Effects of *Lactobacillus casei* Shirota intake on caries risk in children

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**Abstract**  
**Background/purpose:** Yakult is a well-known probiotic beverage consisting of a single live bacterial species, *Lactobacillus casei* Shirota. However, the potential cariogenic/carciostatic effects of Yakult intake among children have not been studied yet. Hence, this study aimed to investigate the clinical effects of short-term Yakult intake on oral biofilm acidogenicity, cariogenic bacterial counts, and caries risk in children.

**Materials and methods:** Eighteen children, 7–11 years of age, consumed standard Yakult daily for 7 days. Prior to and after intervention, functional oral biofilm acidogenicity characterized by the Stephan curve, *Lactobacillus* and *Streptococcus mutans* counts, and caries risk were determined.

**Results:** Probiotic intervention demonstrated significant increase in minimum pH from 4.88 to 5.14 (P = 0.02), 18.2% reduction in area under the Stephen curve [area under the curve (AUC)], and 29.3% decrease in pH recovery time, although these two differences were not statistically significant. No difference was observed in *S. mutans* and *Lactobacillus* counts or caries risk after intervention (all P > 0.05). However, on subgroup analysis using “reduction of AUC” to separate “responders” from the “nonresponders”, the significant cariostatic effects on oral biofilm acidogenicity, among “responders”, were revealed by an increase in minimum pH (P = 0.005) and a reduction in pH recovery time (P = 0.003).

**Conclusion:** There may be a potential cariostatic effect of short-term Yakult intake in reducing functional biofilm acidogenicity in children with certain oral biofilm and risk profile. Further studies may be needed to validate this probiotic effect. Quality risk assessment may be critical prior to prescribing/recommending Yakult as an adjunct caries-preventive treatment for children.

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Introduction

Probiotics are defined as living microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Clinical studies support the use of specific Lactobacillus and Bifidobacterium bacterial strains for the management of rotavirus and Clostridium difficile diarrhea, bladder cancer, allergic hypersensitivity, lactase deficiency, and surgical infections. There has also been increasing interest in the potential usage of probiotics for prevention of dental caries. A double-blind, randomized, placebo-controlled clinical trial demonstrated that a 6-week intake of lozenges, containing Lactobacillus brevis CD2, effectively reduced plaque acidogenicity and salivary Streptococcus mutans concentration in high caries risk (CR) school children. Preliminary clinical data suggest that Lactobacillus rhamnosus and Lactobacillus salivarius may offer protection against dental caries in young children. In a double-blind placebo-controlled trial conducted in 2- to 3-year-old children, daily intake of three probiotic Streptococcus strains significantly reduced 1-year caries increments by 4-fold. Therefore, probiotics may be considered an adjunct to the current caries control measures such as tap water fluoridation and restriction of dietary sugar intake.

Yakult, a well-known probiotic product with more than 50 years of market history in Japan and Taiwan, is a dairy beverage consisting of a suspension of a single live bacterial species, Lactobacillus casei Shirota, in artificially sweetened skimmed milk. Yakult has been shown to be safe with no adverse effects in both healthy and immunocompromised children when administered daily for up to 55 months, and L. casei Shirota has been classified as a "generally recognized as safe" additive by the United States Food and Drug Administration. In addition, the inclusion of sweeteners in Yakult improves palatability and compliance when administered to children. Yakult intake has been correlated with relief of chronic idiopathic constipation symptoms and increase in natural killer cell capacity when administered to children. Yakult intake may be considered an adjunct to the current caries control measures such as tap water fluoridation and restriction of dietary sugar intake.

Materials and methods

Clinical study

Ethical approval for this study was granted by the Institutional Review Board of Chang Gung Memorial Hospital (reference number 99-1697B). The clinical study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and is registered at ClinicalTrials.in.th (identification number TCTR20150922002). With written parental permission and child’s assent, participants were recruited from the Children’s Dental Clinic of Kaohsiung Chang Gung Memorial Hospital based on the following inclusion criteria:

1. Schoolchildren of at least 7 years of age
2. Cooperative disposition
3. Absence of any systemic disease
4. No habitual consumption of Yakult
5. No restorations on maxillary canines or premolars

Both at baseline and after 1-week Yakult consumption, caries risk assessment (CRA; using Carigram), oral bacterial counts, and functional biofilm pH characterization (plotting of Stephan curves) were performed as detailed below. Study participants abstained from oral hygiene procedures 2 days prior, and food intake 2 hours prior, to all measurements. Because L. casei Shirota is not a first colonizer, gargling might increase the colonization of the probiotic bacteria; hence, participants were instructed to gargle with one bottle (100 mL) standard Yakult (Yakult Co. Ltd., Taipei, Taiwan), for 1 minute prior to swallowing the contents, daily after the evening meal for 7 consecutive days.

Based on the study design, this project is a single-group pilot clinical study (without control and randomization). Dependent variables include three parameters representing biofilm acidogenicity (3BA) derived from the “Stephan curve” [including lowest pH reached, pH recovery time, and the area under curve (AUC) below the critical pH 5.5], caries risk (CR), salivary S. mutans, Lactobacillus, and buffering capacity (BC). The independent variables include consumption of Yakult (before/after), and response to Yakult consumption based on AUC change (respondent/nonrespondent). All participants were recruited from outpatient pool as a convenient sample and were informed via doctors.

Oral examination and salivary tests

Oral examinations were conducted by an experienced pediatric dentist (C-Y.S.H.), using mirrors and explorers under focused flashlights in a conventional dental chair. Caries rate was assessed using deft and defs indices based on the World Health Organization diagnostic criteria. Oral hygiene status was evaluated for six index teeth (1E, 1B, 2D, 3E, 3B, and 4D) according to the Silness-Löe Plaque Index. Salivary S. mutans and Lactobacillus counts, and buffer capacity were semiquantified using Dentocult S. mutans Strip mutans, Dentocult Lactobacillus, and Dentobuff test kits (Orion Diagnostica, Espoo, Finland), respectively.
Plaque pH (acidogenicity) characterization

Plaque pH measurements were performed using a Beetrode NMPH-3 (World Precision Instruments Inc., Connecticut, Sarasota, FL, USA) 0.1-mm-diameter palladium touch microelectrode connected to a portable Orion PerpHect Model 370 unit (Thermo Fisher Scientific, Beverly, Essex, MA, USA). To create a reference salt bridge, participants immersed a finger in 3M KCl solution containing a DRIREF-5 4.7-mm-diameter porous glass reference electrode (World Precision Instruments Inc., Connecticut, Sarasota, FL, USA) connected to the pH meter unit. Electrodes were sterilized in 2.5% w/v glutaraldehyde and recalibrated against pH 4.0 and 7.0 standard buffers between each reading. Oral biofilm pH was measured at the distal surfaces of the maxillary canines, as previously described. 23 Participants were instructed to rinse with a 10% sucrose solution for 1 minute. pH measurements were taken immediately prior to and after rinsing, then at 2 minutes, 5 minutes, and subsequently at 5-minute intervals henceforth until a stabilized pH was reached. A Stepman curve24 was generated for each participant at each visit, with three parameters derived from the curve, namely, the lowest pH reached, pH recovery time, and the AUC below the critical pH 5.5.

Caries risk assessment

The caries risk of each participant was assessed using Cariogram as described previously.25,26 Questionnaire data, caries experience, oral hygiene status, and biological parameters (salivary S. mutans, Lactobacillus, and BC) were entered into the Cariogram software. For each participant, the Cariogram output of “actual chance to avoid new caries” was subtracted from 100% to obtain the percentage chance of developing caries. Participants were subsequently categorized into caries risk groupings of very low risk (0–20%), low risk (21–40%), moderate risk (41–60%), high risk (61–80%), and very high-risk (81–100%).

Statistical analyses

Statistical analyses were performed using the SPSS statistical package, version 20.0 (International Business Machines, Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to test for normality, and Levene’s test was used to test for homogeneity of variance, prior to selection of appropriate parametric/nonparametric tests. The overall data were analyzed using two-tailed paired-sample t tests regarding CR and 3BA derived from the Stepman curve, with the subgroup analysis using Wilcoxon signed-rank test to assess the CR and 3BA outcome variables and the chi-square test for S. mutans, Lactobacillus, and BC dependent variables. For all analyses, P < 0.05 was considered statistically significant.

Results

Twenty-three children were recruited, but five of them did not complete the study. Eighteen children, seven boys and 11 girls, aged between 7 and 11 years (mean, 9.17 ± 1.15 years) participated throughout the entire clinical study (Figure 1). The basic information of the 18 study participants is shown in Table S1. The questionnaire revealed no existing oral or caries-related diseases among participants. Of the 18 participants, 14 children (77.8%) consumed 4–5 meals/d, and all used fluoride toothpaste. The caries risk of study participants was assessed using Cariogram and categorized based on percentage risk. At baseline, the mean caries risk of all participants was 40.4% ± 23.8%. Based on the caries risk categorization, 44.4% of participants had low or very low caries risk, 38.9% had moderate caries risk, and 16.7% had high or very high caries risk at baseline.

After 1 week of Yakult consumption, the mean lowest pH displayed a significant 5.2% increase (P = 0.02: Table 1). Nonsignificant decreases in mean pH recovery time (29.3%) and AUC (18.2%) were also observed compared to baseline data (Table 1). The subgroup analysis using “reduction of AUC” to separate “responders” from the “nonresponders”, AUC revealed that responders (N = 12, Table 1)
experienced significant 74.1% decrease in AUC (P = 0.002), substantial reduction in recovery time by 74.0% (P = 0.003), and increase of lowest pH by 0.41 (P = 0.005). These results indicated significantly decreased functional biofilm acidogenicity after 1-week consumption of Yakult. However, nonresponders (N = 6) underwent negative changes in all three parameters, indicating the increase in functional biofilm acidogenicity (Table 1). The data of Yakult effect on plaque acidity in all participants are shown in Table S2.

Overall, participants had higher S. mutans scores than Lactobacillus scores, both prior to and after Yakult intervention. Twelve (before) or 13 participants (after) had high S. mutans scores ranging from 2 to 3, compared to only four participants with similarly high Lactobacillus scores (Table 2). S. mutans and Lactobacillus counts were not significantly changed in children after 1-week Yakult consumption (Table 2). The data of Yakult effect on oral bacterial counts prior to and after intake in all participants are shown in Table S3.

Discussion

The present study demonstrated that the probiotic effects of Yakult intake in children may vary significantly depending on individuals’ biofilm ecology prior to treatment. After a 7-day consumption of Yakult, an acidic sweet drink, significant cariostatic effects in children with certain biofilm profile were demonstrated, whereas children with “low” caries risk may experience potential cariogenic/detrimental effects after consuming the probiotic drink.

Recently, the cariostatic effects of regular long-term (10 months) Lactobacillus intake in high-caries preschool children has been demonstrated, in which the percentage of new individuals who developed cavitated lesions (9.7%) in the probiotic group (consuming milk supplemented with L. rhamnosus SP1) was shown to be substantially lower than 24.3% of the control group (consuming standard milk).

Another clinical study found that a 12-week intake of two strains of Lactobacillus reuteri (D 17938 and ATCC PTA 5289) considerably reduced the prevalence of high Candida counts in frail elderly patients. Furthermore, in vitro studies have proven the effectual inhibition of S. mutans growth and biofilm formation by L. casei Shirota, and other L. casei strains. Interestingly, a previous study found that L. casei Shirota and B. breve strain Yakult, introduced into hepatectomy patients in formulation with prebiotic galacto-oligosaccharides, had successfully colonized patients and persisted 14 days after probiotic treatment, suggesting that combined probiotic and prebiotic formulations (“synbiotics”) may promote probiotic establishment and retention in vivo.

Theoretically, consumption of Yakult can be cariogenic because of its acidity, sweet contents (fructose/glucose), and acid-producing bacteria; however, our study discovered that the cariostatic effects of Yakult surprisingly outweighed its cariogenic risk in children with certain biofilm ecology and/or caries risk. Hence, being sweet and palatable, Yakult may be a promising caries-preventive agent especially for children with high caries risk and those “addicted” to sugar-containing food. As such, further clinical investigations on the specific cariogenic/cariostatic effects of Yakult are of significant importance.

Considering that standard Yakult contains 17% w/w carbohydrates, including the cariogenic fermentable sugars, namely, sucrose, glucose, fructose, and maltodextrin, L. casei Shirota/Yakult may have a decreased cariostatic effect on oral biofilms when administered in such a sweet and acidic beverage. Alternative vehicles for probiotic administration have been proposed, including cheese, tablets, yoghurt, and straws, and may be more effective for L. casei Shirota as an adjunct caries-preventive treatment. Furthermore, certain patients with established oral biofilms may inhibit colonization of probiotic species and thus hinder probiotic activity. In children aged 6–12 years, rinsing the oral cavity with a chlorhexidine-containing antimicrobial solution prior to probiotic intake was correlated with increased oral L. rhamnosus GG colonization and reduced counts of S. mutans for up to 5 weeks after treatment. A similar procedure may potentially improve L. casei Shirota uptake and persistence in children.

Although the exact mechanism(s) of probiotics in caries prevention remain unknown, there have been a few speculations, including the coaggregation of S. mutans, bacteriocidic effects on S. mutans, reduced production of insoluble extracellular polysaccharides in biofilm formation, and reduction of salivary counts of S. mutans. Nevertheless, probiotic effect has been demonstrated without reduction of S. mutans level in biofilm, similar to our findings. Our recent laboratory study found the decreased acid production of S. mutans via reduction of gtfB, gtfC, and ldh expressions in the presence of L. casei Shirota without significant change in S. mutans counts. Therefore, it is substantiated in our study that bacterial counts in saliva or biofilm might not be a reliable outcome parameter to assess the probiotic effect.

Based on the findings of subgroup analysis, this study highlighted the importance of preintervention patient selection via a valid CRA program. Although Carigogram has demonstrated good validity among Swedish children, the results of this study suggested that other methods may be more appropriate in certain settings. Future studies should explore the impact of specific probiotic strains and delivery vehicles on caries risk in children with varying biofilm ecology.

Table 1 Effects of probiotic beverage on plaque acidity.

| N  | Mean (SD) | P       |
|----|-----------|---------|
|    | Before    | After   |
|----|-----------|---------|
| Area under the curve | 18 | 5.97 (4.86) | 4.88 (6.44) | 0.396 |
|    | 12 | 5.67 (5.32) | 1.47 (3.58) | 0.002* |
|    | 6 | 6.55 (4.18) | 11.71 (5.38) | 0.028* |
| Lowest pH | 18 | 4.88 (0.27) | 5.14 (0.49) | 0.020* |
|    | 12 | 4.93 (0.29) | 5.34 (0.46) | 0.005* |
|    | 6 | 4.80 (0.22) | 4.74 (0.27) | 0.6   |
| Recovery time | 18 | 16.18 (9.75) | 11.44 (13.04) | 0.121 |
|    | 12 | 14.42 (9.22) | 3.75 (4.65) | 0.003* |
|    | 6 | 19.83 (10.67) | 27 (9.84) | 0.075 |
| CRA (%) | 18 | 40.42 (23.17) | 40.31 (21.76) | 0.976 |
|    | 12 | 39.79 (27.82) | 37.50 (26.31) | 0.126 |
|    | 6 | 41.67 (15.06) | 45.92 (11.21) | 0.465 |

CRA = caries risk assessment by Carigogram; SD = standard deviation.
*Statistically significant at P < 0.05.
Results from two-tailed paired t test.
Results from Wilcoxon signed-rank test.
schoolchildren, its performance on Asian children appeared less satisfactory and inferior to the National University of Singapore Caries Risk Assessment (NUS-CRA) program. It is substantiated in this study that Cariogram was not sensitive enough to show the probiotic/beneficial effect of Yakult among the respondents, as shown in Table 2 that the risk % of the respondents did not significantly change (P > 0.05). Therefore, to maximize the cariostatic benefit while preventing the cariogenic effect of Yakult among Asian children, it may be prudent to carefully select participants using an appropriate CRA system, such as NUS-CRA community-screening model published earlier, prior to recommendation and/or administration of Yakult, before an ideal/perfect CRA program is developed.

As the first study to investigate the cariogenic/cariostatic effect of Yakult among children, this study has multiple limitations. First, it is not a randomized clinical trial with the control group receiving placebo. Therefore, the results need to be interpreted cautiously, taking into consideration the potential Hawthorne effect and potentially weak external validity. Second, the sample size is rather small and may be underpowered. However, the significant effects shown in the respondent group indicates that the potential effect may warrant further investigation. Nevertheless, to assess the validity of the result, the post hoc power calculation was carried out. With a sample size of 18, the detectible difference between before and after treatment will be 70% of the standard deviation of the difference. The estimated standard deviation for the difference in lowest pH, before and after treatment, is about 0.4 unit. Hence, with 80% power, the detectible difference is about 0.28 (which is slightly more than what we observed 0.25). Assuming the mean difference is 0.25, the power of detecting the difference is about 70%, which is reasonably acceptable. Third, the clinical effects may be further attenuated by potential noncompliance of children such as insufficient rinsing duration and daily intake of the probiotic beverage or inability to refrain from brushing prior to the clinical measurements. However, these non-compliant factors are likely to reduce the cariostatic effects observed in our study. Therefore, the actual cariostatic effect of Yakult may be underestimated in this study. Future studies may enlist parental oversight in ensuring compliance by the children and increase the observation period in a randomized clinical trial. Fourth, the duration of intervention time may be too short for the probiotic effect to take place. In the future, longer intervention time with randomized controlled design and stratified caries risk groups may be considered.

In conclusion, there may be a potential cariostatic effect of short-term Yakult intake in reducing functional biofilm acidogenicity in children with certain oral biofilm and risk profile. Future randomized clinical trials may be needed to validate the potential cariostatic effect observed in our study. Quality risk assessment may be critical prior to prescribing/recommending Yakult as an adjunct caries-preventive treatment for children.

Conflicts of interest

The authors declare that they have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jds.2016.09.005.

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