

Effects of Probiotic Bacteria on *Candida* Presence and IgA Anti-*Candida* in the Oral Cavity of Elderly

Fabio Henrique Boarini Pacheco MENDONÇA¹
Silvana Soléo Ferreira dos SANTOS¹
Ivan da Silva de FARIA¹
Célia Regina GONÇALVES e SILVA¹
Antônio Olavo Cardoso JORGE²
Mariella Vieira Pereira LEÃO¹

¹Basic Institute of Biosciences, UNITAU - University of Taubaté, Taubaté, SP, Brazil

²São José dos Campos Dental School, UNESP - Univ Estadual Paulista, São José dos Campos, SP, Brazil

Imbalance in the resident microbiota may promote the growth of opportunistic microorganisms, such as yeasts of *Candida* genus and the development of diseases, especially in aged people. This study evaluated whether the consumption of the probiotic Yakult LB[®] (*Lactobacillus casei* and *Bifidobacterium breve*) was able to influence on the specific immunological response against *Candida* and on the presence of these yeasts in the oral cavity of 42 healthy aged individuals. Saliva samples were collected before and after the probiotic use for 30 days, 3 times a week. The samples were plated in Dextrose Saboraud Agar with chloramphenicol, the colony-forming units (CFU/mL) were counted and the *Candida* species were identified. Anti-*Candida* IgA analysis was conducted using the ELISA technique. ANOVA and Student's t-test were used for normally distributed data and the Wilcoxon test was used for data with non-normal distribution ($\alpha=0.05$). The results showed a statistically significant reduction ($p<0.05$) in *Candida* prevalence (from 92.9% to 85.7%), in CFU/mL counts of *Candida* and in the number of non-*albicans* species after consumption of the probiotic. Immunological analysis demonstrated a significant increase ($p<0.05$) in anti-*Candida* IgA levels. In conclusion, probiotic bacteria reduced *Candida* numbers in the oral cavity of the elderly and increased specific secretory immune response against these yeasts, suggesting its possible use in controlling oral candidosis.

Key Words: *Candida*, probiotics, geriatrics, elderly.

INTRODUCTION

Yeasts of *Candida* genus are normal constituents of the human flora, found as commensal on skin, genitourinary and gastrointestinal tracts (1). These microorganisms can be isolated in the oral cavity of 20% to 80% of healthy individuals (2,3). However, some factors, such as immunosuppression and use of broad-spectrum antimicrobial drugs, can favor their overgrowth and the development of candidosis (4). Oral candidosis is a common problem among the elderly, denture wearers are more susceptible to the disease (5,6).

Products known as probiotics have been consumed for many years for therapeutic and prophylactic reasons. The presence of probiotic microorganisms in mucosa can prevent the growth of other bacterial species by

production of acid and antimicrobial products (7) or by interference on the mucosa immunity by modulating IgA synthesis and mucus production (8).

Experiments performed with probiotic bacteria, *Lactobacillus acidophilus* and *Lactobacillus fermentum* in rats infected with *Candida albicans* showed a significant reduction in amounts of yeasts in the oral cavity, suggesting a therapeutic capacity of these bacteria in candidosis (9).

Hatakka et al. (10) also observed a reduction of 32% on *Candida* prevalence in the oral cavity of elderly who consumed a cheese enriched with probiotic bacteria.

With the increase in life expectancy, geriatric problems became more frequent. Being candidosis a common disease among the elderly, the search for products that could help in its treatment is important

Therefore, the aim of this study was investigate the effect of Yakult LB[®] consumption in *Candida* spp. presence and in anti-*Candida* IgA levels in the oral cavity of elderly.

MATERIAL AND METHODS

Studied Population

After approval of the research project by the institutional Ethics Committee (Protocol #057/08), 42 institutionalized women aged 65 or older who lived in the city of Taubaté, SP, Brazil, were clinically healthy and had not used antibiotics or immunosuppressors for the previous 3 months were selected for the study. The sample size was determined considering: the total number of clinically healthy aged women of the institutions (n=50), 5% of error, 95% of confidence level and 80% of expected prevalence of *Candida* based on previous pilot study. Preliminary analyses regarding the oral and dental conditions were performed, including presence of periodontal disease, periodontal pockets and mucosal lesions. When these lesions were detected, the patients were included in the study and treated only after enrolment to avoid interference in the results. Patients with emergency conditions were not included and were treated immediately. If any new fact that could influence the results (e.g.: antibiotics use) emerged during the course of the study, the samples of that patient were also excluded. All participants and their caregivers were fully informed about the objectives and methodology of the study and signed an informed consent form.

Saliva Samples and Probiotic consumption

Samples of volunteers' saliva were collected without stimulation, about 2 h after oral hygiene, in the morning or in the afternoon, depending on the rules designated by the institution. The samples were stored in sterile disposable collectors and kept on ice up to 3 h. Then, a fraction of the samples was plated in culture media for isolation and identification of *Candida* yeasts. Another fraction was kept in a vial added of phenylmethylsulfonyl fluoride 0.1 mM (Sigma Co., St Louis, MO, USA) and sodium azide 0.1% (Difco Laboratories Inc., Detroit, MI, USA) at -20°C, for assessment of IgA levels.

The caregivers were instructed to mix 1 g (content of 1 envelope) of the probiotic Yakult LB[®] (Yakult S/A Industria e Comércio, Caçapava, Brazil) (*Lactobacillus*

casei and *Bifidobacterium breve*, 2x10⁷ to 10⁹ and 5x10⁷ to 10⁹ CFU/mL, respectively) with some kind of juice and give to the elderly 3 times a week, at the same hour, for 30 days. After this period, another saliva sample was collected from each participant.

Isolation, Counting and Identification of Candida Genus

The original samples or samples diluted 10-fold serially were plated in duplicate in Saboraud Dextrose Agar (Oxoid, Lawrence, KS, USA) with chloramphenicol (1 mg/mL) (Inlab, São Paulo, SP, Brazil) and incubated at 37°C for 48 h at room temperature for 5 days. The number of colony forming units *per* mL of saliva (CFU/mL) was counted. Suggestive colonies from each plate were confirmed by smear tests stained by the Gram technique. The *Candida* species were identified by phenotypic (germinative tube formation and chlamydoconidia production) and biochemistry tests (fermentation and assimilation of carbohydrates and urase test).

IgA Analyses

The levels of anti-*Candida* IgA were also analyzed using the ELISA technique. Sensitization of ELISA plates was performed using surface antigens from *Candida* cells, prepared as described by Koga-Ito et al. (11). The plates were then incubated with diluted saliva (1:10), followed by an anti-human IgA labeled with horseradish peroxidase. The reaction was developed with orthophenylenediamine and H₂O₂ as substrate. Absorbance was measured at 450 nm.

Statistical Analysis

First, the normality of the data (CFU/mL counts and IgA anti-*Candida* levels, before and after the use of probiotics) was evaluated using the D'Agostino test. ANOVA and Student's t-test were used for normally distributed data while the Wilcoxon test was used for data with non-normal distribution. A significance level of 5% was established for all analyses.

RESULTS

The results showed a reduction in the prevalence of *Candida* in oral cavity of elderly, since 39 (92.9%) individuals carried the microorganism before probiotic

consumption and after that just 36 (85.7%). A statistically significant reduction in CFU/mL counts ($p=0.02$, Wilcoxon test) and in the log of CFU/mL counts (Fig. 1) ($p=0.0$, ANOVA test; $p=0.03$, Student's t-test) was also observed. There was a reduction in *Candida* numbers in 57.1% of the elderly and total elimination of the yeasts in 11.9% of them.

Among the identified species, *C. albicans* was the most frequently isolated one before and after the probiotics use. The species profile was slightly modified, since a reduction in the numbers of non-*albicans* species was observed (Table 1).

Table 1. *Candida* species identified from oral cavity of elderly individuals before and after probiotics use.

Specie	Before		After	
	Strains	%	Stains	%
<i>C. albicans</i>	28	57.1	25	69.4
<i>C. tropicalis</i>	8	16.3	5	13.9
<i>C. guilliermondii</i>	6	12.2	3	8.3
<i>C. glabrata</i>	3	6.1	2	5.6
<i>C. lipolytica</i>	1	2.0	-	-
<i>C. krusei</i>	1	2.0	1	2.8
<i>C. kefyr</i>	1	2.0	-	-
<i>C. parapsilosis</i>	1	2.0	-	-
Total	49	100	36	100

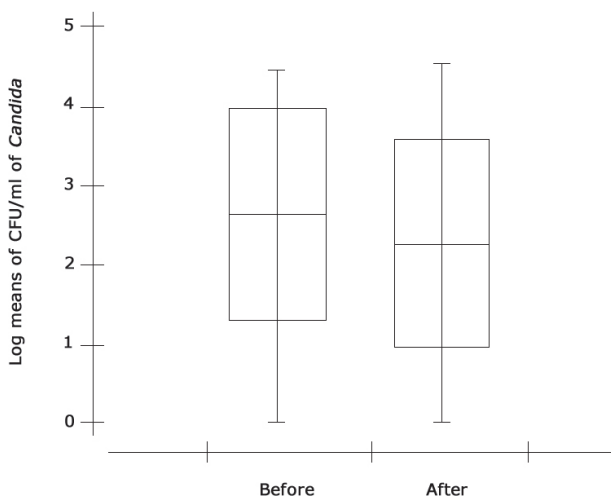


Figure 1. Log means of CFU/mL of *Candida* from the oral cavity of elderly before and after use of probiotics ($p=0.0$, ANOVA test; $p=0.03$, Student's t-test).

Immunological analysis showed a significant increase in the levels of anti-*Candida* IgA ($p=0.00$, ANOVA test and $p=0.01$, Student's t-test) (Fig. 2). The results showed that 26 (61.9%) individuals had their IgA levels increased, and from them 17 (65.4%) had a reduction in amounts of *Candida* in the oral cavity.

DISCUSSION

With the increase in life expectation, works involving geriatrics are becoming more and more important. Being the aged population more susceptible to *Candida* colonization, it was expected to find a high prevalence of the fungus in the oral cavity of the studied individuals. Nevertheless, the results showed a reduction in the prevalence and counts of *Candida* after probiotic use. Santos et al. (12) had already observed a similar effect with the same product (Yakult LB®), but in a younger population. Hatakka et al. (10), also studying elderly, observed a decrease from 30% to 21% in the prevalence of *Candida* in oral cavity after the consumption of a cheese enriched with probiotic bacteria. Although *Candida* presence could be influenced by many factors, it is important to highlight that the older people of the present study maintained the same habits and were accompanied throughout the experiment by the caregivers of the institution, who administered the probiotic in a blind manner, i.e., without knowledge of the elderly. With this, the placebo effect was minimized, a study with a placebo group would be appropriate. The

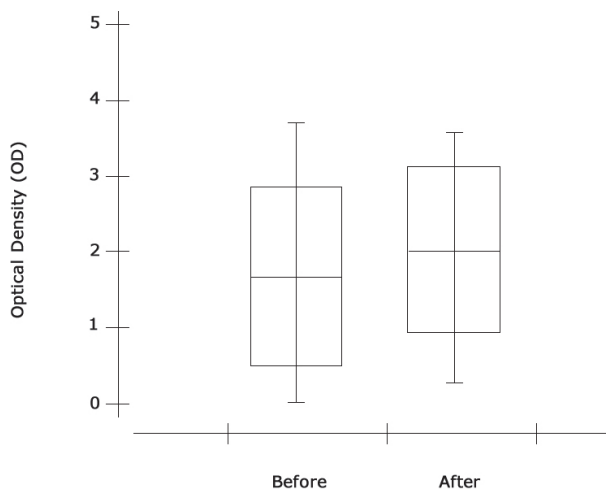


Figure 2. Optical density (OD) corresponding to IgA anti-*Candida* levels of the oral cavity of elderly individuals before and after use of probiotics ($p=0.0$, ANOVA test; $p=0.01$, Student's t-test).

quality of the samples could also contribute to variations. However, in the whole experiment, it was maintained the same time for the collection of the samples and the maximum of 3 h for processing them, reducing the risk of interferences.

The identification of analyses showed that *C. albicans* was the most prevalent species before and after the probiotic use. Its predominance in the oral cavity has been demonstrated (6,12,13). Several non-*albicans* species were also isolated, a phenomenon identified in long-term antibiotic users, immunosuppressed and chronic pathologies patients (14-16). For example, Alberth et al. (15) observed the substitution of *C. albicans* for non-*albicans* species (*C. kefyr*, *C. lusitaniae*, *C. sake*, *C. tropicalis*) in children with cancer, especially in the stages of severe neutropenia. Costa et al. (16) observed an increase in non-*albicans* species in HIV-infected patients, although *C. albicans* was still the most frequent. Thus, the elderly of this work had a profile of *Candida* species more similar to patients with a predisposing factor. However, with the probiotic use there was a reduction in non-*albicans* species frequency, approximating the elderly to a healthy profile.

Controversial results regarding the role of secretory antibodies in the prevention of *Candida* infections and the correlation of *Candida* and anti-*Candida* IgA levels have been reported. Silva et al. (17) observed that patients with vaginal candidiasis showed lower levels of anti-*Candida* IgA in saliva when compared with the control group (absence of candidosis). In contrast, Kantardjiev and Popova (18) reported that the saliva samples of patients with denture stomatitis, mainly associated with *Candida*, showed higher titers of anti-*Candida* antibodies.

The present results revealed that after the period of probiotics use, a significant increase in the levels of specific IgA was observed. According to Boirivant and Strober (19), probiotics can improve the defense function of epithelial cells by the induction of cytokine secretion and the production of immunoglobulins and antimicrobial substances. Since some individuals in this study did not present the yeast or showed diminished amounts in the oral cavity after probiotics use, it is likely that *Candida* elimination could have occurred due to secretory immunological stimulation.

The maintenance and re-establishment of the internal balance, frequently compromised in older people, are the best alternative to avoid the development of candidosis and other opportunistic diseases.

The findings of this study suggest that the periodic consumption of probiotics could help recovering this equilibrium and eliminating less adherent *Candida* species, but with pathogenic potential.

RESUMO

Desequilíbrios na microbiota residente podem promover o crescimento de microrganismos oportunistas, como as leveduras do gênero *Candida*, e o desenvolvimento de doenças, especialmente na população idosa. Este estudo investigou se o consumo do probiótico Yakult LB® (*Lactobacillus casei* e *Bifidobacterium breve*) era capaz de influenciar na resposta imune secretória anti-*Candida* e na presença destes microrganismos na cavidade bucal de 42 idosas saudáveis. Amostras de saliva foram coletadas antes e depois do consumo do probiótico por 30 dias, 3 vezes por semana. As amostras foram semeadas em Agar Sabouraud Dextrose com cloranfenicol, as unidades formadoras de colônias (UFC/mL) foram contadas e as espécies de *Candida* foram identificadas. A análise de IgA anti-*Candida* foi realizada pela técnica ELISA. Os resultados demonstraram redução na prevalência de *Candida* (de 92,9% para 85,7%), na contagem de UFC/mL ($p \leq 0,05$) e no número de espécies não-*albicans*, depois do consumo do probiótico. As análises imunológicas mostraram um aumento significativo dos níveis de IgA anti-*Candida* ($p \leq 0,05$). Concluindo, as bactérias probióticas reduziram significativamente a quantidade de *Candida* na cavidade bucal dos idosos e aumentaram a resposta imune secretória específica para esta levedura, sugerindo a possibilidade de sua utilização no controle da candidose bucal.

ACKNOWLEDGEMENTS

The authors thank Yakult S/A Indústria e Comércio (Caçapava, SP, Brazil) for the donation of Yakult LB® samples.

REFERENCES

1. Miranda LN, van der Heijden IM, Costa SF, Sousa AP, Sierra RA, Gobara S, et al.. *Candida* colonization as a source for *Candidaemia*. *J Hosp Infect* 2009;72:9-16.
2. Oksuz S, Sahin I, Yildirim M, Gulcan A, Yavuz T, Kaya D, et al.. Phospholipase and proteinase activities in different *Candida* species isolated from anatomically distinct sites of healthy adults. *Jpn J Infect Dis* 2007;60:280-283.
3. Tsang CS, Chu FC, Leung WK, Jin LJ, Samaranyake LP, Siu SC. Phospholipase, proteinase and haemolytic activities of *Candida albicans* isolated from oral cavities of patients with type 2 diabetes mellitus. *J Med Microbiol* 2007;56:1393-1398.
4. Vecchiarelli A, Pericolini E, Gabrielli E, Pietrella D. New approaches in the development of a vaccine for mucosal candidiasis: progress and challenges. *Front Microbiol* 2012;3:1-7.
5. Pinto TMS, Neves ACC, Leão MVP, Jorge AOC. Vinegar as an antimicrobial agent for control of *Candida* spp. in complete denture wearers. *J Appl Oral Sci* 2008;16:385-390.
6. Pires F, Santos E, Bonan P, De Almeida O, Lopes M. Denture stomatitis and salivary *Candida* in Brazilian edentulous patients. *J Oral Rehabil* 2002;29:1115-1119.
7. Sobel JD. Vulvovaginal candidosis. *Lancet* 2007;369:1961-1971.

8. Matsuzaki T, Takagi A, Ikimura H, Matsuguchi T, Yokokura T. Intestinal microflora: probiotics and autoimmunity. *J Nutr* 2007;137:798S-802S.
9. Elahi S, Pang G, Ashman R, Clancy R. Enhanced clearance of *Candida albicans* from the oral cavities of mice following oral administration of *Lactobacillus acidophilus*. *Clin Exp Immunol* 2005;141:29-36.
10. Hatakka K, Ahola AJ, Yli-knuuttila H, Richardson M, Poussa T, Meurman JH, et al.. Probiotics reduce the prevalence of oral *Candida* in the elderly - a randomized controlled trial *J Dent Res* 2007;86:125-130.
11. Koga-Ito CY, Unterkircher CS, Watanabe H, Martins CA, Vidotto V, Jorge AO, et al.. Caries risk tests and salivary levels of immunoglobulins to *Streptococcus mutans* and *Candida albicans* in mouthbreathing syndrome patients. *Caries Res* 2003;37:38-43.
12. Santos AL, Jorge AOC, Santos SSF, Silva CRG, Leão MVP. Influence of probiotics on *Candida* presence and IgA anti-*Candida* in the oral cavity. *Braz J Microbiol* 2009;40:960-964.
13. Weckwerth PH, Carnietto C, Weckwerth AC, Duarte MA, Kuga MC, Vivan RR. *In vitro* susceptibility of oral *Candida albicans* strains to different pH levels and calcium hydroxide saturated aqueous solution. *Braz Dent J* 2012;23:192-198.
14. Querido SMR, Back-Brito GN, Santos SSF, Leão MVP, Koga-Ito CY, Jorge AOC. Opportunistic microorganisms in patients undergoing antibiotic therapy for pulmonary tuberculosis. *Braz J Microbiol* 2011;42:1321-1328.
15. Alberth M, Majoros L, Kovalecz G, Borbás E, Szegedi I, J Márton I, et al.. Significance of oral *Candida* infections in children with cancer. *Pathol Oncol Res* 2006;12:237-241.
16. Costa CR, de Lemos JA, Passos XS, de Araújo CR, Cohen AJ, Souza LK, et al.. Species distribution and antifungal susceptibility profile of oral *Candida* isolates from HIV-infected patients in the antiretroviral therapy era. *Mycopathol* 2006;162:45-55.
17. Silva CRG, Melo KE, Leão MVP, Ruis R, Jorge AOC. Relationship between *Candida* in vaginal and oral mucosae and salivary IgA. *Rev Bras Ginecol Obstet* 2008;30:300-305.
18. Kantardjiev TV, Popova EV. Anti-*Candida* antibodies in serum and saliva of patients with denture stomatitis. *Folia Med (Plovdiv)* 2002;44:39-44.
19. Boirivant M, Strober W. The mechanism of action of probiotics. *Curr Opin Gastroenterol* 2007;23:679-692.

Received October 15, 2011
Accepted October 31, 2012