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A Multispecies Probiotic Reduces Oral *Candida* Colonization in Denture Wearers

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Abstract

Purpose: The prevalence of *Candida* infections has been rising with an increasingly aging population and a larger population of immunocompromised individuals. The use of probiotics may be an alternative approach to antifungal agents in the prevention and treatment of oral candidiasis. This study aimed to evaluate the short-term effect of probiotics in reducing the infection level of oral *Candida* in candidiasis-asymptomatic elderly denture wearers.
Materials and Methods: In a double-blind randomized study, 59 denture wearers harboring *Candida* spp. in the oral cavity with no clinical symptoms were allocated into two groups: probiotic and placebo. All patients were instructed to clean the denture daily. The probiotic group poured a capsule containing lyophilized *Lactobacillus rhamnosus* HS111, *Lactobacillus acidophillus* HS101, and *Bifidobacterium bifidum* daily on the palatal surface of the maxillary denture, whereas the placebo group was submitted to the same regimen using placebo capsules. *Candida* spp. infection levels were evaluated in palate mucosa samples obtained before and after a 5-week experimental period.
Results: All patients harbored *Candida* in the palate mucosa at baseline. Fifty-five individuals completed the experimental period. The detection rate of *Candida* spp. was 92.0% in the placebo group after the experimental period, whereas it was reduced to 16.7% in the probiotic group. The reduction promoted by the probiotic regimen was independent of baseline characteristics such as *Candida* infection level and colonizing species, age of denture, and other variables.
Conclusion: The probiotic product was effective in reducing the colonization of the oral cavity with *Candida* in candidiasis-asymptomatic elderly denture wearers, suggesting that this multispecies probiotic could be used to prevent oral candidiasis. *Clinical implications*: Colonization of oral surfaces by *Candida* is considered a risk factor for invasive fungal infections. The use of a product with *L. rhamnosus, L. acidophilus*, and *B. bifidum* may represent an alternative treatment for reduction of *Candida* infections in elderly denture wearers.

Elderly individuals who use dental prostheses (complete denture, removable partial denture) are susceptible to denture stomatitis (DS) associated with *Candida.*[1] *Candida albicans* is not only able to adhere to the mucosa surfaces, but also to stick to the acrylic resins of dental prostheses. Both the plaque accumulated on the denture and poor oral hygiene contribute to the virulence of *Candida*, offering the clinical picture of *Candida*-associated DS.[2] For the treatment of DS, correct denture and oral hygiene measures for biofilm control are recommended,[1] while prophylactic measures are necessary to prevent *Candida* infection.

In clinical practice the over- or under-diagnosis of *Candida* infection is a problem leading to therapeutic errors, indiscriminate use of antifungal drugs, and consequent resistance to antimicrobial agents.[3] Furthermore, miconazole interacts with other drugs and induces hepatic alterations, whereas nystatin has an unpleasant taste, and some individuals present gastrointestinal (GI) discomfort such nausea, vomiting, and diarrhea, possibly leading to poor patient compliance.[4] Therefore, it is desirable to promote health through natural or alternative therapies, such as by using probiotics.

Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host.[5] Probiotic bacteria in the human gut have beneficial influences, with competitive, antagonistic, and immunological effects against pathogenic microorganisms. Candidiasis results from dysbiosis, that is, an imbalance of the resident microbiota, with overgrowth of the *Candida* spp. On the other hand, probiotic cultures stimulate the proliferation of beneficial bacteria rather than potentially harmful organisms, enhancing the natural host defense mechanisms.[6] Their effect has been observed regarding pathogenic bacteria, but they are also effective against yeast of the genus *Candida* in the vagina[7] and oral cavity.[8-10]

L. paracasei and *Lactobacillus rhamnosus* isolated from the oral cavity of healthy volunteers inhibited *C. albicans* and *Streptococcus mutans* due to the production of organic acids, hydrogen peroxide, and bacteriocins.[9] A commercial probiotic composed of *Lactobacillus plantarum* and *Lactobacillus reuteri* displayed the strongest in vitro inhibition of *C. albicans*, due to low pH and hydrogen peroxide production.[10] In vivo, a cheese containing the probiotic bacteria *L. rhamnosus GG, L. rhamnosus LC705,* and *Propionibacterium freudenreichii* ssp. *sermanii JS* reduced the prevalence of *Candida* in elderly individuals.[8] Due to the evidence of a protective role of probiotics in mucosal surfaces, there is a need for studies aiming to prevent or treat oral diseases, specifically targeting applications, formulas, and forms of administration of probiotics.[11]

Elderly people (>60 years) are more susceptible to *Candida* infection[12,13] owing to host systemic factors (diabetes, nutrition deficiency, xerostomia) that depress the mechanisms of defense and local factors (poor hygiene, traumas caused by ill-adapted dentures) that facilitate the adhesion and penetration of the yeast.[2] Furthermore, dentures are a relevant reservoir of *Candida* due to the facilitation of biofilm formation,[14] especially when oral hygiene is poor.[15]

Therefore, our study aimed to test the effect of a product with multispecies probiotic bacteria on the colonization of *Candida* in candidiasis-asymptomatic elderly denture wearers. For this purpose, a product containing *L. rhamnosus* HS111, *Lactobacillus acidophillus* HS101, and *Bifidobacterium bifidum* was evaluated in a double-blind, randomized, controlled trial, and the influence of different factors on *Candida* reduction, such as *Candida* infection level and *Candida* colonizing species, denture hygiene, age, and ethnic background, was determined.

Materials and methods

This study was approved by the Research Ethics Committee of the School of Dentistry, University of São Paulo, Brazil, protocol number 124/08.

Participants

Volunteers were selected among 158 patients seeking dental treatment (complete denture) at the School of Dentistry, University of São Paulo, São Paulo, Brazil, from 2008 to 2010. After written informed consent, participants were given a questionnaire on personal data, health conditions, prostheses state, and dietary habits. The participants' dentures were classified as a clean denture or a poor hygiene denture. A poor hygiene denture was characterized by the presence of dental plaque, tartar, bad odor, and pigments.

Exclusion criteria comprised inability to perform/understand the experimental procedures, use of antifungal agents and/or antiseptic mouth rinses in the previous 6 months, report of consumption of probiotics, intolerance to lactose or milk derivatives, report of severe GI disorders, heart disease, recent transplant recipients, AIDS, and clinical manifestations of oral candidiasis. Inclusion criteria were detectable levels of *Candida* in palatal mucosa without clinical symptoms of candidiasis, and use of dentures. *Candida* prevalence was determined by culture of a sample obtained with the aid of a cervical brush (Kolplast Ltda, Itupeva, Brazil) from the palatal mucosa.

Fifty-nine individuals (mean age 61.6 years SD = 9.8) met the inclusion criteria, and 55 completed the intervention period. Patients were randomly allocated in one of two groups: probiotic (n = 30) and placebo (n = 29). The clinical evaluation, sample collection, and microbial analysis and patients' orientation were conducted by an investigator blind to the patients' group allocation.

Trial product preparation

Commercially available lyophilized cultures were obtained from HardiStrain® – Probiotics (Future Ceuticals, Momence, IL). *L. rhamnosus* HS111, *L. acidophilus* HS101, and *Bifidobacterium bifidum* were combined in equal amounts, reaching 10^8 CFU (3.3×10^7 CFU of each) per capsule, with added strawberry flavor (Kraft Foods, Brazil). The placebo product had the same characteristics as the probiotic product, but did not contain the probiotic bacteria. Viability tests were performed, and bacterial cells were shown to maintain their viability for at least 120 days after manipulation.

Experimental protocol

Participants used the probiotic or placebo product daily (1 capsule/day) for 5 weeks. Patients were instructed to pour the capsule contents (probiotic or placebo) in the palatal region of the previously cleaned maxillary denture, and to use the denture in close contact with the mucosa of the palate, as usual.

Candida infection levels

At baseline, a palatal sample was obtained from each participant, and the infection level of *Candida* sp. was evaluated by viable counts. Samples obtained by rubbing a cervical brush for 10 seconds on the palatal mucosa surface were serially diluted in phosphate-buffered saline and inoculated on the surface of Sabouraud dextrose agar (SDA) plates with 100 mg chloramphenicol/L (Difco Laboratories, Detroit, MI) added. After incubation for 24 to 48 hours, colonies were counted, and the number of CFU/ml was determined. The same procedure was performed at the end of the 5-week experimental period. The treatment was considered successful when no yeasts were detected at the end of the experimental period.

Candida species were identified by phenotypic methods according to Kurtzman and Fell[16] by testing of tube germ, microcultive, auxanogram, and zymogram. *C. dubliniensis* and *C. albicans* were differentiated by growth in hypertonic

Table 1 Distribution and characteristics (mean ± SD) of participants allocated to the placebo and probiotic groups, at baseline, according to variables: *Candida* infection level and colonizing species, age, ethnic group, gender, type of denture, denture hygiene, and denture age. Only participants who completed the intervention period (N = 55) are shown

Variables		Groups		
		Placebo n = 25	Probiotic n = 30	Total N = 55
Distribution				
Ethnic groups	Caucasian	14	14	28
	Afro-descendant	9	15	24
	Others	2	1	3
Gender	Male	8	6	14
	Female	17	24	41
Denture	Bimaxillary	24	24	48
	Unimaxillary	1	6	7
Denture hygiene classification	Poor	21	25	46
	Clean	4	5	9
Candida species	*C. albicans*	10	11	21
	non-*C. albicans*	11	18	29
	Both	4	1	5
Characteristics		Mean ± SD		
Candida infection level (log_{10} CFU/ml)		3.6 ± 1.1	3.4 ± 1.1	3.5 ± 1
Denture age (years)		6.4 ± 3.8	5.6 ± 3.9	5.9 ± 3.8
Age (years)		62.1 ± 9.4	61.6 ± 7.8	61.8 ± 8.5

broth (SDA broth medium supplemented with 6.5% sodium chloride).[17] Only *C. albicans* grew significantly in the 96-hour incubation period.

Data analysis

Differences in the baseline characteristics between the two groups were assessed by Student's *t*-test and chi-square test. The effect of treatment on *Candida* prevalence and infection levels was evaluated using chi-square test (asymptotic), exact chi-square test, or Fisher's exact test depending on the characteristics of each variable.[18] Logistic regression analysis was used to evaluate the effect of ethnic background, age, denture age, and gender and variables at baseline such as *Candida* species and denture hygiene on the detection rate of *Candida* at the end of the experimental period. The level of significance was set at 5%. Statistical analyses were performed using SAS 9.2 software (Cary, NC).

Results

At baseline, all individuals harbored *Candida*, and there were no differences in *Candida* infection levels between the placebo and probiotic groups, ranging from 1×10^2 to 1×10^7 CFU/ml. The distribution of the individuals according to detection of *C. albicans* or other species of *Candida* at baseline is shown in Table 1. There were no differences in social variables (age, ethnic background, gender) between individuals allocated to the placebo or probiotic group. Furthermore, other characteristics such as percentage of bimaxillary denture wearers, age of denture, and denture hygiene were also similar between the groups (Table 1).

The intervention period was completed by 30 participants of the probiotics group and by 25 participants of the placebo. Four patients of the placebo group were excluded, due to refusal to continue participating (1 patient) and lack of use of the placebo product (3 patients). At the end of the experimental period, *Candida* was detected in only five patients (16.7%) for the probiotics group, whereas 23 patients (92.0%) of the placebo group still showed detectable levels of *Candida* in the palatal mucosa surface samples. Thus, the probability of treatment success, that is, no detectable levels of *Candida* in palatal samples, was significantly higher in the probiotics group than in the placebo group (95% CI = 2.7-39.7, $p < 0.0001$; chi-squared test).

At the end of the 5-week experimental period, the five participants whose samples were still positive for *Candida* of the probiotic group exhibited a yeast load of 3 log CFU (SD = 1.0, 95%, IC = 1.7-4.2)/ml, whereas the mean infection levels for the 23 participants with detectable *Candida* at the end of the experimental period of the placebo group were of 3.7 log CFU (SD = 1.1, 95%, IC = 3.2-4.1)/ml. Treatment with probiotics was equally effective for the patients, independent of the *Candida* levels at baseline (Table 2).

Among five patients of the probiotic group whose samples remained positive after the experimental period, two were bimaxillary and three were unimaxillary denture wearers, and this factor did not influence the treatment success rate. Furthermore, these five patients presented poor denture hygiene at baseline. Few patients from either group exhibited a denture classified as clean at baseline (Table 1), and this factor did not

Table 2 Distribution of participants allocated in the placebo and probiotic groups, according to *Candida* infection levels at baseline and detection of *Candida* after the 5-week experimental period, in palate mucosa samples

Candida infection level at baseline (CFU/ml)	Detection of *Candida* after the experimental period			
	Placebo n = 25		Probiotics n = 30	
	Positive	Negative	Positive	Negative
$10^2 \geq x < 10^3$	4	0	0	8
$10^3 \geq x < 10^4$	6	2	3	5
$10^4 \geq x < 10^5$	8	0	1	7
$>10^5$	5	0	1	5
Total	23	2	5	25

exert any effect on the reduction of *Candida* promoted by the probiotics at the end of the experimental period, when compared to the placebo group. There were no differences in treatment success rate according to ethnic background, gender, age (logistic regression, $p > 0.05$, NS), or denture age (Student's *t*-test, $p > 0.05$).

Other factors, such as the *Candida* species at baseline, did not interfere with the probiotic treatment success rate. At baseline, *C. albicans* was present in 52.1% of the patients, *C. guilliermondii* in 31.3%, *C. tropicalis* in 16.7%, and *C. glabrata* in 16.7%. Only 6.3% of patients harbored *C. dubliniensis*, *C. famata*, or *C. parapsilosis*. At the end of the experimental period in the probiotic group, *Candida* was detected in 3 of 13 patients harboring *C. albicans* at baseline (23.1%) and in 2 of 17 patients harboring other *Candida* species (11.8%).

Discussion

Candida species are major human fungal pathogens that cause both mucosal and deep-tissue infections. The incidence of fungal infections has increased in recent years due to use of broad spectrum antibiotic therapy, immunosuppressive, and cytotoxic therapy, acquired immunodeficiency, and diabetes *mellitus*. *Candida* species do not only promote a local disease in mucosal surfaces, but are associated with invasive opportunistic mycoses and are a major cause of nosocomial bloodstream infection.[19]

C. albicans remains the most common agent associated with invasive candidiasis regardless of age.[20] Although these yeasts are commensal organisms in the oral cavity, GI tract, and vagina of healthy humans,[19] prior fungal colonization of mucosa surfaces is considered a major risk factor for the development of *Candida* bloodstream infection.[21,22]

Prophylaxis of *Candida* infection among critically ill patients is usually made with azoles or oral use of nystatin, and recent reviews concluded that both treatments have a beneficial effect in reducing *Candida* invasive infections.[21,23] Although these local and systemic antifungals have been proven to be effective in reducing fungal colonization and invasive fungal infections, their use is not without harm.[19] Furthermore, the increased number of yeasts resistant to antifungal drugs indicates a need for new targets for new antifungal agents.[24]

Few studies on probiotics against oral *Candida* in the oral cavity have been performed in nonasymptomatic patients. In fact, most studies focused on patients with oral candidiasis, and the probiotic was not the only treatment to reduce *Candida*, but rather an adjuvant of the conventional therapy. A recent study[25] reported that the use of a probiotic product formed by *Bifidobacterium longum*, *Lactobacillus bulgaris,* and *S. thermophilus* in conjunction with oral local antifungal agents (nystatin) was more effective in the treatment of *Candida*-associated stomatitis than the conventional therapy.

In the present study, the use of a probiotic product with *L. rhamnosus* HS111, *L. acidophilus* HS101, and *Bifidobacterium bifidum* was effective in reducing the prevalence of oral *Candida* in asymptomatic elderly denture wearers in up to 83.3% of the participants. Our data indicated that this probiotic product can be indicated as a prophylactic agent to displace already colonizing pathogens such as *Candida* on the oral mucosa surface. Many studies involving adult patients have identified people at risk of developing invasive candidiasis by using scores based on sites of *Candida* colonization.[26] Other studies reported that probiotics containing *Lactobacillus* and *Bifidobacterium* species were able to reduce rectal colonization with *Candida* and candiduria in children receiving broad spectrum antibiotics.[27] Our group has previously shown that a probiotic composed of *L. acidophilus* and *L. rhamnosus* was able to prevent colonization by *C. albicans* in an immunosuppressed rat model.[28] Together with the present data, the tested probiotic product may represent an alternative method to reduce *Candida* colonization, thus preventing *Candida* infections.

Our data also demonstrated that the tested probiotic product was able to reduce yeast occurrence in the oral mucosa regardless of the *Candida* species and *Candida* infection level. These observations are particularly important due to the increased incidence of non-*C. albicans* in invasive mycoses, especially from the species *C. tropicalis*, *C. glabrata*, and *C. guilliermondii*, which are resistant to antifungal agents like the azoles.[29] One of these species, *C. glabrata*, was detected at baseline in the probiotics group and not detected at the end of the experimental period. This species often colonizes elderly individuals, regardless of denture use.[13] Another species for which the treatment with probiotics was successful was *C. guilliermondii*, which is a relatively uncommon agent of candidemia increasingly reported in Latin America.[30]

The use of dentures is a predisposing factor to the onset of pathologies related to *Candida* sp., such as angular cheilitis and DS. In both clinical pictures, local factors such as inadequate occlusal vertical dimension, trauma caused from an ill-fitting denture, poor denture hygiene, and systemic diseases and deficiencies of the immune system are usually associated with the *Candida* infection.[31] Thus, prevention of oral candidiasis includes the replacement of dentures every 5 years and frequent biofilm control involving the denture sanitization.

The present data indicate that a reduction in *Candida* detection rate by probiotics was also independent of the hygiene of the oral prosthesis. A clean prosthesis was very seldom found at baseline among the studied population in both groups; however, volunteers from both groups received instructions on

denture hygiene and were motivated to clean their dentures daily. On the other hand, the age and consequent roughness of the prosthesis may have hampered the prosthesis cleaning effect in both groups.

The mechanisms of probiotics against oral *Candida* may involve a combination of factors,[7] such as competition for adhesion sites and nutrients,[32] production of antimicrobial compounds,[7,33] stimulation of cytokine production and phagocytosis,[34,35] induction of IgA secretion,[36] maintenance of the epithelial barrier of defense, and modulation of the innate and adaptative immune response.[37,38]

In this study, the probiotic product was used on the upper prosthesis and was in close contact with the mucosa of the palate. Thus, besides its local activity, a systemic immunological effect might have also been possible due to swallowing of the product dissolved in saliva, which in turn may have promoted stimulation of the immune system, altering levels of salivary IgA antibodies and consequently preventing microbial adherence.[39]

L. rhamnosus, *L. acidophilus*, and *B. bifidum* are commonly used as probiotic agents, and are part of the microbiota of the oral cavity and the gut.[40,41] Although lactobacilli are related to caries lesions, their emergence is dependent on dietary factors, and species of this genus are usually found in deep dentin lesions.[40] Thus, their use as probiotics in the oral cavity should not introduce any harm, even in dentate patients; however, their role in altering oral microbiota diversity should be further investigated. Since the prevention of *Candida* colonization is considered as a key strategy in reducing the incidence of invasive candidiasis, the present data indicate that the use of probiotics to reduce *Candida* levels in the oral mucosa may help prevent oral candidiasis and even other invasive *Candida* infections.

Conclusions

The probiotic product was effective in reducing the colonization of the oral cavity with *Candida* in candidiasis-asymptomatic elderly denture wearers, suggesting that this multispecies probiotic could be used to prevent oral candidiasis. Colonization of oral surfaces by *Candida* is considered a risk factor for invasive fungal infections. The use of a product with *L. rhamnosus, L. acidophilus*, and *B. bifidum* may represent an alternative treatment for reduction of *Candida* infections in elderly denture wearers.

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