Original Article

THE INFLUENCE OF ORAL PROBIOTICS ON THE AMOUNT OF ORAL BIOFILM FOUND IN SALIVARY FLUID

Dr. Vivek Pawar[1], Dr. Reshu Rastogi[2], Dr. Neelam Makkad[3], Dr. Manish Kumar[4*], Dr. Rupashree Chatterjee[5], Dr. Shikha Sharma[6]

[1] Professor, SMBT Institutes of Dental Sciences and Research, Dhamangoan, Nashik, Maharashtra, India.
Email: vivekpawar27@gmail.com
[2] Department of Oral and Maxillofacial Surgery, Kothiwal Dental College and Research Centre, Moradabad, India.
Email: dr.reshurastogi88@gmail.com
[3] Consultant Oral Medicine Radiologist, Andheri West, Mumbai.
Email: drneelam.odmr@gmail.com
[4] Assistant Professor, Department of Dentistry, Government Medical College & Hospital, Ratlam (M.P).
Email: drmanishagarwalmds@gmail.com
[5] PG Student, Department of Pediatric and Preventive Dentistry, TIDSHRC, Bodri, Bilaspur, Chhattisgarh.
Email: dr.rupashree.chatterjee@gmail.com
[6] Senior lecturer, Department of Conservative Dentistry and Endodontics, Sri Sai College of Dental Surgery, Vikarabad Telangana, India.
Corresponding author: Dr. Manish Kumar, Assistant Professor, Department of Dentistry, Government Medical College & Hospital, Ratlam (M. P).
Email: drmanishagarwalmds@gmail.com

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Abstract

The purpose of this study was to evaluate the impact that oral probiotics containing the Streptococcus salivarius K12 strain had on the amount of salivary secretory immunoglobulin A, the pace at which saliva was produced, and the amount of oral biofilm. In this double-blind, placebo-controlled, two-arm, parallel-group investigation, thirty-one consenting patients who met the inclusion criteria were recruited. These patients were then randomly allocated into two groups: one receiving probiotics (n = 20) and the other receiving a placebo (n = 20). After 4 weeks of intervention and 2 weeks of washout, the unstimulated salivation rate, concentration of salivary secretory immunoglobulin A, Turesky index, and Papillary-Marginal-Attached index were evaluated. Thirty patients were successful in completing all of the study's requirements. When we compared the probiotic group to the placebo group, we did not find any significant differences in salivary flow rates or levels of salivary secretory immunoglobulin A. The salivary secretory immunoglobulin A concentrations (mg/L) for the probiotic group at baseline and at the end of the study were 230 and 190, respectively, whereas the values for the placebo group were 210 and 180, respectively. At both 4 and 6 weeks, the probiotic group showed a statistically significant reduction in the amount of plaque that had accumulated. Within the confines of the present study, it is possible to draw the conclusion that the consumption of probiotics (Streptococcus salivarius K12) does not influence salivation rates or levels of secretory immunoglobulin A in the saliva, but that it does have a beneficial impact on the accumulation of plaque.

Keywords: Probiotics, Streptococcus salivarius K12, dental biofilm, salivary secretory immunoglobulins A, and unstimulated salivary flow rate.

Introduction

The mouth cavity is home to the microbiome that is the second most abundant in the human body. This community of microorganisms, which is home to approximately 700 different species [1,] is founded not only on the interactions of microbes with the environment of the host, but also on the interactions of microorganisms with one another [2, 3]. A healthy oral cavity is distinguished by a dynamic equilibrium between commensal bacteria, which are non-infectious, and opportunistic microorganisms, which are cariogenic. This balance may be thrown off by eating a diet heavy in carbohydrates, practicing poor dental hygiene, taking certain drugs, or suffering from a systemic ailment [3]. Dental caries are the most common non-communicable disease in the world [4], and they are primarily caused by an imbalance in the oral microbiome, also known as a predominance of cariogenic microorganisms [5]. These cariogenic microorganisms include different types of streptococci and lactobacilli, actinomyces, bacteroides, and bifidobacteria. In this context, one of the potential approaches to preventing dental caries is to switch out the cariogenic germs that cause cavities with commensal bacteria. Oral biotics and probiotics are two examples of medications (supplements) that may be used to accomplish this goal [6]. These medications help to restore the natural balance of the microflora in the gut.

According to the World Health Organization (WHO), probiotics are defined as living bacteria that, when given to a host in sufficient quantities, are able to improve that host's overall health [7]. The Russian Nobel laureate Élie Metchnikoff [8] was the first person to propose the notion that the consumption of certain microbes would have the potential to be helpful to the gastrointestinal system. Since that time, several studies have shown the efficacy of probiotics in the treatment and prevention of gastrointestinal [9], allergy [10], and respiratory [11,12] disorders. [9,10] [11,12]

One of the first investigations into the use of a variety of microbes for dental reasons was carried out by Miller and colleagues [13]. In later years, a number of studies demonstrated that consumption of probiotics led to a reduction in the amount of oral pathogens. In instance, a significant reduction in the numbers of Streptococcus mutans and Candida albicans has been seen in a number of investigations [6,14,15,16,17]. It is anticipated that the oral microflora will change into one that is more diverse, with commensals taking the lead as the dominant species. It is generally believed that cariogenic microbes and probiotic bacteria compete for adhesion sites or dietary substrates, and that probiotic bacteria impact immunological processes by secreting antimicrobial compounds [18]. Consequently, taking probiotics may lead not only to a decrease in particular infections but also to changes in the overall makeup of the oral microbiome [19,20].

In order for caries to develop, it is necessary for there to be adequate contact time between the microbe and the sensitive tissues [21]. This is in addition to the existence of cariogenic bacteria and nutritional substrates, which are meals that are rich in carbohydrates. Increased salivary flow rate [22] brought about by the use of probiotics decreases the amount of time that microbes spend in touch with the tooth surface, which in turn lowers the risk of dental caries.

In addition, studies have observed that the administration of probiotics might potentially raise the levels of secretory immunoglobulin A (sIgA) in the saliva [23,24,25]. Immunoglobulins reduce the ability of microbes to cling to surfaces, protect the host from the absorption of antigens from mucosal surfaces, decrease the effects of inflammation, increase phagocytosis, and neutralize the effects of microbial toxins and invasive pathogens [26]. [27] Research has shown that one of the primary immunoglobulins found in saliva, known as secretory immunoglobulin A, plays a significant part in the fight against dental caries. It is possible to postulate that the modification of the oral microbiota, increase in salivary flow rate, and increase in secretion of sIgA brought about by probiotics might bring about a decrease in the rate at which plaque is formed [15].

Lactobacillus casei, Lactobacillus paracasei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus gasseri, Lactobacillus reuteri, Bifidobacterium bifidum, Bifidobacterium infantis, and Bifidobacterium subtilis are some of the most common types of probiotics used in dentistry [28]. Other types of probiotic However, these strains often fail to colonize oral tissues, which is why a new generation of streptococci-containing probiotic strains has recently been produced. These strains are more likely to be effective in colonization oral tissues.

There are several different types of streptococci that may be found in the oral microbiome [29], including mitis, sanguinis, anginosus, salivarius, downei, mutans, pyogenic, and bovis streptococci. The bacteria known as Streptococcus salivarius is of special significance and is the focus of a great deal of study because it plays a significant part in maintaining a healthy equilibrium among the microbial communities that reside in the gastrointestinal tract [30]. It is one among the first bacteria to colonize oral mucosa in the first few days after birth [31], which is when a newborn's mouth is most susceptible to infection. It has been shown that this significant commensal may prevent the development of key cariogenic streptococci (Streptococcus mutans and Streptococcus sobrinus) by inhibiting their ability to colonize tooth sites during the earliest stages of oral colonization [32,33]. Additionally, S. salivarius was isolated from the dental plaque biofilm of a kid who did not have dental caries and had healthy oral tissues [34]. Miller et al. demonstrated a reduction in plaque formation when S. mutans, the main cariogenic microorganism, was co-cultured with S. salivarius, Streptococcus faecalis, or L. casei compared to pure cultures of S. mutans [13]. This reduction in plaque formation was observed when comparing pure cultures of S. mutans to co-cultures of S. mutans with these other microorganisms. S. salivarius generates bacteriocins, prevents Aggregatibacter actinomycetemcomitans from colonizing the epithelium, and defends against the invasion of Candida albicans by reducing adhesion via processes that are independent of its antimicrobial action [33]. It has also been proven that this microbe has the ability to influence the immune response by suppressing the inflammatory pathways that are triggered by certain infections [33]. S. salivarius may be effectively utilized to prevent and cure ENT (ear, nose, and throat) inflammatory illnesses [35], halitosis [36], candidiasis [37], and dental caries [38,39,40] due to the qualities that it has. [35] K12 and M18 are the two strains of S. salivarius that contain probiotics that have the highest potential, according to [31].

In spite of the enormous number of research that have been conducted, there is still no general agreement about the manner in which probiotics alter indices of oral health. In addition, there is a dearth of published research on the use of S. salivarius (especially the K12 strain) as a dental probiotic. Our research is designed to investigate the impact that oral probiotics containing the Streptococcus salivarius K12 strain have on the amount of salivary secretory immunoglobulin A (sIgA), the pace at which saliva is produced, and the amount of oral biofilm.

Materials and methods:

This clinical investigation was granted permission to proceed by the Local Ethics Committee. This study did not receive any money from outside sources. The experiment's design was based on the updated Helsinki's code for human clinical research (2013) and the CONSORT 2010 recommendations for reporting randomized clinical trials. Both sets of guidelines were used to determine how the trial should be conducted.

The purpose of this research was to investigate the impact that oral probiotics containing Streptococcus salivarius K12 have on oral biofilm, the pace at which saliva is produced, and the amount of secretory immunoglobulin A (sIgA) that is present in the saliva. Between the months of April 2022 and August 2022, a randomized, two-arm, parallel-group trial with double blinding was carried out.

General itineraries and a breakdown of the study program

Patients who went to the Dental Institute were asked to take part in the research and given the opportunity to do so. One of the authors of the research recruited thirty-one healthy adult volunteers between the ages of 20 and 24 and then allocated them to one of the therapies (DS). All of the patients were required to provide their written informed permission before they could take part in the study or have their data published for the purposes of research and education. It was suggested to the patients that they wash their teeth twice day with a pea-sized quantity of toothpaste that did not include any antibacterial or antiplaque components using the standardized approach known as the Bass technique.

The Criteria for Inclusion

• a dentition that is permanent; • the presence of more than 20 teeth; • the absence of systemic and chronic disorders.

The Criteria for Exclusion

• Having more than five cavities that need to be treated; • Refusing to sign an informed consent form; • Taking supplements or lozenges containing probiotics or prebiotics three weeks before the study; • Having taken antibiotics in the month prior to the study; • Having orthodontic and prosthetic treatment; • Having an allergy to the components of the drugs that were used in the study; • Having used other hygiene products, immunostimulants and antibacterials, probiotics, or prebiotics

Random assignment to one of the following research groups was performed for participants who fulfilled all inclusion criteria and none of the exclusion criteria: Lozenges were given to Group 1 that included a probiotic (Streptococcus salivarius K12), whereas Lozenges were given to Group 2 that were a placebo. The concealment of the allocation was accomplished by using containers with numbers that were assigned by a "third party" (person who did not participate in the study). The lozenges were put in bottles that were not labeled and then placed in the containers. Both the probiotic lozenges and the placebo lozenges had the same flavor, color, texture, and size; the only difference was that the placebo lozenges did not contain any live bacteria. At the time of enrollment, a package of lozenges was given to every volunteer. Neither the people who participated in the study nor the researchers had any idea what kind of lozenges were being used.

All of the subjects took either probiotics or a placebo for a total of four weeks, one lozenge each day (Table 1). The intervention was followed by a washout period of two weeks, during which time the prescription lozenges were not consumed by the participants. This time frame was included in order to determine whether or not the findings obtained were consistent.

Table 1

Group	Dietary Supplement Composition	Intervention
Group 1—probiotic (“Bactoblis”)	*Streptococcus salivarius* K12 (≥1 × 10^9 CFU in 1 tablet), fructose (sweetener), maltodextrin, silicon dioxide, magnesium stearate (vegetable), flavoring (strawberry)	Dissolve the lozenges in the mouth once a day for 4 weeks
Group 2—placebo	Fructose (sweetener), maltodextrin, silicon dioxide, magnesium stearate (vegetable), flavoring (strawberry)	

Both the concentration of secretory immunoglobulin A in saliva and the unstimulated salivary flow rate were considered to be primary outcome measures. The Turesky version of the Quigley-Hein plaque index (TQHPI) and the papillary marginal attached index were used as secondary outcome measures (PMA). The assessments were carried out by a single operator at the beginning of the study as well as after 4 and 6 weeks (DS).

We obtained the TQHPI, PMA, and DMFT (decayed, missing, and filled teeth index) in the same manner as is reported previously [41,42,43]. The salivary concentration of sIgA was measured with the assistance of ELISA and a salivary secretory IgA indirect enzyme immunoassay kit (8668 IgA secretory ELISA-BEST kit, VectorBest, Novosibirsk, Russia), both of which were utilized in accordance with the instructions provided by the manufacturer.

Participants were instructed to abstain from eating, drinking, smoking, and engaging in any oral hygiene activities for at least ninety minutes prior to having their saliva collected for the purpose of measuring unstimulated salivary flow. All of the measurements were done first thing in the morning to account for the diurnal fluctuations that

might occur in saliva production. Participants were instructed to avoid swallowing saliva and asked to lean forward and spit all of the saliva they produced every two minutes into a graduated test tube. After a few minutes of relaxation, participants were instructed to remain seated comfortably and were then trained to avoid swallowing saliva. It was determined how much saliva had been collected during the course of the previous ten minutes. Following is the formula that was used to calculate the flow rate: Salivation rate (ml/min) = saliva volume (mL)/saliva collecting time (min).

This pilot study's sample size was determined by comparing it to the sample sizes of other studies that were conceptually comparable [44,45,46]. Each and every analysis was carried out using the per-protocol population. We performed an analysis on every participant in the study provided that they did not significantly depart from the protocol, which was to be evaluated on an individual basis by the research's principle investigator (KB) just prior to the database being locked.

Depending on the types of variables being analyzed, the data were either displayed as means and standard deviations, confidence intervals with a 95% level of accuracy, medians, 25th and 75th percentiles, or percentages. The Shapiro–Wilk test and the Levene test were used to determine whether or not the continuous variables had a normal distribution and whether or not they had a spherical distribution, respectively. If the data satisfied the conditions of normality and sphericity, a repeated measures mixed ANOVA was carried out, and then the post hoc Tukey test, which included a correction for multiple comparisons, was carried out afterwards. In the event that the aforementioned assumptions were not satisfied, the Mann–Witney U-test was used to evaluate the differences between the groups, and the Friedman test with post hoc comparisons was utilized to evaluate the differences that existed within the groups at the various study timepoints. The studies of categorical and ordinal variables both made use of the same non-parametric tests to determine results. In order to determine the frequencies of categorical variables within the groups, the Fisher's exact test was used.

Results

The participants in the research study ranged in age from 20 to 24 years old, with a mean age of 22 years. Using a random sequence generator, they were split into two groups: the placebo group, which consisted of 13 females and 7 men, and the probiotic group, which consisted of 15 females and 5 man. There was not a significant difference between the groups in terms of age, the distribution of gender, DMFT values, or the decay component of DMFT values (Table 2).

Table 2 Characteristics of subjects at inclusion.

Parameter	**Total (*n* = 40)**	**Probiotics (*n* = 20)**	**Placebo (*n* = 20)**	**Statistical Significance**
Sex				
Female	28	15	13	1.0
Male	12	5	7	
Age				
m (sd)	21.1	21.3	20.6	0.2
DMFT				
Median [Q1; Q3]	8	9	10.5	0.42
min-max	0–20	0–20	2–17	

Decay				
Median	2.3	3.4	2.1	0.39
min-max	0–5	0–5	0–5	

Table 3 displays the salivary IgA baseline value, as well as the outcome value and washout value. It also displays the unstimulated salivary flow rate and dental indices. When comparing the participants who took probiotics to those who took a placebo, we did not find any statistically significant changes in the levels of salivary secretory immunoglobulin A (sIgA), nor did we find any variations in the unstimulated salivary flow rates. Following an intervention with probiotics that lasted for four weeks, followed by a washout period of two weeks, the participants in the study had TQHPI values that were considerably lower than those of the controls. At the beginning of the study, seven people had PMA index values that were higher than 0; of those seven, three were in the probiotic group and four were in the control group. These changes did not reach the level of statistical significance, but the PMA values did trend to drop in the probiotic group at both the outcome and washout timepoints.

Table 3 Summary of evaluated parameters.

Parameter	**Probiotics (*n* = 20)**	**Placebo (*n* = 20)**	**Statistical Analysis**
sIgA, mg/L, mean			
Baseline	224	210	0.45
Outcome	201	180	0.58
Washout	225	191	0.75
Salivation, mL/min, m (sd)			
Baseline	0.45	0.46	0.99
Outcome	0.54	0.51	0.07
Washout	0.52	0.53	0.82
TQHPI, median [Q1; Q3]			
Baseline	2.7	2.8	0.54
Outcome	2.4	2.7	0.01
Washout	2.6	3.1	0.009
Within-group comparisons	0.023	0.12	
PMA > 0 units	*n* = 2	*n* = 5	
Baseline	4.1	2.3	0.47
Outcome	0	3.4	0.24
Washout	0	3.4	0.17
Within-group comparisons	0.061	0.56	

There were no statistically significant differences in the distribution of the study subjects across the levels of salivary sIgA and PMA in the probiotic and placebo groups at all study timepoints (Table 4).

Table 4 Distribution of sIgA levels (low, normal, high) [a] and PMA (zero or greater than zero) in the study groups.

	Probiotics (*n* = 20)	**Placebo (*n* = 20)**	**Significance [b]**
Level of sIgA			
Baseline, *n*			
Low	7	4	0.83
Normal	10	12	
High	3	4	
Outcome, *n*			
Low	6	7	0.89
Normal	10	12	
High	4	1	
Washout, *n*			
Low	5	4	0.69
Normal	10	12	
High	5	4	
PMA			
Baseline, *n*			
PMA = 0	15	13	1.0
PMA > 0	5	7	
Outcome, *n*			
PMA = 0	16	15	0.65
PMA > 0	4	5	
Washout, *n*			
PMA = 0	20	15	0.26
PMA > 0	-	5	

Discussion

In the current research, we investigated the impact that oral probiotics containing Streptococcus salivarius K12 had on salivary levels of secretory immunoglobulin A, salivation rates, and oral biofilm in people who were otherwise healthy. Comparing the probiotic group to the placebo group, we did not find any rise in the levels of

salivary secretory immunoglobulin A (sIgA), nor did we find an increase in the flow rates of saliva. However, after two and four weeks of use of probiotics, we saw a statistically significant reduction in the amount of plaque that had accumulated in the probiotic group. In the group that took probiotics, there was a reduction in the PMA index; nevertheless, the differences between the groups did not approach the level of statistical significance. This might have been caused by a relatively low percentage of individuals who had gingivitis.

IgA in the saliva is an essential protein that plays a role in the protection of the mouth against many illnesses. Because it may be collected without causing any discomfort to the patient and is regarded as a sign of both health and illness [47], the assessment of sIgA levels is used extensively in the field of dental science. Even while research on the impact of probiotics on sIgA levels have produced mixed findings, there is evidence that probiotics have a favorable influence on the immunological response of the host [48]. After taking probiotics, several research observed higher levels of salivary sIgA in children [47,48], elderly patients [52], and adults [44,47,49,50,51]. Other studies, however, were unable to substantiate these results [23,53,54,55,56]. One research even revealed that consumption of a probiotic containing Bifidobacterium led to a reduction in the amount of salivary sIgA [57]. Our findings are consistent with those of Ebrahimpour et al., who conducted a meta-analysis and found that taking probiotics did not have a significant impact on the levels of salivary sIgA when compared to taking a placebo [58]. The results of the current study's analysis of variance demonstrated that neither the time factor (baseline/outcome/washout) nor the group factor (probiotic/placebo) had an effect on the levels of salivary sIgA.

The rate of salivary flow is another important characteristic that must be monitored carefully in order to preserve dental and overall health [59]. There is some evidence to suggest that the flow rate of saliva might be affected by probiotics [59,60]. On the other hand, the findings of other investigations [61,62,63,64] did not support this effect of probiotics. Our findings are in line with those of more recent research in that we did not see an increase in the unstimulated salivary flow rate after the consumption of probiotics as opposed to the placebo.

The age of the participants in a study may be used to help explain differences in the impact that probiotics have on different health markers. Antibody responses in the investigations that included older persons could have been different from those of healthy adults [58]. Additionally, both intra- and inter-individual changes in saliva volume and its contents are impacted by a range of circumstances. Some of these factors include smoking cigarettes [47,54], chronic and acute stress [47,54,58], depression [47,54], and circadian fluctuation [47,65].

There is a possibility that the immune-modulatory effects of probiotics, both in general and for individual species, are strain-specific [54]. There were no other published reports that we could find that were directly similar to ours, as far as we know. The only research that was even somewhat similar to ours was that of Ferrer et al., who investigated the impact of a topical application of Streptococcus-containing probiotics on plaque buildup, saliva quality, and salivary flow [22]. They observed that the group that received probiotics had a substantial increase in salivary flow rate at day 15 compared to the group that received a placebo. In addition, the probiotic group had a reduction in the quantity of dental plaque and gingival irritation, while the placebo group did not experience any of these changes [22]. A similar effect on plaque formation was reported by Burton et al., who demonstrated that the probiotic strain S. salivarius M18 administered twice daily caused a significant reduction in plaque formation in children [66]. These researchers found that the probiotic strain S. salivarius M18 was effective in preventing plaque formation in children. After two and four weeks of use of probiotics, we found that the probiotic group had a statistically significant reduction in TQHPI compared to the control group.

The ability of probiotics to reduce plaque may, however, depend on the particular strain as well as the product used. A meta-analysis conducted by Nadelman and colleagues [67] found that dairy probiotics led to an increase in plaque buildup, which may have been caused by an increase in the quantity of carbs.

One may reasonably anticipate that a reduction in the plaque index would lead to a corresponding reduction in gingivitis (PMA score). Numerous studies have shown that the use of probiotics may considerably improve a variety of gingival health indicators, such as a reduction in gingival indices and the amount of bleeding that occurs during probing [22,42,68,69]. [Citation needed] Even though the differences between the groups did not meet the criteria for statistical significance, our research showed that the probiotic group had a lower PMA index than the control group. It is possible that this occurred because the majority of patients in both groups practiced either

excellent or moderate levels of oral hygiene, which resulted in a small proportion of patients developing gingivitis. In a similar vein, Montero and colleagues [70] showed modest changes in the mean gingival index overall, despite the fact that it considerably decreased at the locations of acute inflammation.

TQHPI values were shown to have a somewhat positive connection with the number of teeth that were decayed. This was the case despite the fact that the rate of plaque buildup is a variable that is subject to fast change and that the progression of caries is a rather gradual process. Although some authors hypothesized that the level of salivary sIgA may serve as a predictor of caries resistance in a patient [27,71,72,73], there was no significant correlation between the value of the decay component of DMFT and the sIgA level. This was the case despite the fact that there was a positive correlation between the two variables.

According to the research that has been done, the levels of sIgA in saliva may be reliant on the changes that occur in salivary flow rates [74,75]. As a result, protein concentrations in saliva may also be affected by these rates. For instance, a rise in salivary sIgA was recorded in patients with xerostomia who were pregnant women [78] and students who were stressed out because of examinations [74]. We discovered that there is a significant inverse relationship between the rates of salivation and the levels of sIgA (p 0.001). In a similar vein, prior research [79,80,81,82,83] has shown that there is an inverse association between the flow rates of saliva and the amounts of sIgA in the saliva.

We are not ashamed to admit that our research has a number of shortcomings. This was a preliminary investigation, and the comparatively low number of participants was determined by comparing it to the sample sizes of other studies that were comparable [44,45,46]. On the basis of the findings obtained from this study, it has been decided to conduct more research using a more extensive sample size. Although a four-week intake of probiotics and a two-week washout period are relatively short periods for the assessment of the effect of probiotics, similar timepoints were used in the previous studies [47,54,84]. Although these timepoints are relatively short, they were used in studies with a similar purpose. Furthermore, despite the fact that probiotics may have an effect on a variety of salivary components [25,58,63,85], we investigated the effect of probiotics on a single salivary protein (sIgA). We did this because we hypothesized that this parameter would be the one that would be most sensitive to probiotic consumption.

Conclusions

Within the confines of this pilot investigation, it is possible to draw the conclusion that the consumption of probiotics (Streptococcus salivarius K12) does not influence the salivation rates of healthy individuals nor does it alter the amounts of secretory immunoglobulin A found in their saliva. On the other hand, there was a discernible reduction in the buildup of plaque.

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