Probiotics Reduce the Prevalence of Oral *Candida* in the Elderly––a Randomized Controlled Trial

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ABSTRACT

Overgrowth of oral yeast is a common problem among the elderly. Probiotic bacteria are known to inhibit the growth of pathogenic microbes. We tested the hypothesis that cheese containing probiotic bacteria can reduce the prevalence of oral *Candida*. During this 16-week, randomized, double-blind, placebo-controlled study, 276 elderly people consumed daily 50 g of either probiotic (n = 136) or control cheese (n = 140). The primary outcome measure was the prevalence of a high salivary yeast count ($\geq 10^4$ cfu/mL) analyzed by the Dentocult® method. The prevalence decreased in the probiotic group from 30% to 21% (32% reduction), and increased in the control group from 28% to 34%. Probiotic intervention reduced the risk of high yeast counts by 75% (OR = 0.25, 95%CI 0.10-0.65, p = 0.004), and the risk of hyposalivation by 56% (OR = 0.44, 95%CI 0.19-1.01, p = 0.05). Thus, probiotic bacteria can be effective in controlling oral *Candida* and hyposalivation in the elderly.

KEY WORDS: probiotics, *Lactobacillus* GG, *Candida*, the elderly, randomized trial, hyposalivation.

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INTRODUCTION

The elderly are vulnerable to *Candida* infection provoked by chronic diseases, medication, poor oral hygiene, reduced salivary flow, or the impairment of the immune system (Shay *et al.*, 1997). As many as 75% of elderly people in Finland harbor oral yeast (Närhi *et al.*, 1993). Even though the colonization by *Candida* may be asymptomatic, heavy growth usually leads to local candidosis, with various types of mucosal lesions and symptoms (Shay *et al.*, 1997). It is important, therefore, that yeast proliferation be controlled.

Probiotic bacteria, such as *Lactobacillus rhamnosus* GG (Saxelin, 1997), may modify the microbial balance of the host by reducing the overgrowth of pathogens, such as *Candida* (Payne *et al.*, 2003). *Lactobacillus* GG reduced the numbers of *Candida* cells in the alimentary tract of immunodeficient mice (Wagner *et al.*, 1997) and, recently, the enteric colonization of *Candida* in pre-term neonates, as measured by colonies isolated from oro-pharyngeal, gastric aspirate, stool, and fecal specimens (Manzoni *et al.*, 2006).

Certain strains of lactobacilli can adhere to the mucosal epithelium, and may thereby compete for adhesion sites with *Candida* (Strus *et al.*, 2005). In addition, *Lactobacillus* species produce different metabolites, such as hydrogen peroxide (Strus *et al.*, 2005), and antifungal cyclic peptides (Ström *et al.*, 2002), which inhibit the *in vitro* growth of *Candida*. In mice inoculated orally with *Candida*, lactobacilli have shortened the duration of *Candida* colonization in the oral cavity, possibly by inducing the production of IL-4 and IFN-γ in lymph nodes and nitric oxide (NO) in the saliva (Elahi *et al.*, 2005).

In the present study, we investigated whether treatment with cheese containing a mixture of probiotics (*Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Propionibacterium freudenreichii* ssp. *shermanii* JS) would reduce the growth of oral *Candida* in the elderly. *Lactobacillus rhamnosus* LC 705 and *Propionibacterium* JS have been previously used as preservatives against yeasts in the manufacture of sour milk products (Suomalainen and Mäyrä-Mäkinen, 1999).

METHODS

Participants

Independent elderly people aged 70-100 yrs were recruited by research assistants from old people's homes and sheltered housing units in the Helsinki area of Finland. Exclusion criteria were the presence of dementia (mini-mental state (MMS) test of over three points) and the taking of current oral yeast medication. In total, 304 elderly participants from 12 old people's homes and 37 sheltered housing units gave their written informed consent. The study protocol was approved by the Ethical Committee of Helsinki University Central Hospital.

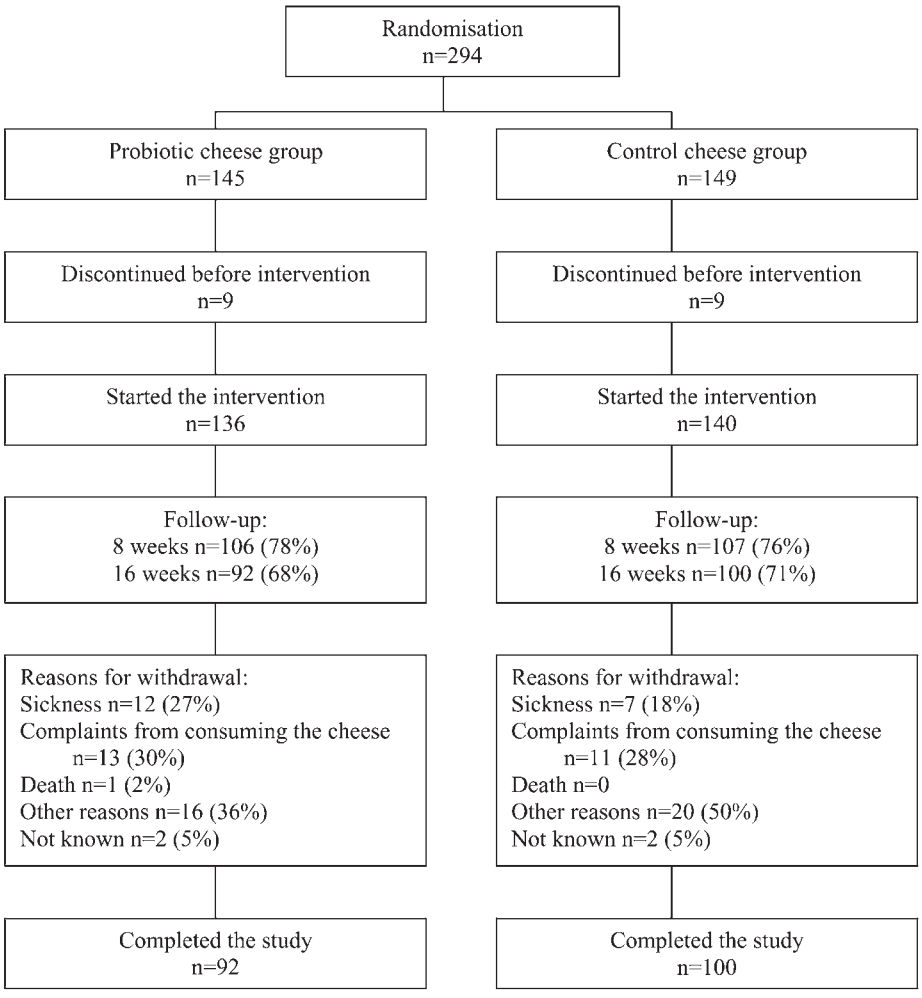

Figure 1. Trial profile during the 16-week study.

Study Protocol and the Intervention

This randomized, double-blind, placebo-controlled study, with two parallel groups, was carried out between January, 2001, and March, 2002. Each participant was randomly allocated to the probiotic group or the control group, according to a computer-generated random permuted blocks method. A block size of four was used, and it was stratified according to the baseline yeast count.

During the 16-week intervention, after a three-week run-in period, the participants consumed daily either 50 grams of Emmental-type probiotic cheese (containing 15% fat) or 50 grams of edam-type control cheese, divided into two portions. In the probiotic cheese, *Lactococcus lactis* and *Lactobacillus helveticus* were used as starter cultures, and 10^7 cfu (colony-forming units)/g of each of the probiotic strains, *L. rhamnosus* GG (ATCC 53103), *L. rhamnosus* LC705, and *Propionibacterium freudenreichii* ssp *shermanii* JS (Valio Ltd, Joensuu, Finland) were added. Control cheese contained only *Lactococcus lactis* as a starter culture, and no probiotic strains were added. For blinding purposes, the packaging of both cheeses was identical. The manufacturer did not reveal the identities of the treatment groups to the investigators until the intention-to-treat (ITT) analysis was performed. The use of other probiotic-containing products was forbidden during the study.

Clinical Examination

The oral and dental status of the participants was examined by an experienced dentist at Weeks 0 and 16, according to WHO criteria (WHO, 1997). The number of decayed, missing, and filled teeth and the number of prosthetic appliances were recorded. The Community Periodontal Index (CPI) was recorded for each sextant (0 = healthy gums; 1 = no periodontal pockets but bleeding occurs on probing; 2 = presence of pockets no deeper than 3 mm; 3 = pockets of 4-5 mm; 4 = pockets 6 mm or deeper) (WHO, 1997). Mucosal lesions were recorded as white oral lesions (leukoplakia), red oral lesions, hyperplasia, ulceration, and pigmented oral lesions. During a monthly visit, a research assistant collected information on each participant's health, medication, oral pain, and sensations of dry mouth.

Saliva Samples

Sampling for oral yeasts was undertaken 4 times (at the beginning of the run-in period, and at Weeks 0, 8, and 16) between 8 and 11 a.m. The participants were told to refrain from eating, drinking (except water), and smoking for 1 hr prior to the investigation. The research assistant sampled for the yeasts by rotating a cotton wool swab in a standardized fashion on the oral mucosa of the cheeks, tongue, and gingival margin (dentate participants) or alveolar ridge (edentate participants), and immediately inoculating it onto a Dentocult® CA slide (Orion Diagnostica, Espoo, Finland). After incubation for 48 hrs at 37°C, the growth was assessed semi-quantitatively according to the manufacturer's visual scale and product instructions, with the following categories, where: 0 represented no visible colonies, 1 was equivalent to a colony density of 10^3 cfu/mL, 2 was equivalent to 10^4 cfu/mL, 3 was equivalent to 10^5 cfu/mL, and 4 was equivalent to 10^6 cfu/mL (the counts were extrapolated to salivary concentrations, cfu/mL). Category 1 (10^3 cfu/mL) was used to distinguish between participants with low ($<10^4$ cfu/mL) and high ($\geq 10^4$ cfu/mL) prevalence. Further identification of the yeast species was made by subculturing them onto CHROMagar *Candida* (Becton Dickinson, Franklin Lakes, NJ, USA), which permitted the presumptive identification of *C. albicans, C. glabrata, C. krusei,* and *C. tropicalis.*

Stimulated and unstimulated salivary secretion rates and salivary buffering capacity were measured at Weeks 0 and 16. The participants were told to collect all their unstimulated secreted saliva in a measuring cylinder for 5 min. A paraffin-wax-stimulated salivary secretion was then collected for another 5 min. An unstimulated salivary flow rate of below 0.1 mL/min and a

stimulated flow rate of below 0.8 mL/min was considered to indicate hyposalivation. The salivary buffering capacity was measured from stimulated saliva by the Dentobuff® Strip method (Orion Diagnostica, Espoo, Finland), and was classified according to the manufacturer's instructions (low, pH ≤ 4.0; intermediate, pH 4.5-5.5; high, pH ≥ 6.0).

Sample Size

Calculation of the sample size was based on the assumption that the consumption of cheese with probiotic bacteria results in a proportional reduction of 24% in the prevalence of yeast (from 75% to 57%). The results of a preventive oral hygiene program (Budtz-Jørgensen *et al.*, 2000) were used as reference data. With a power of 80% and at a significance level of 0.05, 108 participants *per* group were needed for the difference between the groups (75% *vs.* 57%, OR 0.44) to be detected. After adjustment for drop-out (30%), 154 participants *per* group were needed.

Statistical Analysis

The primary outcome variable was the high yeast count (≥ 10^4 cfu/mL = categories 2-4) after the intervention. The secondary outcome variables were hyposalivation, salivary buffering capacity, dry mouth, mucosal lesions, oral pain, and decayed teeth. We used logistic regression analysis to study the association between the intervention and the high yeast count, and the baseline yeast count (yes/no) was included as a categorical covariate. The possible confounding factors (baseline yeast category, age, diabetes, salivary flow rate, buffering capacity, denture wearing, number of drugs used daily, gender, and type of housing) were introduced to the stepwise multivariable model (criterion for entering, $p < 0.15$). The results are given as odds ratios (OR) with 95% confidence intervals (CI). The effect of the probiotic intervention on the secondary outcome variables was analyzed by separate logistic regression analysis, with the corresponding baseline included as a categorical covariate. The Student *t* test for independent samples and the chi-square test were used to compare the baseline characteristics between the groups.

All analyses were based on the intention-to-treat (ITT) population. In addition, the primary outcome variable was analyzed by the *per* protocol (PP) population, excluding participants who did not fulfill the inclusion criteria (18 participants were under 70 yrs old, and two participants used medication for *Candida*), who dropped out of the study, or who had cheese-eating compliance of under 80%. Thus, the number of participants included in the PP analysis was 151 (54.7%). Statistical analyses were performed with the use of SPSS software (Version 11.5, SPSS Inc., Chicago, IL, USA).

Table 1. Baseline Characteristics of the Participants

Characteristic		Probiotic Group (n = 136)		Control Group (n = 140)	
Mean age (range)		78.9 (58.7-95.2)[b]		79.2 (65.4-94.7)	
Females		100	(73.5)	110	(78.6)
Number of diseases	0	12	(8.8)	7	(5.0)
	1-3	81	(59.6)	83	(59.7)
	≥ 4	43	(31.6)	49	(35.3)
	Median (range)	3	(0-10)	3	(0-9)
Diabetes		30	(22.1)	22	(15.7)
Number of drugs used daily	0	14	(10.3)	11	(8.0)
	1-3	49	(36.0)	51	(37.2)
	≥ 4	73	(53.7)	75	(54.8)
	Median (range)	4	(0-18)	4	(0-25)
Type of housing	Old people's home	35	(25.7)	32	(22.9)
	Sheltered housing unit	101	(74.3)	108	(77.1)
Smoking	Non-smokers	88	(64.7)	89	(63.6)
	Ex-smokers	44	(32.4)	45	(32.1)
	Smokers	4	(2.9)	6	(4.3)
Use of regular	Antibiotic medication	11	(8.1)	6	(4.3)
	Lactic acid bacteria products	50	(36.8)	53	(37.9)
Yeast count (cfu/mL)	0	39	(28.7)	33	(23.6)
	10^3	51	(37.5)	61	(43.6)
	10^4	25	(18.4)	24	(17.1)
	10^5	21	(15.4)	21	(15.0)
	10^6	0		1	(0.7)
Dentition	Own teeth	31	(23.1)	41	(29.3)
	Partial dentures	57	(42.5)	54	(38.6)
	Full dentures	46	(34.3)	45	(32.1)
CPI[a] score	0 (healthy gums)	2	(1.5)	4	(2.9)
	1 (bleeding, no periodontal pockets)	0		1	(0.7)
	2 (periodontal pockets ≤ 3 mm)	14	(10.4)	17	(12.1)
	3 (periodontal pockets 4-5 mm)	9	(6.7)	15	(10.7)
	4 (periodontal pockets ≥ 6 mm)	8	(6.0)	6	(4.3)
Hyposalivation	Unstimulated secretion	41	(31.1)	31	(23.0)
	Stimulated secretion	57	(44.2)	60	(46.2)
Salivary buffering capacity	Low	19	(15.0)	26	(20.0)
	Intermediate	35	(27.6)	30	(23.1)
	High	73	(57.5)	74	(56.9)

[a] CPI = Community Periodontal Index.
[b] Figures are numbers of participants (percentages in parentheses) unless otherwise stated.

RESULTS

Participant Follow-up and Health Status at Baseline

A total of 304 persons volunteered to participate in the study, and 294 were included and randomized to the two study groups (Fig. 1). Of these, 276 participants began the study,

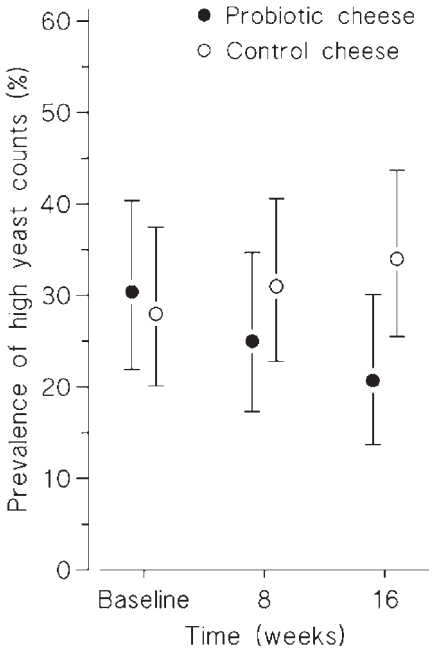

Figure 2. Percentage (95% confidence interval) of participants with high yeast counts (≥ 10^4 cfu/mL) in the probiotic (n = 92) and control (n = 100) groups at baseline, in the middle, and at the end of the intervention.

Table 2. Associations between Probiotic Intervention and Hyposalivation, Dry Mouth, High Salivary Buffering Capacity, Mucosal Lesions, Oral Pain, and Decayed Teeth after 16 Weeks of Intervention

Variable		Probiotic Group, n (%)	Control Group, n (%)	OR	95% CI	p-value
Hyposalivation	Baseline	21 (23.6)*	18 (19.4)			
	Week 16	16 (18.0)	25 (26.9)	0.44	0.19 to 1.01	0.05
Dry mouth	Baseline	47 (51.6)	61 (61.6)			
	Week 16	48 (52.7)	68 (68.7)	0.54	0.27 to 1.06	0.07
High salivary buffering capacity	Baseline	55 (64.7)	52 (59.1)			
	Week 16	53 (62.4)	62 (70.5)	0.51	0.24 to 1.09	0.08
Mucosal lesions	Baseline	22 (25.0)	22 (23.7)			
	Week 16	23 (26.1)	25 (26.9)	0.35	0.04 to 3.44	0.37
Oral pain	Baseline	10 (11.0)	15 (15.0)			
	Week 16	11 (12.1)	20 (20.0)	0.58	0.25 to 1.34	0.20
Decayed teeth	Baseline	34 (38.6)	24 (25.8)			
	Week 16	28 (31.8)	23 (24.7)	0.92	0.39 to 2.2	0.85

* The results are based on separate logistic regression analyses, where the corresponding baseline information is included as a categorical covariate. Only participants who completed the study are included (probiotic group, n = 85-92; control group, n = 88-100).

but 84 (30%) subsequently dropped out. Those who dropped out carried more yeast (p = 0.08) and were older (p = 0.06) than those who completed the study. Pre-treatment characteristics and baseline oral health status are given in Table 1. Those who had regularly used lactic acid bacteria-containing products before the study had high yeast counts (≥ 10^4 cfu/mL) less often than those who did not (25% *vs.* 38%; p = 0.03).

Effect of Probiotic Cheese on the Prevalence of Yeast and on Different *Candida* Species

At baseline, 30.4% of the probiotic group and 28.0% of the control group had high yeast counts (≥ 10^4 cfu/mL) (Fig. 2). After 8 and 16 wks of the intervention, the prevalence of high yeast counts in the probiotic group had diminished to 25.0% and 20.7%, respectively, *i.e.*, a 32% reduction in the prevalence by the end of the study. In the control group, the prevalence increased: The corresponding figures were 31.0% and 34.0%. At the end of the intervention, the risk of high yeast counts had decreased in the probiotic group compared with the control group (OR = 0.39, 95%CI 0.18 to 0.83, p = 0.01). When adjusted for baseline yeast counts, salivary buffering capacity, and denture wearing, the odds ratio was reduced even more (adjusted OR = 0.25, 95%CI 0.10-0.65, p = 0.004).

Sixty-one percent of the participants in both groups had a cheese-eating compliance of at least 80%. According to the PP analysis, the risk of having high yeast counts was reduced in the probiotic group (adjusted OR = 0.23, 95%CI 0.07-0.71, p = 0.01). The results were also analyzed according to whether participants wore dentures or not. No association was found between denture wearing and the effects of the probiotic therapy (data not shown).

There were minor fluctuations, but *C. albicans* was the predominating species recovered during the entire study, found in 94% of the positive participants at the beginning of the study, and in 90% at the end. In the probiotic group, two participants who carried *C. albicans* at the beginning had been colonized by *C. glabrata* by the end. In the control group, *C. glabrata* was found in two participants at the beginning, and in seven participants at the end. *C. tropicalis* was found in only one person in the probiotic group, and *Saccharomyces cerevisiae* in two participants in the control group.

Effect of Probiotic Cheese on Secondary Outcome Variables

The median unstimulated salivary flow increased in the probiotic group, from 0.18 mL/min at the beginning to 0.22 mL/min at the end, and decreased in the control group, from 0.22 mL/min to 0.18 mL/min. Both the number of participants with hyposalivation and the risk of hyposalivation were reduced in the probiotic group (Table 2). Probiotic treatment tended to lower the risk of dry mouth, high salivary buffering capacity, mucosal lesions, and oral pain, although not significantly.

DISCUSSION

We carried out this study to evaluate the effectiveness of probiotic treatment on the prevalence of oral yeast in the elderly. The average baseline prevalence of yeast was 74%, with high counts in 33%. After 16 wks of intervention, the prevalence of a high yeast count diminished by 32% in the probiotic cheese group, while it increased by 21% in the control group. Probiotic treatment reduced the risk of high yeast counts by 75%. Previous regular use of probiotic bacteria also seemed

to prevent high counts of yeast.

In an earlier study, probiotic cheese containing *Lactobacillus* GG and *Lactobacillus rhamnosus* LC705 tended to reduce the level of salivary yeasts in healthy adults (Ahola *et al.*, 2002). This finding was confirmed in our study, and could possibly be explained by the addition of the strain *Propionibacterium* JS. The combination of *Propionibacterium* JS and *Lactobacillus rhamnosus* LC705 has been used as an active preservative against yeast in the manufacturing process (Suomalainen and Mäyrä-Mäkinen, 1999). In a recent study (Manzoni et al, 2006), oral supplementation with *Lactobacillus* GG also reduced the gastrointestinal colonization of *Candida* (23% *vs.* 49%) in pre-term neonates.

One interesting observation in our study was that probiotic treatment reduced the prevalence of hyposalivation and a subjective feeling of dry mouth. The gustatory stimulus caused by chewing cheese twice a day does not explain the observation, since hyposalivation increased in the control group. Based on the present study, the explanation for the reduced prevalence of hyposalivation in the probiotic group remains unclear. However, it could be hypothesized that probiotics might have somehow affected the composition of saliva, such as the concentrations of mucins and salivary immunoglobulins, as has been shown in animal (Negretti *et al.*, 1997) and in *in vitro* (Mack *et al.*, 2003) studies, thereby affecting the nature of saliva secreted.

Unlike in the probiotic group, yeast carriage was found to have increased in the control group. The major factors predisposing to oral candidosis, such as denture wearing (Närhi *et al.*, 1999), or the use of steroid inhalers, systemic corticosteroids, and wide-spectrum antibiotics (Shay *et al.*, 1997), did not differ between the groups, and cannot be the causative factors. The use of products containing lactic acid bacteria prior to the intervention was fairly common in both groups, and also a protective factor against *Candida* colonization. Therefore, the absence of the protective effects of probiotics, together with increased hyposalivation, might explain the increase in *Candida* growth in the control group.

It has been suggested that oral *Candida* incidence increases with age (Lockhart *et al.*, 1999), possibly because of impaired immunity. Several elements in the immune system, such as T-lymphocytes, granulocytes, NK-cells, mast cells, and macrophages, account for the protection against *Candida* infections (Peterson, 1992). *Lactobacillus* GG and *Propionibacterium* JS cause enhanced T-cell and B-cell proliferation in mice (Kirjavainen *et al.*, 1999). Probiotics have also stimulated the production of IFN-α, enhanced phagocytic capacity (Arunachalam *et al.*, 2000), and increased the proportions of helper T-lymphocytes and the activity of natural killer cells in elderly patients (Gill *et al.*, 2001). In animal studies, lactobacilli have attenuated *Candida* infection by inducing the production of IL-4 and IFN-γ (Elahi *et al.*, 2005), and by attenuating the production of pro-inflammatory IL-1β and TNF-α (Brzozowski *et al.*, 2005). Probiotics may also inhibit the *Candida* growth by producing antimicrobial compounds (Ström *et al.*, 2002; Strus *et al.*, 2005), and may inhibit its adhesion to epithelial cells (Reid *et al.*, 1995). In an *in vitro* model mimicking gastrointestinal conditions, *Lactobacillus* suppressed the growth of *Candida* after antibiotic treatment (Payne *et al.*, 2003), possibly by competing for the same receptor sites.

Our primary hypothesis, that probiotic bacteria can reduce the prevalence of oral *Candida* in the elderly, was confirmed in the present study. Oral candidosis is common in elderly people, and under certain circumstances can contribute to disseminated candidiasis (Shay *et al.*, 1997). Probiotics could be regularly used as a prophylactic or therapeutic means, without side-effects, of reducing *Candida*. Probiotics also diminished the risk of hyposalivation and the feeling of dry mouth, and can therefore be considered beneficial to oral health in general.

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