Microbiology

Influence of consumption of probiotics on presence of enterobacteria in the oral cavity

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Abstract: Clinical benefits of probiotics have been clearly reported in different gastrointestinal disorders, many of them caused by enterobacteria. The oral cavity is a port of entry and can be an important reservoir of these microorganisms. This work evaluated whether consumption of probiotics was able to influence the presence of enterobacteria in the oral cavity and the specific secretory response against these microorganisms. Saliva samples of healthy individuals were collected and plated in Mac-Conkey agar. Carriers of Gram-negative, rod-shaped microorganisms in the oral cavity were selected and instructed to use the probiotic Yakult LB for 20 days. Saliva was then collected and enterobacteria species were identified using the API 20 E system and by ELISA using anti-entero-bacteria IgA. The results showed reduction in the prevalence of entero-bacteria, but no significant changes in enterobacterial counts (log CFU/mL; $p = 0.3457$). The species most frequently isolated were *Enterobacter cloacae* and *Klebsiella oxytoca*, both before and after probiotic consumption. No significant changes were observed in anti-enterobacteria IgA levels. In conclusion, probiotic consumption had some influence on enterobacterial presence in the oral cavity, but did not affect enterobacterial counts or the specific immune secretory response against them.

Descriptors: *Lactobacillus casei*; *Bifidobacterium*; Enterobacteriaceae; Probiotics.

Introduction

Probiotics are food supplements containing live microorganisms or microbial cell components that promote host health.[1] Clinical benefits of probiotics have been clearly documented for different gastrointestinal disorders (e.g., lactose intolerance, viral and bacterial gastroenteritis, and inflammatory bowel disease[2]) and in immunotherapy against cancer and allergies.[3] The presence of probiotic microorganisms in intestinal mucosa can prevent the colonization of pathogens, probably via competitive effects, production of antimicrobial substances, and/or modulation of mucosal immunity.[4-6]

Shu *et al.*[7] observed that the consumption of probiotic bacteria induced a reduction in diarrhea caused by *Escherichia coli* toxin in pigs. The authors also observed an increase in phagocyte activity and in levels of IgA antibodies, reducing the mortality caused by *E. coli* 0157:H7 in mice.[8] Jespersen[9] observed an inhibitory effect of probiotics in pathoge-

nicity of *Salmonella typhimurium*, *Shigella flexneri*, and *Clostridium difficile* in infant acute gastroenteritis.

The oral cavity is a port of entry for many microorganisms and can be an important reservoir of enterobacteria. These microorganisms are potential agents in gastrointestinal disorders, hospital infections, and aggravation of oral disease.[10] An increased knowledge about products that may inhibit the adherence or growth of these bacteria is important and essential. Thus, the objective of the present study was to analyze whether consumption of the probiotic Yakult LB influences the presence of enterobacteria in oral cavity and the specific immune secretory response against these microorganisms.

Methodology

The study was based on Santos *et al.*[11] and was approved by the Local Research Ethics Committee according to the Declaration of Helsinki. Healthy young individuals were selected, excluding those who had used antibiotics for a period of 3 months, who were smokers, or who wore oral prosthesis or orthodontic devices. All participants were fully informed concerning the objectives and methodology of the study and provided written free and informed consent.

Initially, 112 individuals were analyzed and saliva samples were collected without stimulation. Carriers of Gram-negative, rod-shaped microorganisms in the oral cavity were selected for a second collection 1 month later. Individuals who tested positive both times were instructed to use the probiotic Yakult LB® (Yakult S/A Indústria e Comércio, Lorena, Brazil) (*Lactobacillus casei* at 2×10^7 to 10^9 and *Bifidobacterium breve* at 5×10^7 to 10^9 CFU/mL) daily for 20 days. After this period, another saliva sample was collected from each subject.

All samples were plated in duplicate in MacConkey agar (Oxoid, Lawrence, USA) and incubated at 37 °C for 24-48 h. The number of colony forming units per milliliter of saliva (CFU/mL) was determined. Suggestive colonies from each plate were confirmed by smear tests stained by the Gram technique. Enterobacteria species were identified using the API 20 E system (BioMérieux, Craponne, France).

Levels of anti-enterobacterial IgA were also analyzed by ELISA. Sensitization of ELISA plates was performed using a pool of enterobacteria cells isolated from the oral cavity of participants, which was killed with formaldehyde and then diluted in 0.1 M carbonate-bicarbonate buffer, pH 9.6, at a final concentration of 10^9 cells/mL. The ELISA plates were then incubated with diluted saliva (1:10), followed by anti-human IgA labeled with horseradish peroxidase. The reaction was developed with orthophenylenediamine and H_2O_2 as substrate. Absorbance was measured at 496 nm.

CFU/mL counts and anti-enterobacteria IgA levels before and after use of probiotics were compared by analysis of variance (ANOVA) for paired samples with a 5% level of significance ($p < 0.05$).

Results

From 112 initial saliva samples, 42 (37.5%) were carriers of Gram-negative rod-shaped microorganisms. Of these, 38 (90.5%) tested positive again 1 month after the first collection. Of these, 20 individuals were included in this study after losses due to voluntary withdrawal, lack of compliance, and use of antibiotics.

After taking probiotic for 20 days, 5/20 individuals no longer tested positive for enterobacteria, indicating a 25% reduction in the prevalence of enterobacteria in the oral cavity. A reduction in enterobacterial counts was also observed in most of the subjects (Figure 1). However, there was no significant difference between the mean log CFU/mL before and after probiotic consumption ($p = 0.3457$) (Figure 2).

Eight species of enterobacteria were identified before probiotic consumption, whereas six were identified after. The most frequently isolated species, both before and after consumption, was *Enterobacter cloacae*, followed by *Klebsiella oxytoca* (Table 1). The use of probiotic did not significantly change the amounts of either species. The species isolated from each individual before and after probiotic consumption are shown in Table 2. *E. sakazaki*, *Pantoa* spp., *E. amnigenus*, and *Serratia odorifera 1* were found only before consumption; *S. marcescens* and *Citrobacter freundii* were found only after consumption.
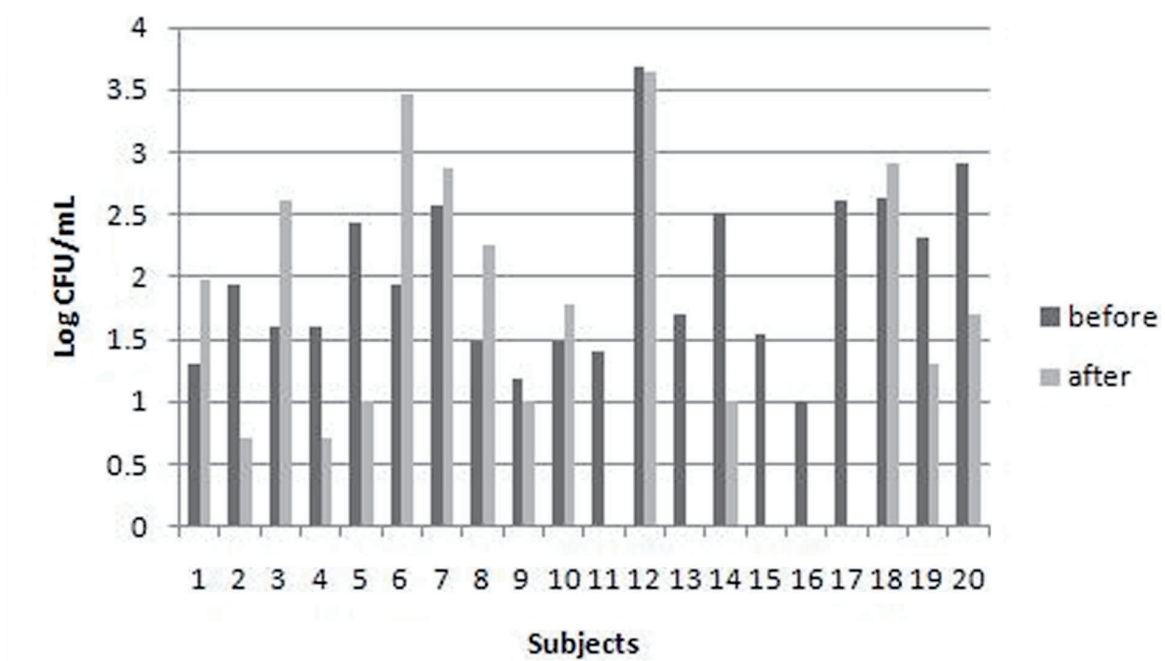
Figure 1 - Comparison between mean log CFU/mL in saliva before and after probiotic consumption for each individual subject.
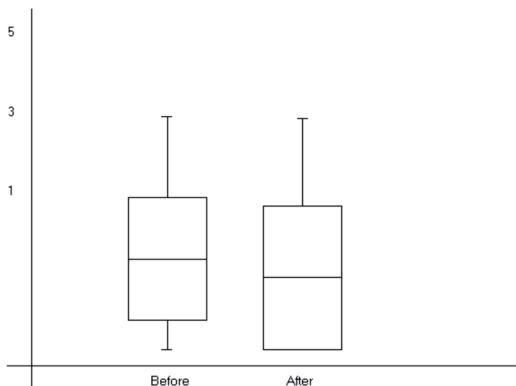

Figure 2 - Mean log CFU/mL of enterobacteria isolated from the oral cavity before and after probiotics ($p = 0.3457$).

Figure 3 - Mean optical density (OD_{496}) corresponding to levels of anti-enterobacteria IgA in saliva before and after probiotics ($p = 0.1215$).

Immunological analysis showed no significant changes in the level of anti-enterobacteria IgA ($p = 0.1215$) (Figure 3) and there was no correlation between IgA levels and CFU/mL counts (data not shown).

Discussion

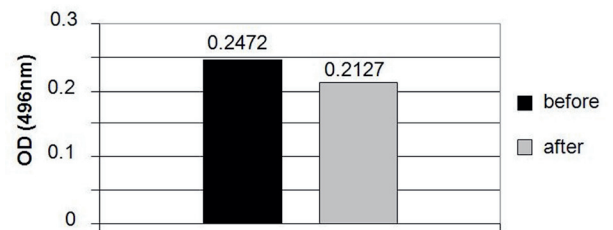Enterobacteria are considered part of the transient microbiota of the oral cavity, but colonization can persist for years and may favor transmission of these microorganisms.[10] Buehlmann *et al.*[10] studied a decolonization regimen of antibiotics or mouth rinse with 0.2% chlorhexidine for eradication of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL) in infected or colonized patients. They observed that 76% of patients became negative for ESBL at follow-up, suggesting that the program could be beneficial in a select group of patients, potentially shortening duration of ESBL colonization and subsequently reducing the risk for transmission. Some *in vitro* studies have demonstrated that probiotics present antimicrobial activity against some bacteria of the Enterobacteriaceae family and can prevent their adhesion to epithelial cells.[12,13]

Table 1 - Log CFU/mL of *E. cloacae* and *K. oxytoca* before and after probiotic consumption

	E. cloacae		K. oxytoca	
	Before	After	Before	After
	0.0000	1.7782	1.3010	1.5441
	1.6021	0.6990	1.6021	2.5250
	2.4393	1.0000	1.9294	2.8451
	2.5740	2.8603	1.3010	0.0000
	0.0000	3.3324	1.3979	0.0000
	0.0000	2.0414	1.6990	0.0000
	1.1761	0.6990	1.9031	1.0000
	1.1761	1.7782	1.0000	0.0000
	3.6812	3.6435	1.3979	1.0000
	2.6233	2.9031		
	2.9031	1.6990		
Mean	1.6523	2.0395	1.5035	0.9905
St dev	1.2938	1.0291	0.3055	1.1195
p value	0.2664		0.2268	

Table 2 - Species and abundance of enterobacteria isolated from the oral cavity of each healthy individual before and after probiotic consumption.

Individual	Species		Log CFU/mL	
	Before	After	Before	After
7	–	E. cloacae	0.000	1.778
	K. oxytoca	K. oxytoca	1.301	1.544
36	E. aerogenes	E. aerogenes	1.929	0.699
44	K. oxytoca	K. oxytoca	1.602	2.525
	–	C. freundii	0.000	1.813
50	E. cloacae	E. cloacae	1.602	0.699
72	E. cloacae	E. cloacae	2.439	1.000
73	K. oxytoca	K. oxytoca	1.929	2.845
	–	E. cloacae	0.000	3.332
74	E. cloacae	E. cloacae	2.574	2.860
75	E. sakazakii	–	1.000	0.000
	–	E. cloacae	0.000	2.041
	–	S. marcescens	0.000	1.845
	K. oxytoca	–	1.301	0.000
81	E. cloacae	E. cloacae	1.176	0.699
	–	S. liquefaciens	0.000	0.699
90	E. cloacae	E. cloacae	1.176	1.778
	Pantoa spp. 2	–	1.176	0.000
96	K. oxytoca	–	1.398	0.000
97	E. cloacae	E. cloacae	3.681	3.643
98	K. oxytoca	–	1.699	0.000
100	K. oxytoca	K. oxytoca	1.903	1.000
	S. liquefaciens	–	2.398	0.000
101	Pantoa spp. 2	–	1.301	0.000
	S. odorifera 1	–	1.176	0.000
102	K. oxytoca	–	1.000	0.000
108	S. liquefaciens	–	2.602	0.000
109	E. cloacae	E. cloacae	2.623	2.903
110	E. sakazakii	–	1.813	0.000
	–	E. aerogenes	0.000	1.000
	K. oxytoca	K. oxytoca	1.398	1.000
	E. amnigenus	–	2.079	0.000
112	E. cloacae	E. cloacae	2.903	1.699
Mean			1.430	1.133

The present work showed that probiotic consumption was able to reduce the number of people carrying enterobacteria in the oral cavity by 25%, suggesting a possible role for the product in decolonization. This phenomenon could be useful in hospital environments to reduce the prevalence of these microorganisms among medical professionals and consequently reduce transmission, since the infections caused by enterobacteria are usually difficult to treat.[10]

Although 25% of tested subjects no longer carried enterobacteria, others showed an increase in counts, so that no significant change was seen in the mean enterobacteria counts before and after probiotics. Brown *et al.*[14] also observed no significant changes in *E. coli*, *Klebsiella*, and *Enterobacter* counts in feces of healthy adults who consumed food containing probiotic bacteria on a daily basis. Thus, a mutually beneficial relationship between the probiotic species and some enterobacteria species cannot be ruled out. Moreover, there is a possibility that probiotic consumption favors the adherence of enterobacteria in the oral cavity of non-carrier individuals, who were not studied, possibly resulting in no net change in prevalence.

Probiotic consumption also had no influence on the amount of each species or the profile of species identified in individuals. The most frequently isolated species before and after probiotics was *E. cloa-*

cae, followed by *K. oxytoca*. The predominance of these species in the oral cavity has already been related,[15] and some authors suggest that the fimbria is an important virulence factor, being responsible for bacterial adherence and persistence.[16]

Although many studies have shown that probiotics can improve cellular and humoral immune responses,[17,18] significant effects of probiotics on the level of secretory antibodies against enterobacteria have not been demonstrated. Previously, only total IgA levels have been shown to be influenced by probiotic consumption. Revolledo *et al.*[17] observed an increase in total IgA levels in serum and intestinal fluid of chickens that received probiotic supplements, and Lara-Villoslada *et al.*[18] observed an increase in total IgA in feces and saliva of children who used products with *Lactobacillus coryniformis* CECT5711 and *L. gasseri* CECT5714.

The presence of antigens in the mucosa induces production of immunoglobulins that interfere with adhesion and facilitate the elimination of pathogens. Thus, immunoglobulin levels increase with antigenic stimulation and decrease after elimination of microorganisms. Although enterobacteria are considered part of the transient microbiota of the oral cavity, these microorganisms are persistent in the intestinal mucosa, promoting an uninterrupted stimulus. It was therefore expected that the specific secretory response could not have changed.

In summary, probiotic consumption may be capable of influencing the prevalence of enterobacteria in the oral cavity. It is possible that probiotics have no effect when there is a balance between enterobacteria and the resident microbiota and the host; however, when the balance is disrupted and the enterobacteria become pathogenic, the consumption of probiotics may induce evident beneficial effects, as other works have shown.[7,9]

Conclusion

Probiotic consumption reduced the prevalence of enterobacteria in the oral cavity, but did not interfere with their amounts or with the specific secretory immune response against them.

Acknowledgements

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