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Effect of *Lactobacillus-Bifidobacterium* Probiotic Yogurt on Oral *Candida* Levels in Older Adults

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Abstract

Objectives: *Lactobacillus* and *Bifidobacterium* are increasingly incorporated in yogurt starters to enhance yogurts' probiotic properties. Since many *Lactobacillus-Bifidobacterium* probiotic strains are recognized for their antifungal activities against oral *Candida* species. The aim of this study was to investigate the effects of daily administering of yogurt containing *Lactobacillus acidophilus, Lactobacillus paracasei*, and *Bifidobacterium lactis* on the levels of oral *Candida* and other factors relating to oral candidiasis.

Methods: The experiment was conducted using a double-blind randomized placebo-controlled study in which 60 older adults were allocated into two groups. After baseline examination and randomization, the control group was given the plain yogurt, while the experimental group was given the probiotic yogurt, twice daily. Saliva samples were collected at baseline and after the administration period of two weeks.

Results: The experimental group demonstrated a significant reduction in oral *Candida* counts (p<0.05), accompanied by significant increases in unstimulated salivary flow rate and salivary pH (p<0.05).

Conclusions: The short-term consumption of yogurt containing *Lactobacillus acidophilus, Lactobacillus paracasei,* and *Bifidobacterium lactis* may provide an alternative method for the prevention of oral candidiasis and xerostomia.

Keywords: *Candida*, older adults, oral candidiasis, probiotics, yogurt

Introduction

Oral health problems constitute a significant concern among older adults due to age-related physiological changes and the presence of comorbid conditions that increase susceptibility to oral diseases.[1-3] Furthermore, older adults often experience limited access to dental care as a result of financial constraints, reduced mobility, and insufficient oral health awareness, highlighting the importance of preventive strategies and regular dental assessments.[4]

Xerostomia, or dry mouth, is a common oral condition in older adults and is frequently associated with medication use. Reduced salivary flow compromises the oral defense system and increases the risk of oral candidiasis.[5,6] In recent years, the prevalence of oral candidiasis has increased substantially, particularly among older adults and immuno-compromised individuals.[7] Oral candidiasis is primarily caused by infection with *Candida* species, most commonly *Candida albicans (C. albicans)*, and is characterized by local discomfort, pain, dysgeusia, and dysphagia, which may ultimately lead to nutritional deficiencies.[8] Moreover, older adults and immunocompromised populations exhibit a higher incidence of invasive candidiasis, which is associated with increased morbidity and mortality.[8]

Antifungal agents such as fluconazole, miconazole, and nystatin remain the mainstay of treatment for oral candidiasis. Although these therapies are generally effective, their use is limited by potential adverse effects and the growing concern of antifungal resistance.[9] Consequently, increasing attention has been directed toward the development of alternative preventive and therapeutic approaches. Natural interventions, including bioactive compounds and probiotics, have demonstrated potential in reducing *Candida* infections through the inhibition of virulence factors and modulation of the oral microbiota.[10-12] Probiotics may be administered orally, commonly through dietary sources, to enhance their therapeutic effects in the oral cavity.

Lactobacillus and *Bifidobacterium* species, particularly *Lactobacillus acidophilus (L. acidophilus), Lactobacillus paracasei (L. paracasei)*, and *Bifidobacterium animalis* subsp. *lactis (B. lactis)*, have been widely incorporated into dairy products, especially yogurt, to enhance probiotic properties. Both *L. acidophilus* and *L. paracasei* have demonstrated inhibitory effects against oral *Candida* species, underscoring their potential role in maintaining oral health.[13-15] Although evidence regarding the direct effects of *B. lactis* on oral *Candida* remains limited, its antimicrobial activity against periodontopathogens has been reported.[16] The effect of administering these probiotic strains with food on the levels of oral *Candida* species and other factors related to oral health is not yet well understood.

Therefore, the present study aimed to investigate the effects of short-term, regular consumption of yogurt containing *Lactobacillus–Bifidobacterium* probiotics, specifically *L. acidophilus, L. paracasei,* and *B. lactis* on oral *Candida* levels and oral parameters associated with candidiasis, including salivary flow rate and salivary pH.

Materials and Methods

Ethical approval

The study protocol was reviewed and approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University (Protocol No. 27/2021; approved June 7, 2021). All participants received comprehensive information regarding the study objectives and procedures and provided written informed consent prior to participation.

Study design and participants

This study employed a randomized, double-blind, placebo-controlled design with two parallel groups over a two-week intervention period. Participants were randomly assigned to either the control or experimental group by research assistants using block randomization. Throughout the intervention, participants consumed 120 g of yogurt twice daily.

A total of 60 individuals aged ≥60 years residing in the Doi Saket District, Chiang Mai Province, Thailand, were recruited. After providing informed consent, participants completed a questionnaire addressing demographic characteristics and medical history, followed by an oral examination and saliva collection. All participants received standardized oral hygiene instructions and were instructed to refrain from using probiotic products, antifungal or antibacterial agents, and antiseptic mouth rinses during the study period (Figure 1).

Inclusion criteria were the presence of oral *Candida* colonization without clinical signs of oral candidiasis or other oral mucosal lesions. Exclusion criteria included uncontrolled systemic diseases (American Society of Anesthesiologists classification III–VI), history of organ transplantation, lactose or dairy intolerance, cancer, history of radiotherapy, smoking, use of topical or systemic antifungal or antibacterial agents or antiseptic mouth rinses within 30 days prior to enrollment, reported probiotic consumption, dementia, and inability to comply with study instructions. Discontinuation criteria were participants who took any probiotic, smoking, use of topical or systemic antifungal or antibacterial agents or antiseptic mouth rinses during the experiment period.

Yogurt preparation and consumption protocol

Both plain yogurt and probiotic yogurt used in this study were supplied by Sunrise Dairy Co., Ltd. (Lat Bua Khao Subdistrict, Sikhio District, Nakhon Ratchasima Province, Thailand). The plain yogurt was produced using a mixed starter culture of *Streptococcus thermophilus (S. thermophilus)* and *Lactobacillus bulgaricus (L. bulgaricus),* with a total viable count of 2.44×10^9 CFU/g. The probiotic yogurt was prepared using a mixed starter culture containing *S. thermophilus, L. bulgaricus, L. acidophilus, L. paracasei,* and *B. lactis,* with a total viable count of 1.51×10^9 CFU/g.

Both yogurt types were specifically manufactured for the study and packaged identically in 120-g white containers. The products were comparable in color, flavor, and texture, ensuring blinding of both participants and investigators.

Participants were instructed to consume one cup of yogurt in the morning after breakfast and one cup in the evening after dinner for two consecutive weeks. They

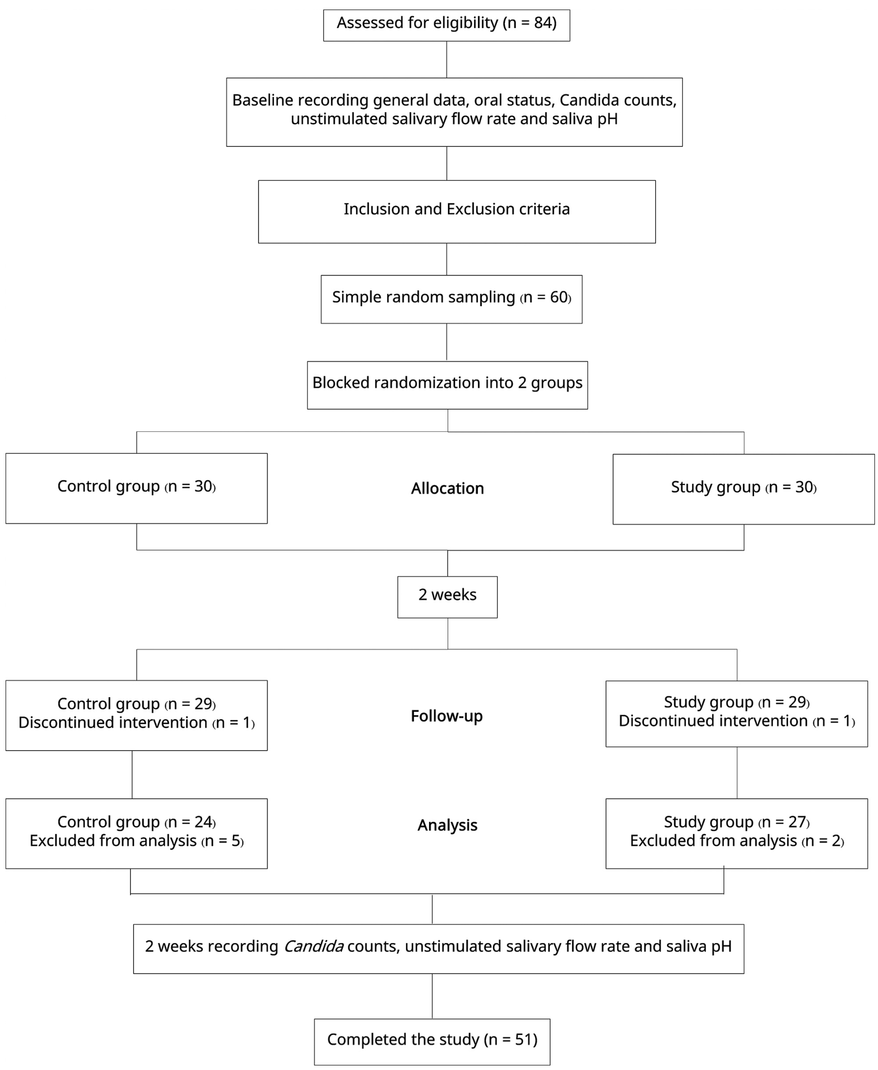

Figure 1: Experimental protocol of the study.

were advised to refrain from drinking water or performing oral hygiene procedures for at least one hour following yogurt consumption.

Saliva collection and oral sampling

Oral *Candida* sampling was conducted at two time points: at baseline prior to yogurt consumption and at the end of the two-week intervention. All samples were collected between 08:00 and 11:00 a.m. Participants were instructed to abstain from eating and drinking (except water) for at least one hour prior to sampling.

Following sample collection, participants rinsed their mouths with 10 mL of water for 60 seconds to remove food debris, followed by a 60-second rinse with 10 mL of phosphate-buffered saline (PBS). The rinse samples were transported under refrigerated conditions to the laboratory and processed on the same day.

Unstimulated salivary flow rate and salivary pH were measured at baseline and at the end of week 2. Participants were instructed to refrain from speaking or chewing during saliva collection. Unstimulated saliva was collected over a 5-minute period by expectorating saliva accumulated in the floor of the mouth into a graduated cylinder. Salivary pH was measured using a LAQUAtwin pH-22 pocket pH meter, which was calibrated prior to each use.

Microbiological analysis

Mouth-rinse samples were centrifuged at 1,700 rpm, 320 g for 10 minutes, and the resulting pellets were re-suspended in 1 mL of PBS. *Candida* quantification was performed using the spread plate technique. A 100-µL aliquot of the suspension was inoculated onto HiCrome *Candida* Differential Agar (HiMedia, India), which allows differentiation of *Candida* species namely *C. albicans, C. krusei, C. tropicalis* and *C. glabrata* based on coloration and colony morphology. Then the samples were incubated aerobically at 37 °C for 48 hours. Colony-forming units (CFU/mL) were enumerated, and all samples were analyzed in duplicate.

Statistical analysis

Data distribution was assessed using the Kolmogorov–Smirnov test. As *Candida* colony counts did not follow a normal distribution, nonparametric statistical tests were applied. The Mann–Whitney U test was used to compare *Candida* colony counts between the control and experimental groups at baseline and post-intervention, while the Wilcoxon signed-rank test was used for within-group comparisons.

Parametric tests were applied to variables with normal distribution, including unstimulated salivary flow rate and salivary pH. The independent samples t-test was used to compare these variables between groups at baseline and after the intervention, and the paired t-test was used for within-group comparisons. Data are presented as mean± standard error (SE). Statistical analyses were performed using SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA), and a p-value<0.05 was considered statistically significant.

Results

Of the 60 participants, 51 completed the experiment protocol (24 in the control group and 27 in the experimental group), resulting in a dropout rate of 15% because of incomplete consuming yogurt as per the research requirements. At baseline, no significant differences were observed between the control (n=24) and experimental groups (n=27) in demographic or oral health parameters (all p>0.05). The mean age was comparable between groups (67.00±0.88 vs. 67.48±0.82 years, p=0.691), and all participants were female. No significant differences were found in BMI, number of systemic diseases, number of teeth, number of decayed teeth, DMFT index, number of occlusal pairs, or denture use, indicating that the two groups were well balanced at baseline (Table 1).

Oral *Candida* colony counts

Mean oral *Candida* colony counts, expressed as log CFU/mL, at baseline and after the two-week intervention period are presented in Table 2 and Figure 2. The mean log *Candida* colony counts were comparable between the control group (1.00±0.12 log CFU/mL) and the study group (1.10±0.09 log CFU/mL) at baseline (p=0.422). After two weeks, the study group demonstrated a significant reduction in *Candida* colony counts (0.70±0.10 log CFU/mL) compared with baseline (p<0.001), whereas no significant change was observed in the control group (baseline: 1.00±0.12 vs. two weeks: 0.96±0.15 log CFU/mL; p=0.796). Although the mean *Candida* level was lower in the study group than in the control group at two weeks, the between-group difference was not statistically significant (p=0.125).

Unstimulated saliva flow rates

Baseline unstimulated salivary flow rates were similar between the control (0.32±0.04 mL/min) and study groups (0.31±0.03 mL/min) (p=0.881). After two weeks, the study group showed a significant increase in salivary flow rate (0.45±0.05 mL/min) compared with baseline (p<0.001). In contrast, the control group showed no statistically significant change (baseline: 0.32±0.04 vs. two weeks: 0.36±0.05 mL/min; p=0.337). The between-group comparison at two weeks did not reach statistical significance (p=0.164). (Figure 3).

Saliva pH

Mean salivary pH values at baseline and after the intervention are illustrated in Figure 4. The baseline salivary pH values did not differ significantly between the control (6.87±0.10) and study groups (6.74±0.11) (p=0.394). After two weeks, the study group demonstrated a significant increase in salivary pH (7.07±0.09) compared with baseline (p=0.003). The control group showed a slight but non-significant increase (baseline: 6.87±0.10 vs. two weeks: 6.99±0.08; p=0.136). No statistically significant difference was observed between groups at the two-week follow-up (p=0.544).

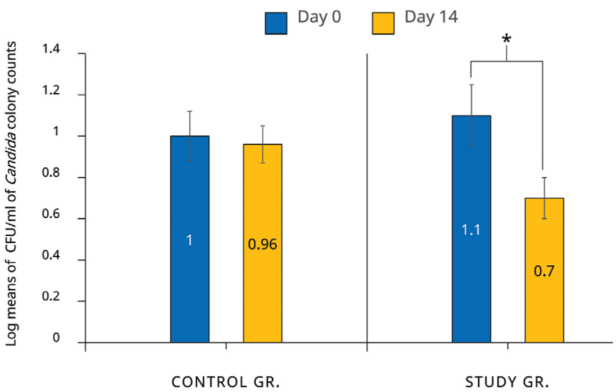

Figure 2: Means and standard error of *Candida* colony counts, *significant difference in *Candida* colony counts (p<0.05).

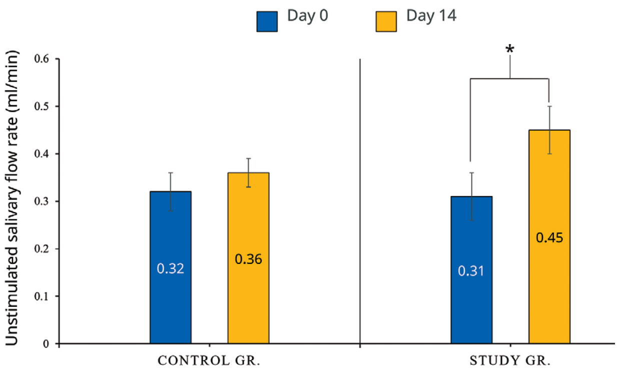

Figure 3: Means and standard error of unstimulated salivary flow rates, *significant difference in unstimulated salivary flow rates (p<0.05).

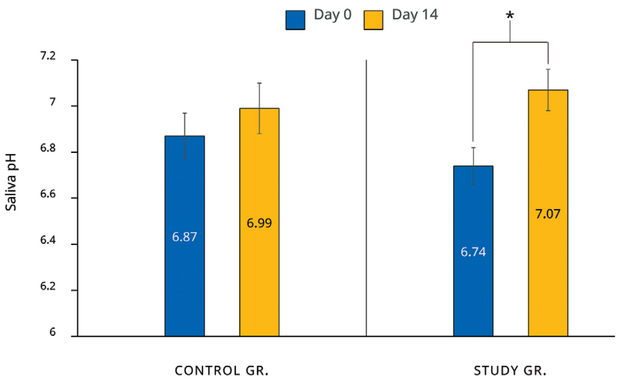

Figure 4: Means and standard error of saliva pH, *significant difference in saliva pH (p<0.05).

Discussion

Older adults exhibit increased susceptibility to oral candidiasis. Antifungal drugs serve as the main treatment; however, the increasing experience of side effects and the prevalence of multidrug-resistant *Candida* species represent a considerable public health concern. Consequently, novel techniques are required to decrease the prevalence of *Candida* infections. Studies indicate that probiotics may serve as an effective alternative treatment for oral candidiasis. From our study, a significant reduction in *Candida* colony counts was observed in the experimental group consuming the probiotic yogurt for two weeks, whereas no significant reduction was noted in the control group consuming the yogurt without probiotics. No significant differences were observed in general data and oral status between the control group and the experimental group across all variables.

Our study indicates that the intake of this specific *Lactobacillus-Bifidobacterium* probiotic yogurt may significantly decrease oral *Candida* levels in older adults. This probiotic formula contains yogurt starters, *S. thermophilus* and *L. bulgaricus,* along with probiotic strains *L. acidophilus, L. paracasei,* and *B. lactis*. The control yogurt contained solely the yogurt starters *S. thermophilus* and *L. bulgaricus*. Previous studies have documented the antifungal effects of probiotic species, including those

Table 1: General statistics and oral health conditions of participants in the control and experimental groups at baseline.

Characteristic	Control group (n=24)	Study group (n=27)	*p*-value
Age	67.00±0.88	67.48±0.82	0.691^{I}
Females, n (%)	24 (100)	27 (100)	
BMI	24.44±0.62	23.83±0.75	0.291^{M}
Number of diseases	1.25±0.24	1.11±0.19	0.715^{M}
Number of teeth	23.83±1.45	23.33±1.38	0.67^{M}
Number of decayed teeth	1.33±0.4	0.56±0.13	0.365^{M}
DMFT	9.83±1.43	10.18±1.42	0.865^{M}
Number of occlusal pairs	4.71±0.48	5.26±0.56	0.212^{M}
Denture wearers	0.12±0.07	0.22±0.08	0.368^{M}

^{I}Independent Samples Test, ^{M}Mann-Whiney U Test; *p-value<0.05 is considered statistically significant.

Table 2: Comparison of the control and study group with respect to *Candida* colony counts, unstimulated salivary flow rates and saliva pH at baseline and two weeks.

Variables		Control group	Study group	*p*-value
Candida colony counts (Log means of CFU/mL)	Baseline	1.00±0.12	1.10±0.09	0.422^{M}
	2 weeks	0.96±0.15	0.70±0.10*	0.125^{M}
	p-value	0.796^{W}	0.000^{W}	
Unstimulated salivary flow rates (mL/min)	Baseline	0.32±0.04	0.31±0.03	0.881^{I}
	2 weeks	0.36±0.05	0.45±0.05*	0.164^{I}
	p-value	0.337^{P}	0.000^{P}	
Saliva pH	Baseline	6.87±0.10	6.74±0.11	0.394^{I}
	2 weeks	6.99±0.08	7.07±0.09*	0.544^{I}
	p-value	0.136^{P}	0.003^{P}	

^{M}Mann-Whiney U Test, ^{W}Wilcoxon Signed Ranks Test, ^{I}Independent Samples Test, ^{P}Pair t-Test, *p-value<0.05 is considered statistically significant.

utilized in the probiotic yogurt formula examined in our study, on *Candida*. A study indicated that probiotic toothpastes with *L. acidophilus* and *L. paracasei* exhibited a more significant inhibitory effect on *C. albicans* than those without probiotics.(13) A separate study demonstrated that *L. paracasei* and *Lactobacillus rhamnosus* exhibited the most significant effects on *C. albicans in vitro*.(17) Furthermore, both the cells and cell-free supernatants of *L. acidophilus* and *Lactobacillus plantarum* exhibited antifungal activity against oral *Candida* species.(18) The main mechanisms of the action of probiotics, such as elimination and inhibition of the growth of pathogenic microorganisms through competition for receptor sites and the secretion of metabolites with antifungal activity, as well as the stimulation of specific and non-specific immune responses by activating T-lymphocytes, and stimulating the production of cytokines, make it possible to also effectively use antifungal.(19)

In an *in vivo* study, the administration of locally applied probiotics (*Bifidobacterium longum*, *L. bulgaricus,* and *S. thermophilus*) demonstrated improvements in specific clinical conditions and a reduction in the prevalence of *Candida* species.(20) A separate study indicated that daily intake of *B. lactis* may lower the risk of oral candidiasis in healthy elderly individuals.(21) The findings indicate a potential role of probiotic *Bifidobacterium* strains in the inhibition of *Candida* species. Nonetheless, the species of *Bifidobacterium* and the methods of probiotic administration varied from those employed in our study. Because the benefits of probiotics are strain specific(22), therefore, each probiotic strain in the yogurt formula utilized in our study requires further evaluation to assess its effects on oral *Candida* in future research.

Our study revealed a notable secondary outcome associated with probiotic yogurt consumption: a significant increase in unstimulated salivary flow rate and saliva pH compared to baseline measurements. Hatakka *et al.,*[23] previously noted an increase in saliva quantity following 16 weeks of daily consumption of probiotic cheese by the participants. The mechanisms underlying these effects are not yet fully understood. A separate *in vivo* study indicated that short-term daily consumption of probiotics through curd in the diet significantly increased salivary pH and decreased salivary counts of *Streptococcus mutans.*[24] One prospective clinical study in edentulous patients found that 60 days of daily probiotic supplementation significantly increased both unstimulated and stimulated saliva volume compared to baseline. This suggests a possible effect on salivary output in individuals dry mouth.[25] Previous studies suggest probiotics can raise salivary pH and buffering capacity, which might support saliva's protective functions, though results vary by strain and study design.[26]

The control group exhibited a slight, non-significant decrease in *Candida* colony counts after two weeks of administration of plain yogurt without probiotics compared to the baseline level. The increases in unstimulated salivary flow rate and saliva pH in this group were also not statistically significant. The additional probiotic strains in the yogurt given to the experimental group demonstrated direct effects on oral *Candida,* unstimulated salivary flow, and saliva pH. It is unclear whether all probiotic strains contributed to these effects or if the observed outcomes resulted from the synergistic effects of the multi-strain combination. Studies indicate that the application of multi-strain probiotics may improve their efficacy in pathogen inhibition. A combination of microbial species exhibiting varying inhibitory effects on pathogens may provide a wider range of action compared to a single strain.[27] Additional investigation is required prior to reaching a conclusion on this specific point.

Conclusions

Daily consumption of a commercially available yogurt containing multiple probiotic strains *(L. acidophilus, L. paracasei,* and *B. lactis)* for two weeks resulted in a significant reduction in oral *Candida* colony counts, along with increases in unstimulated salivary flow rate and salivary pH in older adults. These findings suggest that probiotic yogurt consumption may represent a promising adjunctive strategy for reducing the risk of oral candidiasis and xerostomia. Nevertheless, further studies involving larger and more diverse populations, as well as longer intervention and follow-up periods, are required before this approach can be recommended for routine clinical practice.

Conflict of Interest

The authors declare that there are no conflicts of interest with any of the companies which manufactured the yogurt employed in this study. The company provided the product used in this research. The company had no role in the study design, data collection, analysis, interpretation, or manuscript preparation.

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