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ORIGINAL ARTICLE

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

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**KEYWORDS**
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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/cariostatic 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.[1] 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.[2–4] There has also been increasing interest in the potential usage of probiotics for prevention of dental caries.[5,6] 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.[7] Preliminary clinical data suggest that *Lactobacillus rhamnosus* and *Lactobacillus salivarius* may offer protection against dental caries in young children.[8] 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.[9] Therefore, probiotics may be considered an adjunct to the current caries control measures such as tap water fluoridation and restriction of dietary sugar intake.[10]

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,[11] and *L. casei* Shirota has been classified as a "generally recognized as safe" additive by the United States Food and Drug Administration.[11] In addition, the inclusion of sweeteners in Yakult improves palatability and compliance when administered to children.[12] Yakult intake has been correlated with relief of chronic idiopathic constipation symptoms[13] and increase in natural killer cell counts among habitual smokers.[14] Furthermore, enteral feeding with Yakult containing a coculture of *L. casei* Shirota and *Bifidobacterium breve* strain significantly reduced postoperative infections among biliary cancer patients.[15] Although some potential oral benefits associated with Yakult are still under investigation,[4] putative effects on caries, especially among children, remain largely unknown. Although there seem to be several risk factors related to Yakult (such as acidity, sugars, and the acid-producing bacteria *L. casei* Shirota), there is no study or evidence demonstrating whether intake of Yakult has potential cariogenic or cariostatic effect among children.

Early childhood caries is a serious chronic oral health problem with an alarmingly high prevalence among children in both developed and developing countries, despite implementation of established caries management measures.[16–19] With the popularity, generally accepted safety, and palatability of Yakult, it may be worthwhile to characterize the unknown probiotic effects of this *L. casei* Shirota-containing probiotic beverage on CR indicators in children. Therefore, this study is aimed to investigate among schoolchildren the potential cariostatic/cariogenic effects of short-term Yakult intake on their oral biofilm acidogenicity, *S. mutans* and *Lactobacillus* counts, and CR.

## 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 Cariogram[20]), 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.[21] Oral hygiene status was evaluated for six index teeth (1E, 1B, 2D, 3E, 3B, and 4D) according to the Silness-Löe Plaque Index.[22] 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.

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