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The use of *Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289 on oral health indexes in a school population: A pilot randomized clinical trial

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Abstract

To assess the effects of a probiotic upon oral health indices in adolescents and to establish relationships between these indices and dietary habits and oral hygiene. Twenty-seven adolescents between 12 and 18 years of age were randomized into two groups. The study group received tablets containing *Lactobacillus reuteri DSM 17938/ATCC 5289* for 28 days, while the control group received tablets without any bacteria. *Streptococcus mutans, Lactobacillus* sp., and salivary pH were assessed at baseline and at 7, 14, 21, 28, and 45 days. The plaque, gingivitis, and bleeding indices were recorded at baseline and at 14, 28, and 45 days. Dietary and oral hygiene habits were also evaluated by means of a questionnaire. A less marked rise in *S. mutans* was recorded in the study group. Improvements were observed in terms of plaque, gingivitis, and bleeding, though statistical significance was not reached. Oral pH increased in the study group, though not to a significant degree. Poorer eating habits were significantly correlated to increased plaque. The study parameters decreased with the two strains of *L. reuteri DSM 17938* and *ATCC PTA 5289*, though the results failed to reach statistical.

Keywords

dental caries, lactobacilli, oral health, probiotics

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Introduction

In 2002, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations defined probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host."[1,2]

Although the mechanisms of action of probiotics are not fully known, it has been suggested that they are able to maintain their viability after coming into contact with gastric acids—reaching and adhering to the intestinal mucosa to prevent

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cellular adhesion and invasion of pathogenic microorganisms, with modulation of the immune response.[2,3]

Probiotics are potentially useful in the management of multifactorial diseases such as periodontal disease and caries. Dental caries and periodontal disease are disorders that can be reverted by eliminating the underlying causal factors. The adoption of preventive measures plays a crucial role in combating these two diseases. Research in recent years has focused on controlling pathogenic oral microorganisms through bacteriotherapy with the use of probiotics.[4–6]

Dental caries is a multifactorial disease involving the interaction of four main factors: the bacterial flora (composed mainly of microorganisms that produce organic acids), the host (the quality and shape of the tooth, characteristics of saliva, oral hygiene, type of diet, and ability to develop an immune response at both systemic and local level), substrates for microorganisms (such as carbohydrates contained in secretions and foods), and time (relatively long for decalcification and short for the remineralization of hard tissues). For this reason, dentists should seek to promote good eating and hygiene habits from an early age.[7–10]

The role of probiotics in the prevention of dental caries was first suggested in 1985, and the recommendations for using them are strain-specific and mostly comprise *Lactobacillus* and *Bifidobacterium spp.*[11,12]

In the last decade, further experimental studies have been carried out on the use of probiotic strains for the prevention of dental caries and periodontal diseases.[3,6]

Ten years ago the role played by probiotics in periodontal disease was not known. Efforts have primarily focused on the effect of probiotics upon clinical parameters (plaque, gingival, and bleeding indices) and their interference with the periodontal microbiota.[13,14]

In this regard, the use of probiotics has been postulated to play an important preventive and/or therapeutic role, and may constitute a nonsurgical alternative in the management of caries and periodontal disease.[15,16]

The present study was carried out to evaluate the effects of a probiotic upon oral health indices in a population of adolescents and to establish possible relationships between these indices and dietary habits and oral hygiene.

Materials and methods

Study population

A double-blind, placebo-controlled randomized design was used. Adolescents belonged to Escolapias school in Gandía (Spain) between 12 and 18 years of age were enrolled for 45 days. In the reviewed bibliography it has been seen that after 3 months the probiotic appears to no longer remain in the oral cavity.[2] We wanted to limit the time further and evaluate if there were probiotic traces after 45 days, that was the reason why the study lasted 45 days.

The exclusion criteria were: (i) subjects under 12 years of age or over 18 years of age; (ii) the use of drugs capable of causing salivary alterations; (iii) subjects with braces; (iv) topical fluoride treatment in the last month; (v) regular consumption of probiotics or xylitol chewing gum; (vi) systemic antibiotic medication in the last 4 weeks; (vii) active or untreated dental caries; and (viii) subjects with gait disturbances and/or unidentified syndromes.

The study was conducted in accordance with the recommendations of the Declaration of Helsinki, and was approved by the local Ethics Committee (registration number: CEI18/067). The adolescents' legal guardians signed written informed consent form to participation in the study. Before the study, the subjects had their teeth cleaned by a dentist and were given detailed instructions concerning oral hygiene and the need to brush their teeth twice a day throughout the study. They also received a questionnaire addressing issues related to dietary and oral hygiene habits.

Sample calculation

In relation to the plaque index, a beneficial change associated with the probiotic of at least 0.33 points was expected (equivalent to 20% of the observed range). A variability in the scale of 0.43 points (estimated standard deviation in a pilot group of 10 children) was assumed, with a power of 80% (beta error 20%) and a confidence level of 95% (alpha error 5%). In relation to *S. mutans* levels a beneficial change associated with the probiotic of at least half a point on the logarithmic scale was expected (equivalent to a change in the linear scale of between 10,000 and 100,000 or between 100,000 and 10,000) with a variability of 0.6 points (standard deviation on the logarithmic scale), with a power of 80% (beta

error 20%) and a confidence level of 95% (alpha error 5%). Adopting a conservative approach, it therefore would suffice to recruit 27 patients in order to detect relevant changes in both indicators.

The participants were chosen randomly, dividing them into two groups:

- A: Experimental group. Each study participant was given a bottle of 28 probiotic tablets.
- B: Control group. Each study participant was given a bottle of 28 placebo tablets.

Each child entered into each group randomly.

- Group of children belonging to the study group: red color.
- Group of children belonging to the control group: blue color.

Randomization was performed following the two basic principles of equiprobabilistic allocation of each subject to one of the two treatments and concealment of random allocation.

The equiprobabilistic allocation of each unit of analysis is assigned using an allocation method based on the alphabetical order of the children's surnames (from A to M one group and from N to Z the other). Both groups were created and drawn by an external researcher through a random process (generation of an odd/even number to assign to one of the two groups).

The researcher did not know to which group each subject would be assigned when asked for informed consent, thus ensuring the concealment of the assignment sequence.

Intervention

The probiotic tablets (BioGaia AB, Lund, Sweden) contained two strains *of Lactobacillus reuteri* (*DSM 17938* and *ATCC PTA 5289*) at a dose of 1×10^8 CFU/tablet, respectively. The placebo tablet was identical in size and composition but contained no probiotic strains. Both types of tablets were provided by BioGaia AB (Lund, Sweden). The two groups were differentiated by a color code. All clinical recordings, as well as the laboratory tests, were carried out without knowledge of the group to which the subjects were assigned. The tablets were packed in identical containers carrying the name of the subject. The tutors of each course were responsible for safekeeping the containers in the staff room of the school. In the morning, the tutor delivered a tablet to each student before the break (11:00 a.m.). On Friday the tutor gave each participant a bag with two tablets for the weekend. This procedure was repeated during the 28 days of the study. Participants failing to take a tablet were withdrawn from the study.

Evaluation of microbial, salivary pH, and periodontal indices

Samples of paraffin-stimulated whole saliva were obtained immediately before (baseline) and at 14, 28 and 45 days (follow-up) to assess salivary pH, *S. mutans* and *Lactobacillus* sp., *Streptococcus mutans* and *Lactobacillus sp.* were evaluated using Dentocult® SM (Orion®) and Dentocult® LB (Orion®), in accordance with the instructions of the manufacturer.[17]

The collection of saliva samples and the measurement of the indices and salivary pH were carried out between 9 and 11 a.m. in classrooms prepared previously with the material on the table. A single operator was responsible for analyzing all the samples obtained, with the collaboration of the students of the Master in Pediatric Dentistry of Universidad Cardenal Herrera-CEU (Valencia, Spain) and duly trained school staff—controlling that the process was carried in the most exact way possible.

The plaque, gingivitis, and bleeding indices were assessed under baseline conditions and at 7, 14, 21, 28, and 45 days. Dietary and oral hygiene habits were also evaluated by means of a questionnaire. For its validation, the questionnaire was handed to five pediatric dentistry professors at CEU-Cardenal Herrera University. They were asked to complete it and indicate whether they considered if any of the proposed questions were difficult to understand, inappropriate, ambiguous, or inadequate. Finally, they were asked to make any suggestions they might have in order to improve the questionnaire.

Once the questionnaire had been corrected with the contributions of the specialists, it was examined by a statistician in order to achieve an optimal data collection that would allow a correct statistical analysis of the data.

After the modifications of the statistician, a pilot test was carried out by giving the questionnaire to five parents, who subsequently did not participate in

the study, to check that the questions were properly understood and could be answered appropriately. At the end of the pilot test, the final changes were made to the wording of the questionnaire, thus concluding its validation.

For measurement of the plaque index, we chose the qualitative Silness and Löe index,[18] which allows recording of the different degrees of intensity of bacterial plaque accumulation. The Ramfjord index teeth was considered for the measurements: 16–21–24–36–41–44. A periodontal probe was passed over these teeth, on the four faces of each tooth (buccal, lingual/palatine, mesial, and distal), with evaluation according to the amount of plaque present.

The Silness and Löe index[18] was also used to measure the gingival index, likewise based on the Ramfjord index teeth: 16–21–24–36–41–44. The procedure was the same as in the case of the plaque index, and the values were recorded according to the degree of inflammation observed.

Lastly, we evaluated the simplified bleeding rate,[18] likewise based on the Ramfjord index teeth.

Statistical analysis

The data were processed with the SPSS® version 18.0 statistical package (Chicago, IL, USA) and subjected to one-way analysis of covariance (ANCOVA). Comparisons of qualitative variables were made using a two-tailed chi-squared test. Statistical significance was considered for $P < 0.05$.

Results

A total of 12 participants in the experimental group and 15 in the control group participants completed the entire study period because 3 children did not attend any of the scheduled check-ups (Figure 1).

Microbial evaluation

The baseline and follow-up levels of salivary *S. mutans and Lactobacillus sp.* are reported in Table 1. A beneficial effect of the probiotic upon *S. mutans* collected in saliva was observed. The probiotic was associated with maintenance of the levels of *Lactobacillus sp*., though no statistically significant results were observed on comparing the two groups ($P > 0.05$).

Salivary pH and periodontal indices

An increase in salivary pH was observed in the probiotic group, though with no statistically significant difference between the two groups (Table 1). The data corresponding to the plaque, gingival, and bleeding indices are reported in Table 2. The effect of probiotic use upon the plaque and gingival indices was found to be beneficial in comparison with the group of adolescents administered placebo tablets—though statistical significance was not reached ($P > 0.05$). With regard to the bleeding index, the results were nonsignificant, since the effect of probiotic use upon this index was variable. As in the case of the gingival index, there was a clearly significant rebound effect.

Oral health indices

The relationship between the oral health indices and dietary and oral hygiene habits is presented in Table 3. Poorer eating habits and oral hygiene were significantly correlated to increased plaque ($P < 0.05$). There were no significant differences in the age of the subjects with regard to eating and hygiene habits, except in the frequency of brushing after meals. In effect, those under 14 years of age brushed their teeth less frequently than those over 14 years of age, with higher levels of bleeding gums.

Discussion

In recent years, research on the use of probiotics for the prevention of dental and periodontal diseases has progressed considerably, with advances in the characterization of specific probiotic strains, and of the quantities and frequency of administration needed to secure beneficial effects in certain oral disorders.[12,14] In our series no statistically significant variations were found in the gingival parameters between the study group and the control group, in coincidence with the observations of Sinkiewicz et al.,[19] and Iniesta et al.[20] It should be noted that the combination of strains used differed between our study and that of Sinkiewicz et al.[19] In effect, Sinkiewicz et al.,[19] used *L. reuteri ATCC 55730* and *ATCC PTA 5289*. Iniesta et al.,[20] in turn used the same strains as in our study (*L. reuteri ATCC PTA 5289* and *DSM 17938*), though with methodological differences versus our own series. Shimauchi et al.,[21] and Mayanagi et al.,[22] studied the effects of *L. salivarius* on periodontal parameters such as

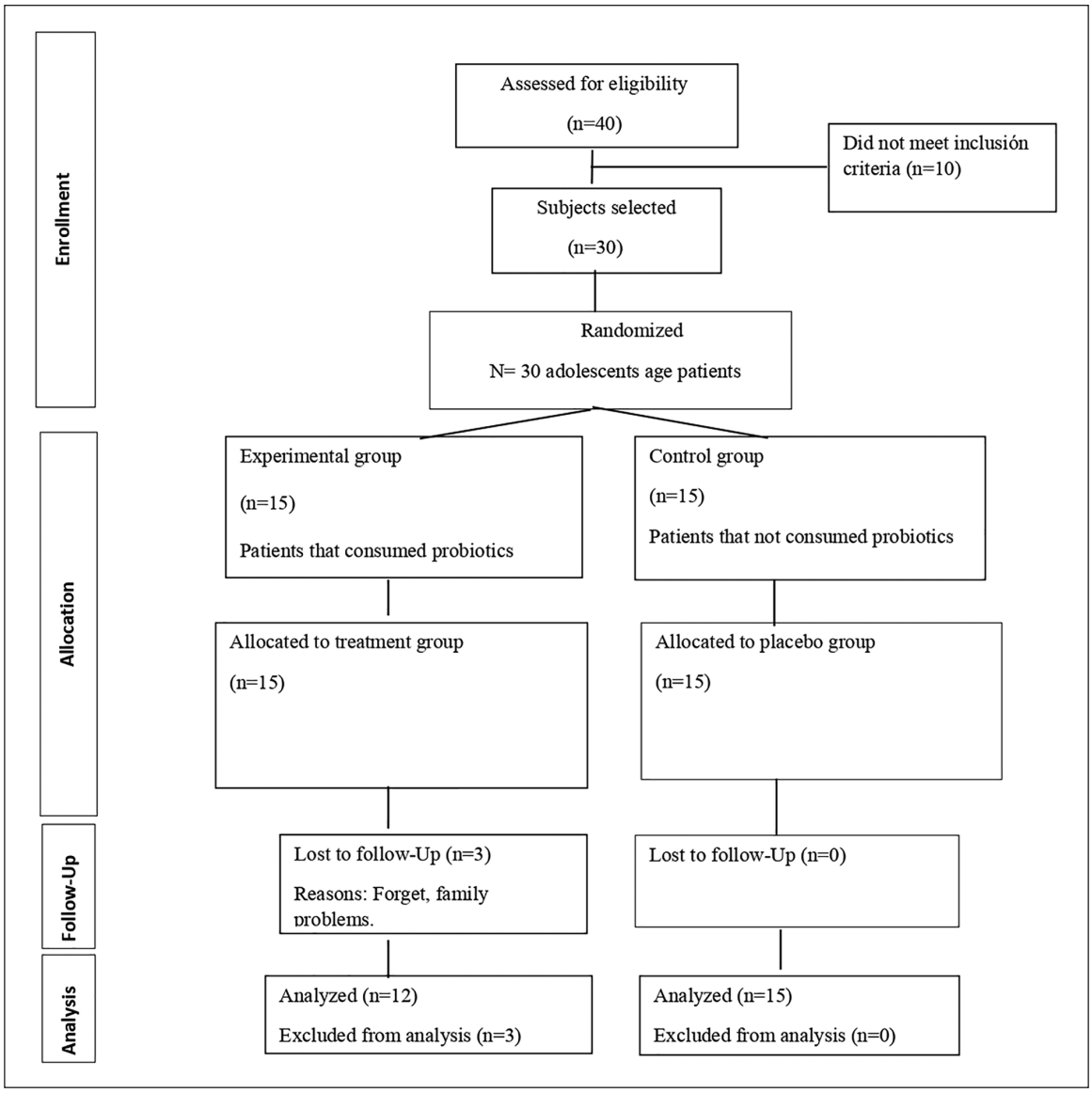

Figure 1. Flowchart of the study, indicating the methodological stages (left) and characteristics of the study groups and their follow-up (right).

probing depth, gingival index, bleeding index, and plaque index. In both studies, improvement in periodontal health was observed among smokers. Similar results have been reported by Krasse et al.,[23] and Vivekananda et al.,[24] where the use of *L. reuteri* also resulted in improvement of the aforementioned parameters in a population of smokers. However, it must be noted that these studies differed from our own in terms of the age of the subjects.

It is not clear how long probiotics remain in saliva once their administration ceases.[2,25] Yli-Knuuttila et al.,[26] observed that after administering the probiotic *LGG* for 14 days, the counts in the oral cavity gradually decreased—indicating that there is no permanent colonization, and that the oral persistence of *LGG* is only temporary. Caglar et al.,[25] likewise showed that the presence of *L. reuteri* in saliva is only temporary and disappears once administration of the probiotic is suspended. In our series, a probiotic tablet prepared with a combination of strains of *L. reuteri DSM 17936* and *ATCC PTA 5289* was used in the study group. Caglar et al.[27,28] used the probiotic *L. reuteri ATCC 55730* in some of their investigations. In one of these studies,[28] they evaluated the effect of chewing gum containing xylitol and probiotic on the salivary counts of *S. mutans* and *Lactobacillus sp*. A statistically significant decrease in *S. mutans*

Table 1. Analysis of the longitudinal effect of the use of probiotics upon the levels of *S. mutans, Lactobacillus sp*., and salivary pH.

Day of study	*S. mutans*				*Lactobacillus sp.*				Salivary pH			
	Probiotic	Placebo	Effect (SEM)	*P*	Probiotic	Placebo	Effect (SEM)	*P*	Probiotic	Placebo	Effect (SEM)	*P*
1	1.39 ± 0.064	1.21 ± 0.80	–		3.70 ± 0.48	3.38 ± 0.47	–		7.23 ± 0.17	7.25 ± 0.21	–	
14	1.42 ± 1.18	1.59 ± 0.91	−0.20 (0.291)	0.41	3.48 ± 0.95	3.36 ± 0.66	0.013 (0.237)	0.96	7.27 ± 0.23	7.35 ± 0.25	−0.040 (0.038)	0.29
28	1.50 ± 0.65	1.63 ± 0.81	−0.059 (0.239)	0.39	3.29 ± 0.47	3.19 ± 0.40	0.091 (0.162)	0.58	7.39 ± 0.14	7.39 ± 0.14	−0.026 (0.056)	0.64
45	1.75 ± 0.75	2.00 ± 0.93	−0.188 (0.309)	0.55	3.17 ± 0.39	3.07 ± 0.26	0.099 (0.127)	0.44	7.28 ± 0.16	7.28 ± 0.16	0.061 (0.060)	0.31

Effect: difference between both groups estimated on the baseline level of the indicator; SEM: standard error of the mean.

Table 2. Analysis of the longitudinal effect of the use of probiotics upon the plaque, gingivitis, and bleeding indices.

Day of study	Plaque index				Gingivitis index				Bleeding index			
	Probiotic	Placebo	Effect (SEM)	*P*	Probiotic	Placebo	Effect (SEM)	*P*	Probiotic	Placebo	Effect (SEM)	*P*
1	0.67 ± 0.44	0.67 ± 0.43	–		0.52 ± 0.36	0.36 ± 0.33	–		0.04 ± 0.05	0.05 ± 0.07	–	
7	0.70 ± 0.29	0.74 ± 0.30	−0.035 (0.071)	0.62	0.78 ± 0.33	0.74 ± 0.40	−0.046 (0.084)	0.59	0.068 ± 0.088	0.087 ± 0.12	−0.013 (0.025)	0.61
14	0.78 ± 0.39	0.68 ± 0.35	0.114 (0.096)	0.24	0.84 ± 0.34	0.71 ± 0.32	0.058 (0.100)	0.56	0.102 ± 0.096	0.091 ± 0.99	0.022 (0.027)	0.41
21	0.63 ± 0.35	0.73 ± 0.34	−0.070 (0.100)	0.49	1.01 ± 0.31	0.98 ± 0.31	−0.026 (0.111)	0.82	0.067 ± 0.089	0.085 ± 0.091	−0.011 (0.032)	0.74
28	0.86 ± 0.36	0.80 ± 0.26	0.035 (0.106)	0.74	1.12 ± 0.29	1.09 ± 0.26	−0.061 (0.099)	0.54	0.085 ± 0.099	0.063 ± 0.077	0.028 (0.032)	0.39
45	0.80 ± 0.32	0.71 ± 0.27	0.067 (0.103)	0.52	1.45 ± 0.26	1.28 ± 0.39	0.065 (0.139)	0.64	0.255 ± 0.149	0.157 ± 0.126	0.105 (0.54)	0.06

Effect: difference between both groups estimated on the baseline level of the indicator; SEM: standard error of the mean.

Table 3. Nonparametric correlations between the scores obtained in the study and the oral health indicators.

	Total score	Diet score	Hygiene score
Plaque index	0.384*	0.283	0.301
Gingivitis index	0.076	0.214	−0.260
Bleeding index	−0.061	−0.061	−0.165
Salivary pH	0.152	0.173	0.235
S. mutans	0.244	0.200	0.043
Lactobacillus sp. log10	−0.261	−0.287	−0.036

*$P < 0.05$.

levels was recorded. However, there were no changes in the salivary counts of *Lactobacillus sp.* In another study by these same authors,[27] the probiotics were administered in two different vehicles: one consisted of a straw containing the probiotic while the other was a tablet containing the probiotic. In this study a statistically significant decrease in *S. mutans* levels was likewise observed. A similar but nonsignificant trend was observed for *Lactobacillus sp.* In coincidence with our own findings, it can be concluded from these studies[27,28] that probiotic use results in a beneficial effect in terms of the salivary counts of *S. mutans*, though in the case of *Lactobacillus sp.* the results vary, and the levels did not always tend to decrease as they did in our series.

Finally, we analyzed the relationship between the oral health indexes and dietary and oral hygiene habits recorded by means of a questionnaire, as well as the relationship between dietary and oral hygiene habits and subject age and sex. Poorer eating habits and oral hygiene were significantly correlated to increased plaque, in coincidence with the observations of Tinanoff et al.[29,30] In conclusion, the study parameters were seen to decrease with the two strains of *L. reuteri DSM 17938* and *ATCC PTA 5289*, though the results failed to reach statistical significance- possibly because of the small sample size involved.

Conclusions

The administration of a daily tablet of two strains of *L. reuteri (DSM 17938* and *ATCC PTA 5289)* for 28 days had an effect on the decrease of the study parameters. Further studies must be carried out to learn more about the sustained action of probiotic bacteria upon the oral cavity, as well as their colonization and biofilm formation capacity, in order to elucidate how they affect the resident flora.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study was conducted in accordance with the recommendations of the Declaration of Helsinki, and was approved by the local Ethics Committee (registration number: CEI18/067).

Informed consent

Written informed consent was obtained from legally authorized representatives before the study.

Trial registration

This randomized clinical trial was not registered because being a pilot study this research reported on feasibility of a larger study.

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Supplemental material

Supplemental material for this article is available online.

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