Brazilian Journal of Microbiology (2009) 40: 960-964
ISSN 1517-8382

INFLUENCE OF PROBIOTICS ON *Candida* PRESENCE AND IgA ANTI-*Candida* IN THE ORAL CAVITY

Agda Lima dos Santos*; Antônio Olavo Cardoso Jorge; Silvana Soléo Ferreira dos Santos; Célia Regina Gonçalves e Silva; Mariella Vieira Pereira Leão

Instituto Básico de Biociências, Universidade de Taubaté, Taubaté, SP, Brasil

Submitted: September 15, 2008; Returned to authors for corrections: April 09, 2009; Approved: June 28, 2009.

ABSTRACT

Probiotics are defined as microorganisms that promote benefits to host health, mainly by regulating resident microbiota. Disequilibrium in microbiota can favor the growth of opportunist microorganisms and the development of pathologies, like candidosis caused by yeasts of the *Candida* genus. This work evaluated whether probiotics consumption was able to influence a specific immunological response to *Candida* and the presence of these yeasts in the oral cavity. Saliva samples were collected from healthy individuals and plated in Dextrose Saboraud Agar with chloramphenicol. Individuals presenting *Candida* in the oral cavity used the probiotic Yakult LB® for 20 days, after which new collections and identifications were performed. Anti-*Candida* IgA analysis was conducted using the ELISA technique. Analysis of the results showed a significant reduction in *Candida* prevalence (46%) and mean *Candida* CFU/mL counts (65%). The *Candida* species identified were *C. albicans* (98%) and *C. tropicalis* (2%), before and after probiotics consumption. Immunological analysis demonstrated a significant reduction in anti-*Candida* IgA levels after probiotics use, probably due to less antigenic stimulation. In conclusion, in the individuals studied, probiotics use significantly reduced the amount of *Candida* in the oral cavity, possibly due to competition between the yeasts rather than by specific secretory immune response stimulation.

Key words: *Candida*; IgA; *Lactobacillus casei*; *Bifidobacterium*

INTRODUCTION

Probiotics are defined as microorganisms that promote benefits to host health, principally by means of resident microbiota regulation (4). The presence of probiotic microorganisms in intestinal mucosa can prevent the colonization of pathogens due to competitive effects or due to the production of compounds that inhibit their development and proliferation (10). An additional function of these products seems to be interference in mucosa immunity by modulating IgA synthesis and mucus production, or alterations in antiinflammatory regulation (20).

There is increasing evidence that probiotics have therapeutic actions, especially in disturbances involving the

***Corresponding Author. Mailing address:** Instituto Básico de Biociências – Universidade de Taubaté – Taubaté, Brazil.; Tel.: +55 12 3629-7909/ +55 12 9781 3344.; Email: ag_lima86@hotmail.com

gastrointestinal (5), genitourinary (17) and immunological systems (16). Hickson *et al*. (8) showed that the **c**onsumption of a probiotic drink containing *Lactobacillus casei, L. bulgaricus* and *Streptococcus thermophilus* was able to reduce the incidence of diarrheas associated with antibiotics use and caused by *Clostridium difficile*. Larsson *et al*. (14) observed that lactobacilli used as a supplement during clindamycin treatment in patients with bacterial vaginosis significantly reduced the recurrence rate and extended the period of recurrence.

Disequilibrium in microbiota can favor the growth of opportunist microorganisms and the development of pathologies, such as candidosis, an infection caused by yeasts of the *Candida* genus (13, 18). These microorganisms can be identified in the oral cavity of 30 to 60% of healthy individuals and the role of secretory antibodies in the prevention of their establishment and proliferation is controversial (9, 19).

Biasoli and Magaró (2) observed a reduction in the adhesion of *Candida albicans* to oral epithelial cells with the introduction of *L. casei* and *L. acidophilus in vitro*. Hatakka *et al*. (7) studied the effect of a cheese enriched with probiotic bacteria on *Candida* prevalence in the oral cavity of elderly individuals and observed a 32% reduction in prevalence. In this work, the influence of probiotics consumption on the levels of anti-*Candida* IgA and on the presence of these yeasts in the oral cavity of healthy young individuals was evaluated.

MATERIAL AND METHODS

After the approval of this project by the local Ethics in Research Committee, healthy young individuals were selected, excluding those who had used antibiotics for a period of three months, who were smokers or wearers of prosthesis or orthodontic devices. All the participants were fully informed concerning the objectives and methodology of the study and provided a written free, informed term of consent.

Initially, 111 individual were analyzed and samples of their saliva were collected without stimulation. Those individuals who presented *Candida* yeasts in the oral cavity were selected and if the microorganisms were identified again one month later, they were instructed to use the probiotics Yakult LB (*Lactobacillus casei* and *Bifidobacterium breve*, 2×10^7 to 10^9 and 5×10^7 to 10^9 UFC/mL, respectively) daily for 20 days. After this period, another saliva sample was collected from each of the 26 participants.

All the samples were plated in duplicate in Saboraud Dextrose Agar (OXOID, Lawrence, KS, USA), to which 1mg/mL of chloramphenicol was added (INLAB, São Paulo, SP, Brazil), followed by incubation at 37ºC for 48 hours and at room temperature for five days. Next, the number of colony forming units per mL of saliva was determined (CFU/mL). Suggestive colonies from each plate were confirmed by smear tests stained by the Gram technique. Next, the *Candida* species were identified by phenotypic (germinative tube formation and chlamydoconidia production) and biochemistry tests (fermentation and assimilation of carbohydrates and urase test).

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*. (13). 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 H_2O_2 as substrate. Absorbance was measured at 450 nm.

Data regarding the CFU/mL counts and IgA anti-*Candida* levels, before and after the use of probiotics, were compared using the Student t test, with a level of significance of $p < 0.05$ (5%).

RESULTS

The results showed a significant reduction in the log mean of CFU/mL counts after the use of probiotics of 65% (Figure 1). A reduction of 46% in the prevalence of *Candida*

in oral cavity was also observed; i.e., out of the 26 individuals selected, *Candida* was no longer identified in 12.

Among the identified species of *Candida*, 98% were *C. albicans* and 2% *C. tropicalis*, both before and after probiotics use, showing that the product did not alter the species profile.

Immunological analysis showed a significant reduction in the levels of anti-*Candida* IgA (Figure 2).

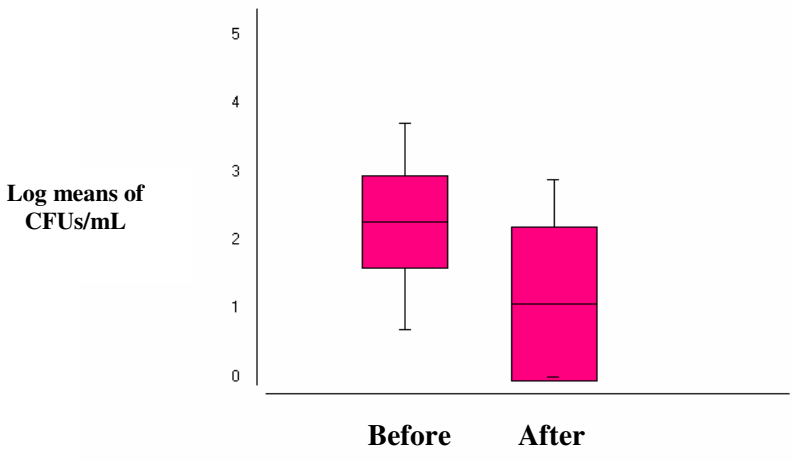

Figure 1. Log means of CFUs/mL of *Candida* before and after probiotics use; Student t test, p = 0.0000.

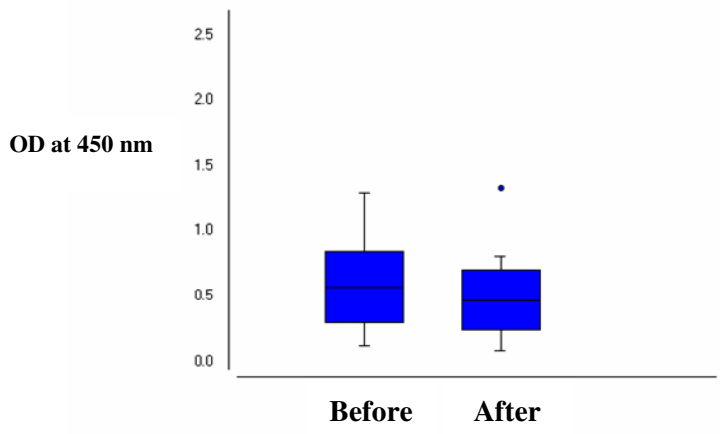

Figure 2. Means of optical density (OD) corresponding to IgA anti-*Candida* saliva levels before and after probiotics use; Student t test, p = 0.028.

DISCUSSION

Analysis of the results showed that the probiotics significantly influenced the amount of *Candida* identified in the oral cavity, since a reduction in CFU/mL counts was observed after probiotics use. A similar reduction in *Candida* levels after probiotics consumption was observed by Elahi *et al*. (6), although in rats with oral candidosis. In the present work, a reduction in the prevalence of *Candida* was also observed. Hatakka *et al*. (7) previously reported that cheese enriched with probiotic bacteria was able to reduce the prevalence of *Candida* in the oral cavity of elderly individuals. However, the reduction observed by the authors was lower, approximately 32%. The difference in prevalence

reduction can be explained by the populations studied, since elderly individuals show a greater predisposition for yeast adherence, due to the use of dental prostheses and other factors.

The most frequently isolated species of *Candida* before and after the use of the probiotics was *C. albicans*. In fact, *C. albicans* is the species most frequently identified in the oral cavity and the species most often reported in relation to pathological situations.

The reduction in the amount and prevalence of *Candida* in the oral cavity after the probiotics consumption could have occurred due to different mechanisms of action. According to Boirivant and Strober (3), probiotics can improve the defense function of epithelial cells by the induction of cytokine secretion and the production of immunoglobulins and antimicrobial substances. According to Matsuzaki *et al*. (15), certain probiotic bacteria have the potential to increase and modify the immune function of the host through the regulation of defense cells. However, the present results revealed that after the period of probiotics use, a significant reduction in the levels of specific IgA was observed.

Controversial results regarding the correlation of *Candida* and anti-*Candida* IgA levels have been reported. Jeganathan *et al*. (11) observed that patients with candidosis showed higher levels of anti-*Candida* IgA compared to the control group (absence of candidosis). Kantardjiev and Popova (12) reported that the saliva samples of patients with denture stomatitis, mainly associated with *Candida*, showed higher titers of anti-*Candida* antibodies compared to controls. Barousse *et al*. (1) observed no significant differences in total and *Candida* specific IgA and IgG concentrations in individuals with and without yeast colonization. In contrast, Vudhichamnong *et al.* (19) and Holmes *et al.* (9) observed an inverse relation between *Candida* CFU/mL counts and specific levels of IgA against this microorganism; i.e., the lower the values of CFU/mL counts, the higher the levels of anti-*Candida* IgA.

Since some individuals in this study, after probiotics use, did not present the yeast or showed diminished amounts in the oral cavity, it is likely that a reduction in antigenic stimulation occurred with a consequent reduction in the production of secretory antibodies.

It cannot be inferred that *Candida* elimination occurred due to secretory immunological stimulation mediated by probiotics use. The results obtained indicate a mechanism of competition and interference in the adhesion of *Candida* to epithelial cells mediated by this product. Given the fact that antibody levels were not monitored throughout the experimental period, we can not negate the hypothesis of an initial stimulation, contributing to the elimination of the microorganism, and a posterior reduction of the response to normal or lower levels, as detected in the research.

It is clear in the literature that IgA plays a role in the defense of the human body against yeasts of the *Candida* genus; however, scarce data is available regarding their role in association with probiotics supplementation. Further studies involving the adherence of probiotics to the epithelial cells of the oral mucosa and specific studies concerning probiotics and *Candida* competition are required to definitively elucidate the effects observed.

REFERENCES

1. Barousse, M.M.; Van Der Pol, B.J.; Fortenberry D.; Orr, D.; Fidel, P.L. JR. (2004). Vaginal yeast colonisation, prevalence of vaginitis, and associated local immunity in adolescents. *Sex. Transm. Infect.* 80(1), 48-53.
2. Biasoli, M.S.; Magaró, H.M. (2003). *In vitro* of carbohydrates and enteric bacteria on adherence of *Candida albicans. Rev. Iberoam. Micol.* 20(4), 160-163.
3. Boirivant, M.; Strober, W. (2007). The mechanism of action of probiotics *Curr. Opin. Gastroenterol.* 23(6), 679- 692.
4. Fuller, R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol.* 66(5), 365-78.
5. Gismondo, M.R.; Drago L.; Lombardi, A. (1999). Review of probiotics available to modify intestinal microflora. *Int. J. Antimicrob. Agents* 12(4), 287-292. *Apud:* Mombelli, B.; Gismondo, M.R. (2000). The use of probiotics in medical practice. *Int. J. Antimicrob. Agents*. 16(4), 531-536.
6. Elahi, S.; Pang, G.; Ashman, R.; Clancy, R. (2005). Enhanced clearance of *Candida albicans* from the oral cavities of mice following

oral administration of *Lactobacillus acidophilus. Clin. Exp. Immun*. 141, 29-36.

7. Hatakka, K.; Ahola, A.J.; Yli-Knuuttila, H.; Richardson, M.; Poussa, T.; Meurman, J.H.; Korpela, R. (2007). Probiotics reduce the prevalence of oral *Candida* in the Elderly – a randomized controlled Trial. *J. Dent. Res*. 86, 125-130.
8. Hickson, M.; D'Souza, A.L.; Muthu, N.; Rogers, T.R.; Want, S.; Raikumar, C.; Bulpitt, C.J. (2007). Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ.* 335(7610), 80.
9. Holmes, A.R.; Bandara, B.M.K.; Cannon, R.D. (2002). Saliva promotes *C. albicans* adherence to human epithelial cells. *J. Dent. Res*. 81(1), 28-32.
10. Jacobsen, C.N.; Nielsen, V.R.; Hayford, A.E.; Moller, P.L.; Michael Sen, K.F.; Paerregaard, A.; Sandström, B.; Tvede, M.; Jacobsen, M. (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strais in humans. *Appl. Environ. Microbiol.* 11(65), 4949-56.
11. Jeganathan, S.; Ufomata, D.; Hobkirk, J.A.; Ivanyi, L. (1987). Immunoglobulin A1 and A2 subclass of salivary antibodies to *Candida albicans* in patients with oral candidosis. *Clin Exp Immunol.* 70(2), 316-21.
12. Kantardjiev, T.V.; Popova, E.V. (2002). Anti-*Candida* antibodies in serum and saliva of patients with denture stomatitis. *Folia Med (Plovdiv).* 44(4), 39-44.
13. Koga-Ito, C.Y.; Martins, C.A.P.; Jorge, A.O.C. (2006). Estudo do Gênero *Candida*. *In:* Jorge, A.O.C. (1ª ed) *Princípios de Microbiologia e Imunologia.* Editora Santos, São Paulo, Brazil.
14. Larsson P.G.; Stray-Pedersen, B.; Ryttig, K.R.; Larsen, S. (2008). Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study. *BMC Womens Health.* 15, 8-3.
15. Matsuzaki, T.; Takagi, A.; Ikimura, H.; Matsuguchi, T.; Yokokura, T. (2007). Intestinal Microflora: probiotics and autoimmunity. *J. Nutr*. 137(3 suppl. 2), 798S-802S.
16. Pelto, L.; Isolauri, E.; Lilius, E.; Nuutila, Salmimen, S. (1998). Probiotic bacteria down-regulate the milk induced inflammatory response in milk hypersensitive subjects but have an immunostimulatory effect in healthy subjects. *Clin. Exper. Allergy*. 28(12):1474-1479.
17. Velraeds, M.M.; Van Der Mei, H.C.; Reid, G.; Busscher, H.J. (1996). Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates. *Appl. Env. Microbiol.* 62(2), 1958-63. *Apud:* MombellI, B.; Gismondo, M.R. The use of probiotics in medical practice. (2000). *Int. J. Antimicrob. Agents.* 16(4), 531-536.
18. Vieira, J.D.G.; Ribeiro, E.L.; Campos, C.C.; Pimenta, F.C.; Toledo, O.A; Nagato, G.M.; Souza, N.A.; Ferreira, W.M.; Cardoso, C.G.; Dias, S.M.S; Araújo, C.A; Zatta, D.T.; Santos, J.S. (2005). *Candida albicans* isoladas da cavidade bucal de crianças com síndrome de Down: ocorrência e inibição do crescimento por *Streptomyces* sp *Rev. Soc. Bras. Med. Trop*. 38(5), 383-86.
19. Vudhichamnong, K.; Walker, D.M.; Ryley, H.C. (1982). The effect of secretory immunoglobulin A on the *in-vitro* adherence of the yeast *C.* albicans to human oral epithelial cells. *Arch. Oral. Biol.* 27(8), 617-21.
20. Wehkamp, J.; Harder, J.; Wehkamp, K.; Wehkamp-Von Meissner, B.; Schlee, M.; Enders, C.; Sonnenborn, U.; Nuding, S.; Bengmark, S.; Fellermann, K.; Schröder, J.M.; Sange, E.F. (2004). NF- κB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. *Infect. Immun.* 10(72), 5750-58.