibiting growth of oral pathogens [13], producing antimicrobial substances [13] and adhering well to human keratinocytes [14]. Probiotic has been further studied in clinical trials to explore the effect on prevention of caries and its safety. It has been demonstrated that for a short-term, 4 weeks, consumption of milk powder containing *L. paracasei* SD1 could reduce salivary MS in adult volunteers [15,16]. A longer-term (6 months) of daily probiotic ingestion significantly reduced the number of MS and caries risk among the school children with high caries [2]. No adverse effect was observed in previous clinical trials, indicating the safety of the strain.

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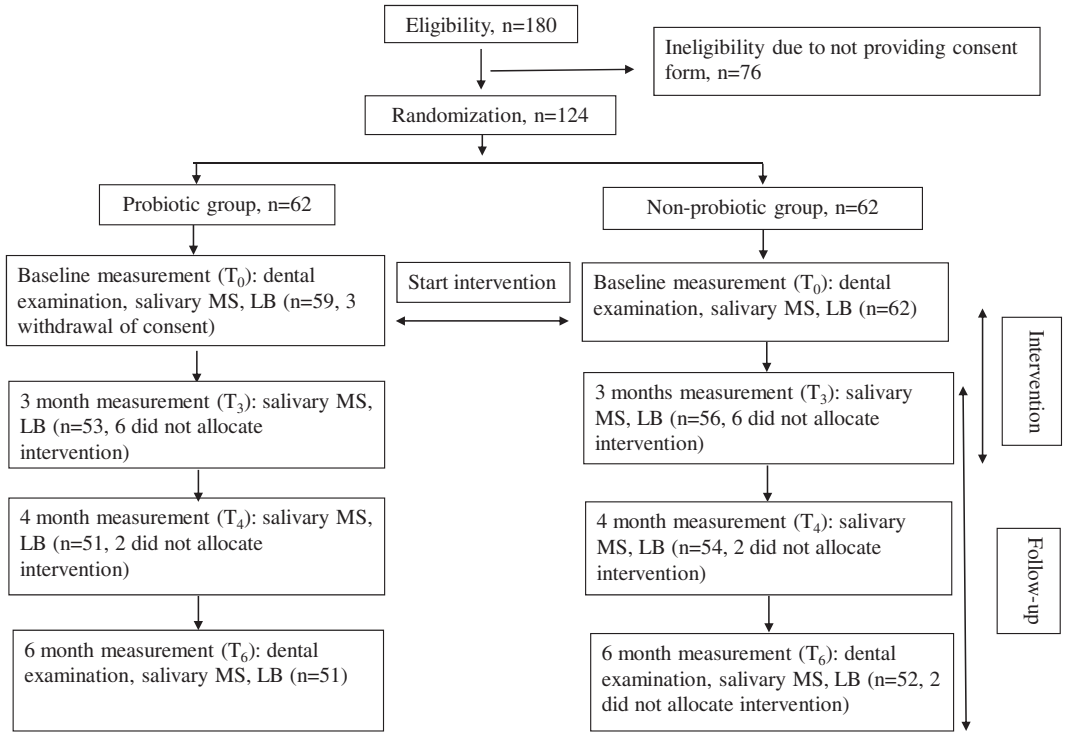

Figure 1. Consort flow diagram of the trial.

It was reported that children with caries lesions are at risk of developing new caries lesions in permanent dentitions [17]. The application of probiotic *L. paracasei* SD1 may be an alternative way for early prevention of dental caries among young children. The aim of this study was to investigate the effect of *L. paracasei* SD1 on the growth of MS as well as dental caries in young children. Any potential side effects of the probiotic were also observed.

Materials and methods

Subjects

The study took place in the small child development centre of Ban Phru, Hat Yai, Songkhla, Thailand. A total of 180 children, aged 1.5–5 years old, were invited to participate in the study. The exclusion criteria were (i) habitual consumption of probiotics or xylitol, (ii) systemic antibiotic medication taken within 6 weeks, (iii) allergic to cows' milk, lactose intolerant or having a severe food allergy, or (iv) having systemic or severe chronic diseases.

The sample size calculation was based on our pilot study; it was calculated that there would be an estimated 80% power at the 0.05 level of significance using two-sided testing for detecting the difference in two proportions of individuals who had caries progressive lesions (37.5% in the probiotic and 66.7% in the control group) over 6 months. Thirty-five participants per group were needed. After adjustment to evaluate the results of the caries increment and allowing for 30% dropout, at least 92 participants of a total sample size was judged as necessary for this study.

The project was thoroughly explained to the children and their parents in a meeting organized at the child development centre and the informed consent form was signed when all individual participants accepted to participate voluntarily in the clinical trial. A flowchart of the study is outlined in Figure 1. All subjects were asked to immediately report any adverse side effects and to fill in the questionnaire form during the 3 months of milk consumption.

Study design

The prospective investigation was a follow-up of a double-blinded, randomized placebo-controlled trial in two parallel groups, with a study period of 6 months. The study was approved by the ethics committee of the Faculty of Dentistry at the Prince of Songkla University, Thailand (EC5702-08-P-HR) and was registered at the Thai Clinical Trials Registry http://www.clinicaltrials.in.th/, being one of WHO's Registry Network, Clinical Trials identifier TCTR20140903001 (registered 2 September 2014, Date of first enrolment 8 May 2014 'retrospectively registered').

Children were randomized to the probiotic or control group by means of drawing lots and this information was kept secret by an independent monitor. This was not unveiled until all data had been analysed. Neither the researchers nor clinicians or healthcare personnel knew whether the children received control or intervention milk during the course of the study.

Intervention

Children in the probiotic group drank 5 g of reconstituted milk powder with probiotic *L. paracasei* SD1 in 50 mL water once daily, whereas children in the control group drank 5 g of reconstituted milk powder without probiotic bacteria in 50 mL water once daily for 3 months. The probiotic milk

Table 1. General characteristics between control and probiotic groups at baseline (T0).

	Total ($n = 103$)	Control ($n = 52$)	Probiotic ($n = 51$)	p value
Age (months)	41.06 ± 9.19	39.69 ± 8.96	42.45 ± 9.29	.113
Gender				
Male	53 (51.46)	31 (59.6)	22 (43.10)	.094
Female	50 (48.54)	21 (40.40)	29 (56.90)	
MS level (CFU/1.5 cm^2)				
0–10	27 (26.21)	16 (30.80)	11 (21.60)	.268
11–50	23 (22.33)	14 (26.90)	9 (17.60)	
51–100	19 (18.45)	9 (17.3)	10 (19.60)	
>100	34 (33.01)	13 (25.00)	21 (41.20)	
Lactobacilli level (CFU/1.5 cm^2)				
0–10	16 (15.53)	8 (15.40)	8 (15.70)	.973
11–50	18 (17.48)	10 (19.20)	8 (15.70)	
51–100	12 (11.65)	6 (11.50)	6 (11.80)	
>100	57 (55.34)	28 (53.80)	29 (56.90)	
Mean of dt	9.72 ± 5.99	8.85 ± 6.09	10.61 ± 5.81	.132

Comparison between control and probiotic groups was performed with Chi-square test (categorical variables) or Mann–Whitney U test (quantitative variables); statistically significant difference at $p < .05$.

powder contained *L. paracasei* SD1 10^7 CFU/g and was prepared according to Teanpaisan et al. [18]. The test and the control milk were delivered in plastic bags labelled with the individual names of the children. All were asked to drink under observation every day by the staff taking care of them. On the holidays or weekends, parents were asked to give the milk at home and then returned the plastic bags.

Compliance was checked by the staff overseeing the children, by completing a logbook every day regarding drinking the milk.

Oral examination

According to the caries management protocol for 3–6 years old, children with moderate and high caries risk should be re-evaluated for oral examination on 6 months and 3 months basis, respectively [19]. Thus, oral examinations in this study were planned to be carried out at baseline and at the end of the study after 6 months by 3 dentists (Piwat S., Akkarachaneeyakorn N., and Chankanka O.). Before the start of the study, the three examination teams were calibrated against each other and inter- and intra-examiner reliability tests were carried out (0.91 and 0.98, respectively) before the baseline and before the re-examination.

Dental caries status was recorded according to the modified Navad criteria for caries assessment [20]; code 0, sound surface; code 1, active non-cavitated lesion; code 2, active cavitated lesion; code 3, inactive non-cavitated lesions; code 4, inactive cavitated lesions; code 5, filling; code 6, filling associated with active caries; and code 7, missing due to caries.

Microbial evaluation

Unstimulated saliva samples were collected at baseline (T0), 3 (T3), 4 (T4) and 6 (T6) months of study using the spatula method [21], and cultured on a Mitis Salivarius Bacitracin agar or a de Man, Rogosa and Sharpe (MRS) agar, being the selective media for MS and lactobacilli, respectively. After 48 h of incubation in anaerobic conditions (80% N_2, 10% CO_2 and 10% H_2) at 37 °C, the number of MS and lactobacilli were counted as colony forming units (CFU)/1.5 cm^2.

Analysis of data

All numerical data were presented as means and standard deviations. The general characteristics of the children (gender, age, number of bacterial counts and mean of decayed teeth) between control and probiotic groups were analysed using the Chi-square test for categorized/dichotomized variables and the Mann–Whitney U test for interval variables. The number of colony counts of MS and lactobacilli were presented as CFU/1.5 cm^2 and were categorized as 0 CFU/1.5 cm^2, 1–50 CFU/1.5 cm^2, and >50 CFU/1.5 cm^2. The mean of decayed teeth and difference of caries increments between the two groups were analysed by the Mann–Whitney U test. Multivariable ordinal logistic regression models were used to assess the association between probiotic consumption and bacterial level at different time points. Also, multivariable logistic regression was used to analyse the risk of caries progression. The odds ratios and their 95% confidence interval were calculated. The software package used was the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL), and the differences were considered as significant when $p < .05$.

Results

From a total of 180 children, 124 children (63 females, 61 males) aged 15–60 months (mean: 41.06 ± 9.18 months) were given informed consent by their parents at the beginning of the study. At the end of the study, 103 children remained in the study indicating the dropout rate was low (17%), and the number of investigated children is given in the flowchart (Figure 1). The baseline characteristics of all 103 children in both groups who participated in the study are presented in Table 1. There were no statistically significant differences between the groups in age, sex, MS and lactobacilli levels at the baseline ($p > .05$). Unerupted teeth were found 23.3% in all children (24 of 103), 25.5% (13 of 51 children) and 21.2% (11 of 52 children) in the probiotic group and the control group, respectively. There was no detection of filled teeth or missing teeth due to caries in any children. Thus, only decayed teeth were analysed in this study. The mean for decayed teeth seemed to be higher in the probiotic than in the control group, however, it was not a significant difference ($p > .05$).

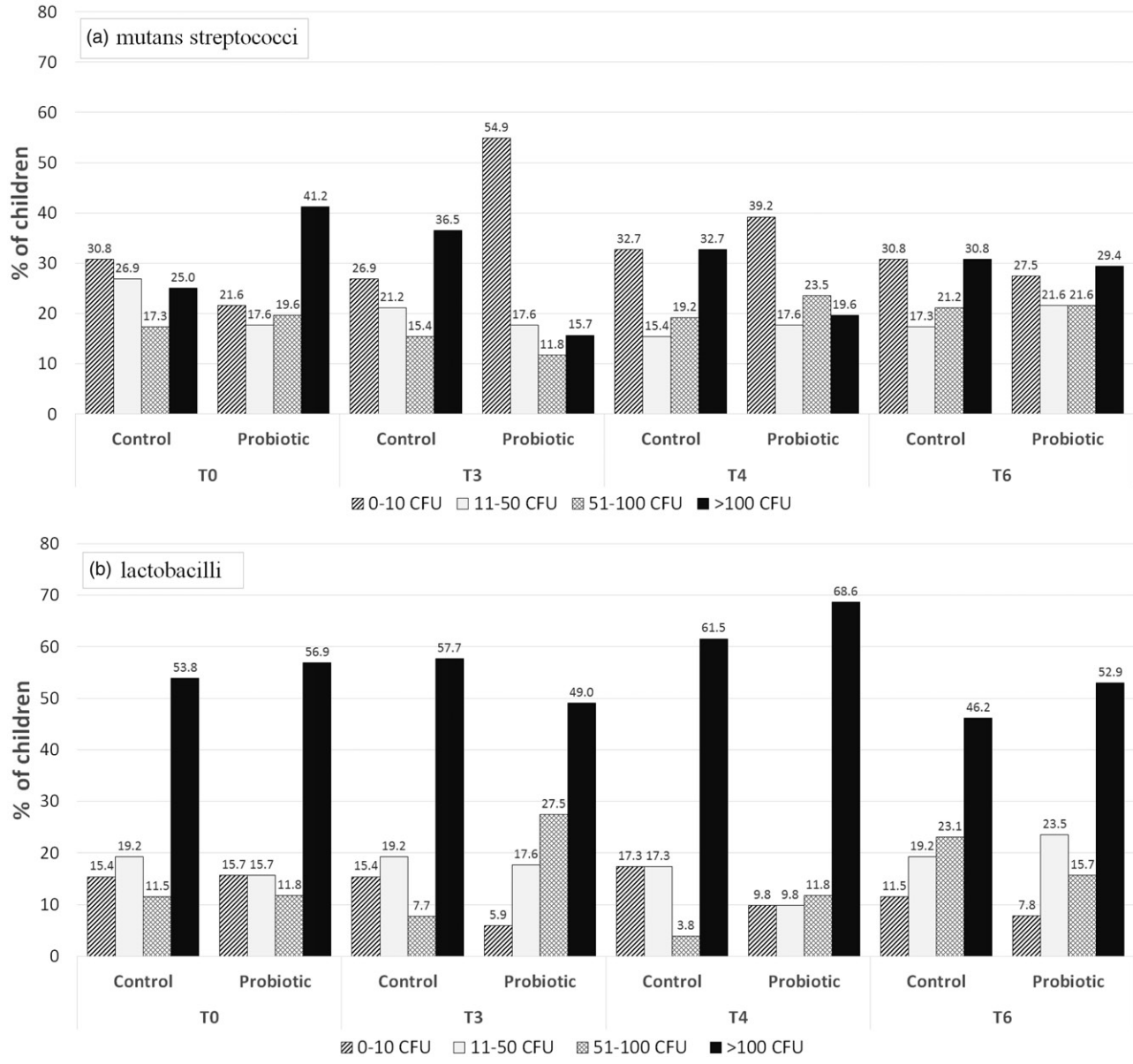

Figure 2. Number of children at each MS or lactobacilli levels by groups and different time points (baseline (T0), 3 (T3), 4 (T4) and 6 (T6) months of study).

The number of children with different levels of salivary MS and lactobacilli at baseline through the end of the study is demonstrated in Figure 2(a,b). After receiving the probiotic milk, a decrease in the number of children with a high MS level (>100 CFU/1.5 cm^2) was observed among children in the probiotic group. For multivariable ordinal logistic regression analyses (Table 2), after receiving the probiotic milk at T3 and T4, children in the probiotic group had significantly lower risk of an increase in MS levels than the control group with an odds ratio of 0.20 (95% CI: 0.09–0.46, $p < .001$) and 0.45 (95% CI: 0.21–0.96, $p = .040$), respectively. Also, probiotic consumption was not significantly associated with lactobacilli levels at any follow-up periods.

Details of tooth surface status examined according to the modified Nyvad criteria are shown in Table 3. Generally, there was no statistically significant difference of individual tooth surface status between the probiotic and control group. A change of individual code over a 6 month time was further carried out using a transitional analysis. Mostly, individual codes still remained at the same status (92.5 and 93.6% in the probiotic and control group, respectively), while the progression of caries (sound or inactive turned to active lesions)

Table 2. The association between groups and MS/lactobacilli levels at different time points (3-, 4- and 6-months of study).

Time point	Parameter	Odds ratio (95% CI)	*p* value
MS level			
T3	Group (probiotic vs. control)	0.20 (0.09, 0.46)	<.001
	MS level at T0	2.35 (1.64, 3.38)	<.001
T4	Group (probiotic vs. control)	0.45 (0.21, 0.96)	.040
	MS level at T0	2.41 (1.70, 3.41)	<.001
T6	Group (probiotic vs. control)	0.70 (0.33, 1.49)	.354
	MS level at T0	2.87 (2.00, 4.12)	<.001
Lactobacilli level			
T3	Group (probiotic vs. control)	1.04 (0.49, 2.19)	.927
	Lactobacilli level at T0	1.74 (1.26, 2.40)	.001
T4	Group (probiotic vs. control)	1.55 (0.67, 3.59)	.309
	Lactobacilli level at T0	2.14 (1.50, 3.05)	<.001
T6	Group (probiotic vs. control)	1.13 (0.54, 2.37)	.751
	Lactobacilli level at T0	1.82 (1.32, 2.52)	<.001

Multivariable ordinal logistic regression models were used to assess the association after adjusting for bacterial levels at baseline (T0); statistically significant difference at $p < .05$.

Table 3. Details of tooth surface status according to Nyvad criteria of children in the probiotic ($n = 51$) and control group ($n = 52$) at baseline and 6 months.

	No. of tooth surface (%) at baseline		No. of tooth surface (%) at 6 months	
Tooth surface status	Probiotic	Control	Probiotic	Control
Unerupted surface	234 (4.10)	219 (3.76)	124 (2.65)	127 (2.66)
Code 0, sound surface	3549 (62.21)	4105 (70.50)	3051 (65.32)	3456 (72.48)
Code 1, active non-cavitated surface	468 (8.20)	458 (7.87)	338 (7.24)	322 (6.75)
Code 2, active cavitated surface	1243 (21.79)	908 (15.59)	878 (18.80)	684 (14.35)
Code 3, inactive non-cavitated surface	85 (1.49)	80 (1.37)	75 (1.61)	73 (1.53)
Code 4, inactive cavitated surface	126 (2.21)	53 (0.91)	205 (4.39)	106 (2.22)
Total surface	5705 (100.00)	5823 (100.00)	4671 (100.00)	4768 (100.00)

was found 4.44 and 4.88%, in the probiotic and control group, respectively. It is noted that reversion of carious lesions (active non-cavitated or active cavitated turned to inactive non-cavitated or inactive cavitated lesions) was found relatively higher in the probiotic group (3.06%) than in control group (1.53). Mean of decayed tooth at baseline and at the end of the study of both groups are shown in Table 4. There were no any filled teeth found either at baseline or at the end of the study. Although there was no statistically significant difference in means of decayed teeth at baseline compared to the end of the study in either group, a significant increase in caries increment was found among children in the control group compared to the probiotic group ($p = .029$). When the main variables were analysed (Table 5), the dependent variables and caries progression were dichotomized scoring as progression and no progression. The model was controlled for probiotic consumption, caries level at baseline, and age. The results showed significant negative association between probiotic consumption and caries progression. The odds of having caries progression in the probiotic group was lower than in the control group (odds ratio 0.34, 95% CI: 0.14–0.82, $p = .016$). While the odds of having caries progression was higher for children with high caries at the baseline ($\geq$6 decayed teeth) (odds ratio 7.06, 95% CI: 1.73–28.83, $p = .006$).

Table 4. Mean ± SD of decayed teeth of an individual's decayed score at baseline and at 6 months of study.

	Control	Probiotic	*p* value
Decayed teeth at baseline (dt0)	8.85 ± 6.09	10.61 ± 5.81	.132
Decayed teeth at 6 months of study (dt6)	10.10 ± 5.55	11.37 ± 5.60	.220
Increase of decayed teeth (Δ dt)	1.25 ± 1.64	0.76 ± 1.29	.029

Statistically significant difference at $p < .05$.

Table 5. Multivariable logistic regression model to estimate the odds of being in the caries progression group relative to the no caries progression group.

Parameter	Odds ratio (95% CI)	*p* value
Probiotic	0.34 (0.14, 0.82)	.016
Caries level at baseline		
0–2 decayed teeth	Ref.	
3–5 decayed teeth	3.826 (0.87, 16.84)	.076
$\geq$6 decayed teeth	7.06 (1.73, 28.83)	.006
Age (month)	0.98 (0.92, 1.04)	.599

Statistically significant difference at $p < .05$.

Discussion

Despite great efforts and achievements in oral health promotion, caries remains a major health problem among childhood [1]. Potential effects of probiotic strains on cariogenic pathogens have been abundantly demonstrated *in vivo* [10–12]. In Thailand, the report of the Ministry of Public Health, Thailand (2012) showed that approximately 60% of children (3-years-old) were affected by dental caries, especially children in the southern part of Thailand. Regional data in Songkhla Province confirmed the high caries development (71.2%) among Thai children in the South of Thailand [2]. The progression of caries increased from 4.2% at 9 months-old to 84.5% at 24 months-old with a mean caries score (dmf) of 0.14 and 5.35, respectively, indicating that the ECC problem among Thai children is severe. Thus, novel complementary strategies for caries prevention are required. In recent years, probiotic applications for prevention and/or treatment of oral diseases, especially dental caries, has been received an attention. Probiotics are defined as live microorganisms, when administrated in adequate amounts that confer a health benefit on the host [22]. In most cases, the MS count number has been used as the indicator to prove the efficacy of probiotics for caries prevention [10–12], while the use of clinical parameters, like the increment of newly developed caries lesions or caries risk was scarce, especially in ECC studies [23–28]. Most studies have shown the efficacy of probiotics in prevention of caries [23,24,27,28]. However, one study did not find any change, neither an increase nor decrease, of caries at 4 years of age in a low caries population after receiving probiotic [25]. The authors explained this finding by the fact that probiotic effects are species and/or strain specific.

This study was designed as a randomized double-blinded clinical 6 months-trial regarding the effects of *L. paracasei* SD1, a human oral-origin strain, on both MS levels and dental caries in young children. This was based on the fact that Thai children are in high caries risk, 3 months for probiotic intervention and 6 months for monitoring caries progression could observe the effect of probiotic strain. The dropout rate from the baseline was low and compliance with the study protocol during the intervention was satisfactory based on the information from the logbook. In previous studies, the follow-up samplings were generally conducted at the termination of probiotic intervention and any information on post-intervention regrowth of the suppressed target bacteria afterward was minimal. This study monitored the MS levels more frequently during the intervention and follow-up time. It was hypothesized that long-term exposure to probiotics could prevent or delay MS colonizing and accordingly

prevent dental caries. Thus, evaluation of the study was based on MS colonization and the increment of newly developed caries lesions between the children receiving probiotic milk and control milk. Our findings revealed that the probiotic was found to be a factor associated with MS levels at follow up periods after adjusting for bacterial levels at baseline, and that the odds of having high levels of MS was significantly low among probiotic subjects at the 3 and 4 months stage of follow-up. The number of children with a high salivary MS level (>100 CFU) in the probiotic group was found to decrease after 3 months of receiving the probiotic milk compared to the baseline, and a decrease of MS could still be observed after cessation of receiving the probiotic milk at least 3 months later (T3–T6). Children taking the control milk seemed to have stable individual MS levels throughout the study compared to the baseline. In previous studies, an increase in the number of children with high salivary lactobacilli levels (>100 CFU) in the probiotic group was observed after consuming probiotics [23,26–28]. However, such increase was not found during the whole period in this study.

There have been only a few reports of randomized controlled clinical trials using caries as the outcome, especially regarding clinical evaluation of probiotic bacteria that originated from the mouth. Most strains isolated and developed for gastrointestinal health have been adopted for use in dental research. The 3-months intervention followed by 3-months observation in this study revealed that *L. paracasei* SD1 strain may have a beneficial effect on delayed caries progression leading to a significantly low caries increment among the children in the probiotic group compared to the control at the end of the study. Whether such findings resulted from a decrease of MS by probiotic effect is still questioned. Given the polymicrobial nature of dental caries, it suggests that preventive strategies directed towards specific bacterial species may not be universally effective, thus future research should consider focusing on the metabolic output of microbial communities for understanding and controlling dental caries [5].

Any residual effect after the taking of products containing probiotics has always been an issue of interest. In this study, there were no reported negative side effects following the probiotic intervention in the young children.

It has been accepted that the effects of probiotic strains are strain specific; not all the *Lactobacillus* are probiotics that possess the ability to confer health benefits for the host [22]. Thus exploring the desirable properties of certain strains is needed. Our previous study revealed that *L. paracasei* SD1 could produce paracasin SD1, a specific antimicrobial protein, against various oral pathogens including cariogenic bacteria [29]. It may support the advantages of this strain with respect to its potential use as a bacterial replacement, which is a means of combatting infections by the administration of non-pathogenic bacteria to displace pathogenic microorganisms. In conclusion, the consumption of milk powder containing *L. paracasei* SD1 has resulted in the reduction of both salivary MS and new caries development. The results indicate that certain probiotics may be an alternative way to be considered for caries prevention of young children in a high-caries population.

Acknowledgements

The authors thank the children and their parents, and all staffs in the child development centre of Ban Phru, Hat Yai, Songkhla, Thailand for their contributions to participate in this research work. Also, authors thank the Oral Microbiology Laboratory, Faculty of Dentistry for facilitating equipment.

Disclosure statement

The authors report no conflicts of interest.

Funding

This research was supported by the National Research Council of Thailand [DEN580217M] and the Postdoctoral Fellowship, Prince of Songkla University for Dr. Nuntiya Pahumunto.

References

[1] Petersen PE. World health organization global policy for improvement of oral health-World Health Assembly 2007. Int Dent J. 2008;58:115–121.
[2] Teanpaisan R, Thitasomakul S, Piwat S, et al. Longitudinal study of the presence of mutans streptococci and lactobacilli in relation to dental caries development in 3–24 month old Thai children. Int Dent J. 2007;57:445–451.
[3] Meurman PK, Pienihäkkinen K. Factors associated with caries increment: a longitudinal study from 18 months to 5 years of age. Caries Res. 2010;44:519–524.
[4] Thenisch NL, Bachmann LM, Imfeld T, et al. Are mutans streptococci detected in preschool children a reliable predictive factor for dental caries risk? A systematic review. Caries Res. 2006;40:366–374.
[5] Simón-Soro A, Mira A. Solving the etiology of dental caries. Trends Microbiol. 2015;23:76–82.
[6] Parvez S, Malik KA, Ah Kang S, et al. Probiotics and their fermented food products are beneficial for health. J Appl Microbiol. 2006;100:1171–1185.
[7] Varankovich NV, Nickerson MT, Korber DR. Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Front Microbiol. 2015;6:685.
[8] Saha S, Tomaro-Duchesneau C, Tabrizian M, et al. Probiotics as oral health biotherapeutics. Expert Opin Biol Ther. 2012;12:1207–1220.
[9] Twetman S, Keller MK. Probiotics for caries prevention and control. Adv Dent Res. 2012;24:98–102.
[10] Jindal G, Pandey RK, Singh RK, et al. Can early exposure to probiotics in children prevent dental caries? A current perspective. J Oral Biol Craniofac Res. 2012;2:110–115.
[11] Cagetti MG, Mastroberardino S, Milia E, et al. The use of probiotic strains in caries prevention: a systematic review. Nutrients. 2013;5:2530–2550.
[12] Laleman I, Detailleur V, Slot DE, et al. Probiotics reduce mutans streptococci counts in humans: a systematic review and meta-analysis. Clin Oral Invest. 2014;18:1539–1552.
[13] Teanpaisan R, Piwat S, Dahlen G. Inhibitory effect of oral *Lactobacillus* against oral pathogens. Lett Appl Microbiol. 2011;53:452–459.
[14] Piwat S, Sophatha B, Teanpaisan R. An assessment of adhesion, aggregation and surface charges of *Lactobacillus* strains derived from the human oral cavity. Lett Appl Microbiol. 2015;61:98–105.
[15] Teanpaisan R, Piwat S. *Lactobacillus paracasei* SD1, a novel probiotic, reduces mutans streptococci in human volunteers: a

randomized placebo-controlled trial. Clin Oral Invest. 2014;18: 857–862.
[16] Ritthagol W, Saetang C, Teanpaisan R. Effect of probiotics containing *Lactobacillus paracasei* SD1 on salivary mutans streptococci and lactobacilli in orthodontic cleft patients: a double-blinded, randomized, placebo-controlled study. Cleft Palate Craniofac J. 2014;51:257–263.
[17] Peretz B, Ram D, Azo E, et al. Preschool caries as an indicator of future caries: a longitudinal study. Pediatr Dent. 2003;25:114–118.
[18] Teanpaisan R, Chooruk A, Wannun A, et al. Survival rates of human-derived probiotic *Lactobacillus paracasei* SD1 in milk powder using spray drying. Songklanakarin J Sci Technol. 2012;34: 241–245.
[19] Ramos-Gomez FJ, Crystal YO, Domejean S, et al. Minimal intervention dentistry: part 3. Paediatric dental care-prevention and management protocols using caries risk assessment for infants and young children. Br Dent J. 2012;213:501–508.
[20] Séllos MC, Soviero VM. Reliability of the nyvad criteria for caries assessment in primary teeth. Eur J Oral Sci. 2011;119:225–231.
[21] Kohler B, Bratthall D. Practical method to facilitate estimation of *Streptococcus mutans* levels in saliva. J Clin Microbiol. 1979;9: 84–88.
[22] Joint FAO/WHO Working Group Guidelines for the evaluation of probiotics in food. [Internet] 2002 [cited 2017 Jan 5]. Available from: ftp://ftp.fao.org/es/esn/food/wgreport2.pdf
[23] Nase L, Hatakka K, Savilahti E, et al. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. Caries Res. 2001;35:412–420.
[24] Stecksen-Blicks C, Sjostrom I, Twetman S. Effect of long-term consumption of milk supplemented with probiotic lactobacilli and fluoride on dental caries and general health in preschool children: a cluster-randomized study. Caries Res. 2009;43:374–381.
[25] Taipale T, Pienihakkinen K, Alanen P, et al. Administration of *Bifidobacterium animal* subsp. *Lactis* BB-12 in early childhood: a post-trial effect on caries occurrence at four years of age. Caries Res. 2013;47:364–372.
[26] Shimada A, Noda M, Matoba Y, et al. Oral lactic acid bacteria related to the occurrence and/or progression of dental caries in Japanese preschool children. Biosci Microbiota Food Health. 2015;34:29–36.
[27] Hedayati-Hajikand T, Lundberg U, Eldh C, et al. Effect of probiotic chewing tablets on early childhood caries-a randomized controlled trial. BMC Oral Health. 2015;15:112.
[28] Rodriguez G, Ruiz B, Faleiros S, et al. Probiotic compared with standard milk for high-caries children: a cluster randomized trial. J Dent Res. 2016;95:402–407.
[29] Wannun P, Piwat S, Teanpaisan R. Purification and characterization of bacteriocin produced by oral *Lactobacillus paracasei* SD1. Anaerobe. 2014;15:17–21.