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Journal of Probiotics & Health

Research Article

Yogurt for the Equilibrium of the Oral Microbiota with the New *Streptococcus salivarius* Probiotic BIO5

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ABSTRACT

This study was carried out to assess whether the use of yogurt prepared with the strain of the probiotic *Streptococcus salivarius* BIO5, could interfere in any way in the oral microbiota of students. Sixty students aged between 4 and 14 years were selected, who took the yogurt three times a week, for a period of 90 days. Student's saliva was collected to check for presence and quantify the following microorganisms: *staphylococci*, total *streptococci*, mutans *streptococci*, *lactobacilli*, *pseudomonas*, yeasts, anaerobes and *enterobacteria*. The results showed that after 90 days of using yogurt, there was a decrease in microorganisms that are not part of the oral microbiota and there was no change in microorganisms considered to be residents.

Keywords: Probiotics; Oral microbiota; *Streptococcus salivarius*

INTRODUCTION

The use of the *Streptococcus salivarius* sbps *salivarius* probiotics has been increasingly studied, an evidence of the interest in the feasibility of the use of this microorganism to control oral microbial diseases and those of oropharynx: lately, prevention or treatment of tonsillitis caused by S. *pyogenes* or Group A *Streptococcus* (GAS) has also been studied, with some special strains able to produce bacteriocin against GAS [1-7].

In children all over the world, sore throat is one of the most common diseases and the infection by GAS is the main concern seeing that the current solution is the use of anti-inflammatory drugs and antibiotics, mainly the penicillin [8,9]. For many years, treatment failure with penicillin has been discussed if it is clinically efficient in eliminating GAS from the throat [10-12].

Tablets with the S. *salivarius* K12 strain were developed in New Zealand, with the aim of controlling halitosis and sore throats caused by GAS [13]. The interest in the S. *salivarius* species occurs because it is a microorganism resident in the oral cavity, especially tongue and cheeks and additionally to the fact that it does not have any degree of toxicity, it can present the capacity of producing bacteriocins against the main agent of bacterial tonsillitis, the GAS species.

We searched for a medium which could prevent tonsillitis and that, at the same time, could be pleasant both for children and adults as well. Thus, we reached the possibility of preparing yogurt with S. *salivarius* strains.

The S. *salivarius* BIO5 strain, showed total absence of toxicity and proved to be functional in the treatment of recurrent tonsillitis [6,14]. It also was verified that the yogurt given routinely, not only did not cause changes which might damage the oral microbiota, but also promoted health to this micro environment by decreasing the transient microorganisms, which are normally the ones that present high resistance to antibiotics and motivate hospital infections.

MATERIAL AND METHODS

This study had the purpose of evaluating whether the introduction of the yogurt prepared with the new S. *salivarius* subsp. *salivarius* strain BIO5 probiotics, might cause any alteration in the composition of the oral microbiota, and whether this alteration might increase or not the number of

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Received date: December 31, 2020; **Accepted date:** January 14, 2021; **Published date:** January 21, 2021

Citation: Fantinato V, De-Carvalho HR, De-Souza ALOP (2021) Yogurt for the Equilibrium of the Oral Microbiota with the new *Streptococcus salivarius* Probiotic BIO5. J Prob Health. 9:232.

resident and transient microorganisms, with the possibility of altering or not the equilibrium of the resident microorganisms of the oral microbiota.

The *Streptococcus salivarius* subps *salivarius* BIO5 strain was isolated from a healthy individual who had never had tonsillitis and its identification was carried out by the PCR test [15].

Sixty school children were selected from Escola Rural Monsenhor Pedro Paulo Farhat, in the City of Bragança Paulista, State of São Paulo, Brazil. This study was approved by the School Ethics Committee, by the Education Secretary and by the Health Secretary of the City, as well as by the Brazilian Health Department. The people responsible for the children whose ages ranged from 04 to 14 attended a detailed oral presentation of the research. Those who agreed to their children's participation signed a "Consent Term" in which all the required information and the permit of the child's participation in the experiment were described.

All the children had their saliva collected twice, within an interval of one week between one collection and the other. The purpose of these collections was to obtain an average of the number of microorganisms of each child's microbiota before the yogurt was used so that a comparison could be made after its use. The collections of saliva carried out before the first use of the yogurt were also necessary for us to verify the possible presence of *Streptococcus* strains with the bacteriocinogenic pattern of BIO5 strain. In order to identify the strain used and differentiate it from the other S. *salivarius* strains, we made fingerprinting using as indicators the standard strains of S. *pyogenes* ATCC 12372, ATCC 12358, ATCC 123249, ATCC 12373, HU-USP 175-11 and HU-USP 756-10 (HU-USP-Hospital Universitário de São Paulo).

Saliva samples were diluted 10-fold in sterile phosphate-buffered saline (PBS, pH 7·5) and then vortex mixed for 10 s. Aliquots were plated onto Mitis-salivarius agar (Difco) for *S. salivarius* enumeration. Incubation was in a 5% CO_2 in air atmosphere for 24 h at 37°C. 25 colonies were collected from each plate and, after incubation and growth, were transferred to sheep blood-agar for storage. From this point onwards the tests of simultaneous antagonism were carried out as previously described by Tagg and Bannister against the six strains of S. *pyogenes* [16]. The children selected were those who did not present bacteria of the positive bacteriocinogenic pattern of BIO5 strain. In none of the children's saliva this pattern of streptococci was found.

Preparation and utilization of the yogurt

The yogurt was prepared with dry whole milk powder (Ninho® with Fe^{++} and vit. A,C,D) Nestlé Brasil, Araçatuba-SP, Brazil; sucrose (Sugar União®), União, São Paulo-SP, Brasil (20 Kcal/5 g); Strawberry base yogurt De Marchi, Jundiaí-SP, Brazil; modified startch, Global food Advanced Food Technology, São Paulo-SP, Brazil. For the production of yogurt, the matrix was fermented with the strain of the *Streptococcus salivarius* BIO5. Each 100 ml portion had nearly 125 Kcal. The count of S. *salivarius* in the freshly yogurt was about 10^8 CFU mL/ml.

Each child received a 100 ml yogurt pot and a disposable spoon on Mondays, Wednesdays and Fridays after the lunch offered by the school. These steps occurred for the period of three months.

Saliva samples

For the follow-up of the colonization and evaluation of the children's oral microbiota, saliva was collected on a monthly basis. Collections were carried out without stimulus before lunch and yogurt intake; each child would spit directly into individual sterile vials which were kept in ice boxes and taken to the lab where the tests were carried out soon afterwards. The maximum time between collection and arrival of the material in the lab was one hour.

Saliva samples were diluted 10-fold and aliquots were plated onto selective culture media to detect and quantify the following microorganisms: MSB Mitis Salivarius agar with bacitracin for the isolation of S. *mutans*, MS agar Mitis Salivarius agar for the isolation of oral total *streptococci*, Sabouraud agar with chloramphenicol at 0.1% for the isolation of *Candida* spp., *Staphylococcus* 110 agar for the detection of *Staphylococcus* spp., Rogosa agar for the isolation of *Lactobacillus* spp., Mc Conkey agar for the detection of *Enterobacteriaceae*, *Pseudomonas* agar, for the isolation of *Pseudomonas* species, and Brucella agar added with defibrinated sheep blood and menadione for the isolation of anaerobic bacteria. The plates were incubated at 37°C/48 h, under aerobiosis, except for the blood agar plates, which were kept at 37°C/5 days, under anaerobiosis. After the incubation period, counts of the number of Colony Forming Units (CFU) and the estimate of number of CFU/ml of each individual's saliva were carried out. The data were subject to statistical analysis so that possible changes in the salivary microbiota could be verified.

RESULTS

In pre yogurt phase, two collections of saliva were carried out so that we could have an average of the variation of the microorganisms studied. The objective was mostly to verify the degree of variation of presence of transient microorganisms, which may vary a great deal from one collection to another, thus interfering in comparative calculations.

The Figure 1 presents the averages of the results from both counts of the saliva microorganisms from volunteers in pre yogurt phase.

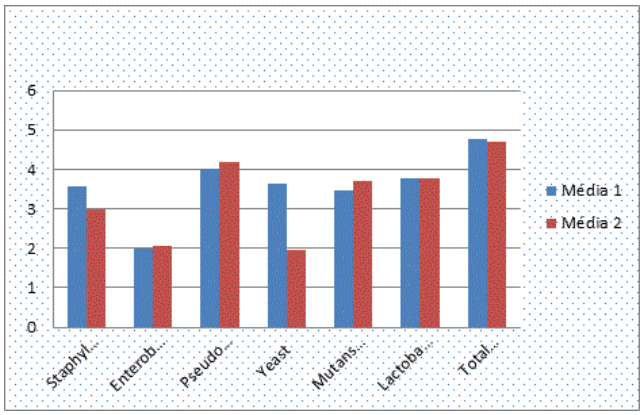

Figure 1: Average (log base 10) of both counts of saliva pre yogurt.

For all the microorganisms studied, we had the count average of each volunteer's saliva in pre yogurt phase and another count average of each volunteer's saliva in post yogurt phase for a three-month period.

Using these averages, we generated the bacteria quantitative graphics per volunteer and the distribution graphics per range of number of bacteria so that we could evaluate the distribution tendency in pre and post yogurt phases.

Microorganism	Moment	Average	Variance	Standard deviation
Enterobacteria	Pre yogurt	2,74254,4733	2,417074628	1,554694384
	Post yogurt	2,152916606	1,553129883	1,246246317
Yeast	Pre yogurt	1,601852327	2,764269826	1,662609343
	Post yogurt	0,821332059	1,062209935	1,030635695
Pseudomonas	Pre yogurt	4,48023646	0,91086512	0,95439254
	Post yogurt	4,183393864	1,182191477	1,08728629
Staphylococci	Pre yogurt	3,099770247	1,089483072	1,043783058
	Post yogurt	2,814622234	0,705263473	0,839799662
Lactobacilli	Pre yogurt	2,344681162	2,692275977	1,640815644
	Post yogurt	2,361241592	2,037599833	1,427445212
Anaerobic Bacteria	Pre yogurt	8,117621466	0,341277535	0,584189639
	Post yogurt	8,05735039	0,209329508	0,457525418
Oral *Streptococci*	Pre yogurt	8,227717499	0,389345381	0,623975465
	Post yogurt	7,688329744	0,277303178	0,526595839
Mutans Group	Pre yogurt	5,234957281	2,688662438	1,639714133
	Post yogurt	3,924010853	2,387510603	1,545157145

Table 1: Results of the means, variations and standard deviation in the bacteria count of the volunteers' saliva, before and after the yogurt.

Enterobacteriaceae

Results of the evaluation of possible changes upon the presence and quantification of *Enterobacteriaceae*, in pre and post yogurt phases, can be viewed in Table 1 and Figure 2 below.

As indicated in the Table 1, *Enterobacteriaceae* count total average in post yogurt phase decreased by over 0.5 (log 10), and variance-ratio of the distance between the lowest and the highest values in relation to the average-decreased by nearly half, meaning that in addition to the fact that the average decreased, the values are less disperse; therefore, more standardized.

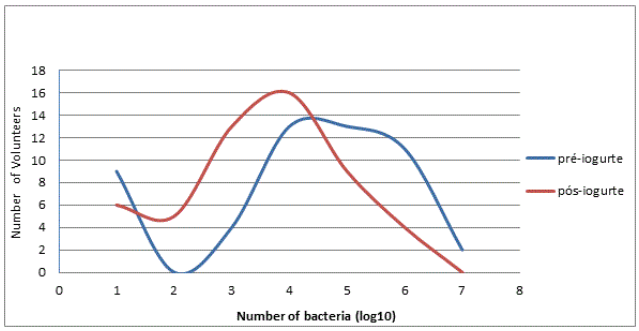

Figure 2: Distribution tendency curves for volunteer quantity over *Enterobacteriaceae* count pre and post yogurt (log 10).

In Figure 2, regarding sampling distribution tendency, it is noticed that the area below the curve represents the number of volunteers with a given number of bacteria.

In pre yogurt phase, the highest concentration of volunteers had a number of *Enterobacteriaceae* within the range between 3 and 7 CFU/ml of saliva (log 10). In post yogurt phase, the highest concentration of volunteers decreased to the range between 2 and 6 CFU/ml of saliva (log 10).

Yeast

Results of the evaluation of possible changes upon the presence and quantification of yeast cells, in pre and post yogurt phases, can be viewed in Table 1 and Figure 3.

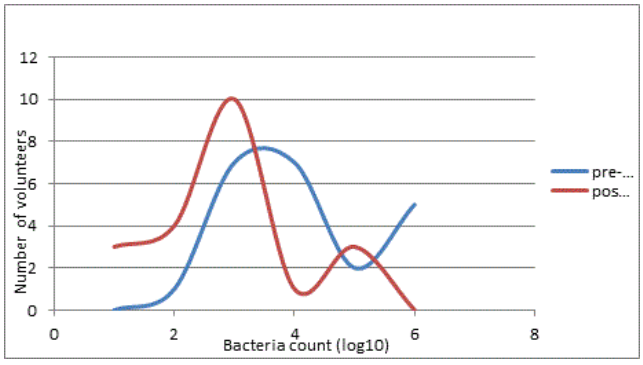

Figure 3: Distribution tendency curves for volunteer quantity over Yeast count pre and post yogurt (log 10).

In pre yogurt phase, the highest concentration of volunteers had a quantity of yeast within the range between 2.5 and 4.5 CFU/ml of saliva (log 10). In post yogurt phase, the highest concentration of volunteers decreased to the range between 2 and 4 CFU/ml of saliva (log 10).

We can detail that within the range between 2 and 3 CFU/ml of saliva, we have nearly 4 volunteers in pre yogurt phase. Within the same range, in post yogurt phase, we have an increase to 8 volunteers. Within the range between 4 and 5 CFU/ml of saliva, in pre yogurt phase, we have 5 volunteers and in post yogurt phase, just two.

This shows that the highest concentration of volunteers, in post yogurt phase, lies within a range of lower number of bacteria.

Pseudomonas

Results of the evaluation of possible changes upon the presence and quantification of the *Pseudomonas* cells, in pre and post yogurt phases, can be viewed in Table 1 and Figure 4. Again, counts total average in post yogurt phase decreased in relation to the average in pre yogurt phase. Despite variance increase, the increase was not as significant as variance was kept low, i.e., the minimum and the maximum values were kept close to the total average.

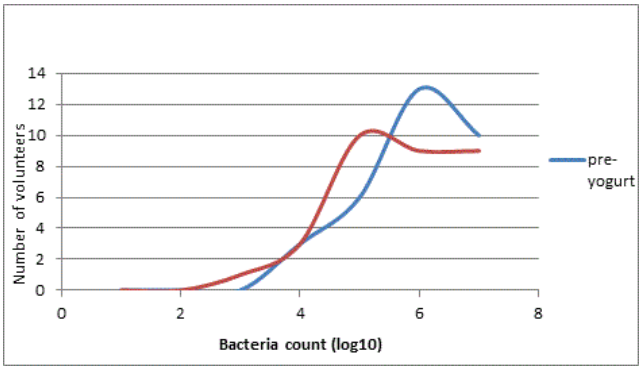

Figure 4: Distribution tendency curves for volunteer quantity over *Pseudomonas* count pre and post yogurt (log 10).

In pre yogurt phase the highest concentration of volunteers had a number of *Pseudomonas* within the range between 5 and 7 CFU/ml of saliva (log 10). In post yogurt phase the highest concentration of volunteers decreased to the range between 4 and 6 CFU/ml of saliva (log 10).

We have nearly 4 volunteers in pre yogurt phase. Within the same range, in post yogurt phase, we have an increase to 8 volunteers. Within the range between 4 and 5 CFU/ml of saliva, in pre yogurt phase, we have 5 volunteers and in post yogurt phase, just two.

We can detail that within the range of 5 CFU/ml of saliva we have nearly 6 volunteers in pre yogurt phase. Within the same range, in post yogurt phase, we have an increase to 10 volunteers. Within the range of 6 CFU/ml of saliva, in pre yogurt phase, we have 13 volunteers and in post yogurt phase this number has decreased to 9.

Staphylococci

Results of the evaluation of possible alterations upon the presence and quantification of *staphylococci*, in pre and post yogurt phases, can be viewed in Table 1 Figure 5. The average here decreased very little in post yogurt phase. Variance also decreased, but again it was not a significant decrease, as the variance is the average of the squared differences from the Mean and a 0.3 variation is little representative.

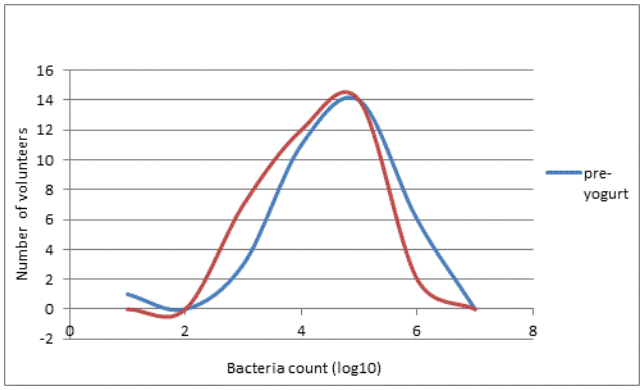

Figure 5: Distribution tendency curves for volunteer quantity over *staphylococci* count pre and post yogurt (log 10).

In this Figure 5 the difference between the pre and post yogurt phases is little noticeable. The most significant area below the two curves remains very close. Even though we can notice few variations at the sides of the curves, clearly the red curve more to the left than to the right, statistically speaking, does not represent a very significant change, although it exists.

In post yogurt phase, the highest concentration of volunteers, which lies within the range between 4 and 5 CFU/ml of saliva, remains practically the same. But within the range of 3 CFU/ml of saliva, we can notice an increase in the number of volunteers from 3 to 7, and within the range of 6 CFU/ml of saliva, a decrease from 6 to 2 volunteers in post yogurt phase.

Lactobacilli

Results of the evaluation of possible changes upon the presence and quantification of *Lactobacilli*, in pre and post yogurt phases, can be viewed in Table 1 and Figure 6. Counts total average in pre and post yogurt phases remains practically the same. This difference by 0.02, being the values in log 10 scale, is practically void and variance decreased.

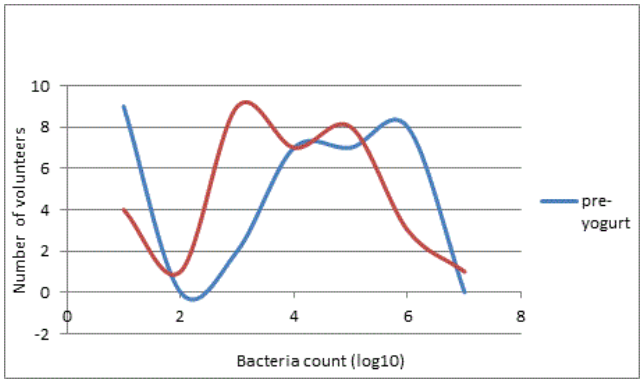

Figure 6: Distribution tendency curves for volunteer quantity over *Lactobacillus* count pre and post yogurt (log 10).

In this Figure it is clear to what extent the tabled values should be noticed more carefully, despite the fact that the total average is practically the same. Change in variance becomes clear in the Figure above. The biggest area below the red curve, clearly more to the left in the Figure, indicates that the concentration of volunteers with lower microorganism count is clearly higher than that of volunteers with higher count, the opposite of what happens with the blue curve.

Anaerobic bacteria

Results of the evaluation of possible changes upon the presence and quantification of total anaerobic count, in pre and post yogurt phases, can be viewed in Table 1 and Figure 7. In this Table variation is not significant in the total averages of pre and post yogurt phases, as much as variation is not significant in variance, which, by the way, is very low. This indicates that anaerobic bacteria are found in all the volunteers in a very standardized way.

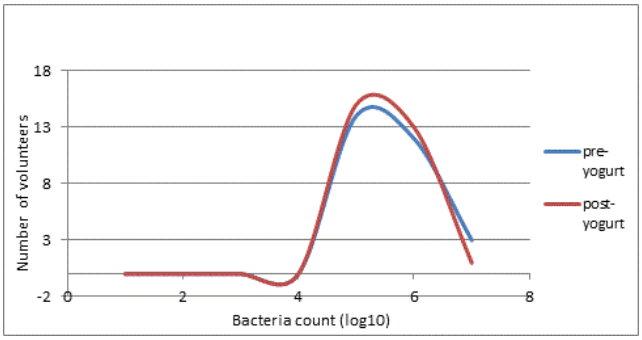

Figure 7: Distribution tendency curves for volunteer quantity over Anaerobic Bacteria count pre and post yogurt (log 10).

In the Figure, we see almost no changes between pre and post yogurt phases and, as mentioned above, the anaerobic bacteria are found in all the volunteers in a very standardized way and ingestion of the yogurt does not change count of these microorganisms.

Oral *streptococci*

Results of the evaluation of possible changes upon the presence and quantification of total streptococci, in pre and post yogurt phases, can be viewed in Table 1 and Figure 8. In total oral streptococci averages there was a little reduction in post yogurt phase. Considering that the values are in log 10 scale, change is not significant, but it is clear. Change in the variance is not significant as the variance itself is very low. This shows that the values found in the counts indicate a very clear standardization in the presence of this microorganism.

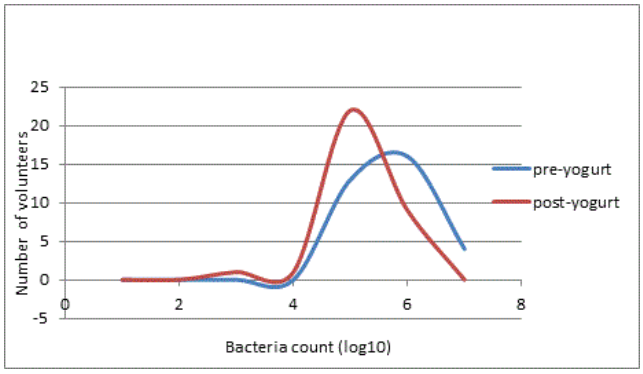

Figure 8: Distribution tendency curves for volunteer quantity over total *streptococci* count pre and post yogurt (log 10).

On verifying the curves in pre and post yogurt phases, it is clear that the microorganism is present within a range which does not suffer a very big change. There is a little reduction of volunteers with high count of microorganisms and an increase of volunteers with lower count thereof, but even so, the count range remains the same in both cases.

In pre yogurt phase, the highest concentration of volunteers had a number of *lactobacilli* within the range between 4 and 7 CFU/ml of saliva (log 10). In post yogurt phase, the highest concentration of volunteers decreased to the range between 4 and 6 CFU/ml of saliva (log 10).

We can detail that within the range of 5 CFU/ml of saliva, we have a variation from 13 to 22 volunteers in post yogurt phase. Within the range of 6 CFU/ml of saliva, in pre yogurt phase, we have 17 volunteers and in post yogurt phase it has decreased to 10.

Mutans *streptococci*

Results of the evaluation of possible changes upon the presence and quantification of streptococci of the mutans Group, in pre and post yogurt phase, can be viewed in Table 1 and Figure 9. There is a significant decrease in the total average of the numbers of the microorganism in question, mainly verifying that the variance remains the same. This means that the average decreased in all individuals in a standardized way.

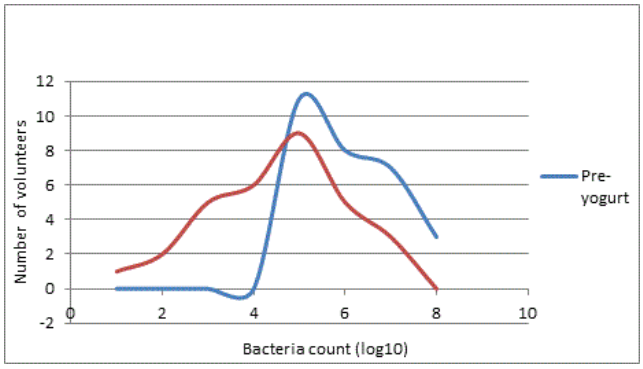

Figure 9: Distribution tendency curves for volunteer quantity over S. mutans count pre and post yogurt (log 10).

This Figures presents, in post yogurt phase, a more homogeneous distribution of the number of volunteers in

relation to the number of bacteria present in each individual, and clearly lower in relation to the results in pre yogurt phase.

In pre yogurt phase, the highest concentration of volunteers presented a number of *lactobacilli* within the range between 4, 5 and 7 CFU/ml of saliva (log 10). In post yogurt phase, the highest concentration of volunteers decreased to the range between 3 and 6 CFU/ml of saliva (log 10).

We can detail that within the range of 3 CFU/ml of saliva, we have a variation from 0 to 5 volunteers in post yogurt phase. Within the range of 7 CFU/ml of saliva, in pre yogurt phase, we have 7 volunteers and in post yogurt phase it has decreased to 3.

DISCUSSION

In reference to the Graphics of distribution and statistical values of the Table 1 we have the total averages in pre and post yogurt phases. In practically all of them total average has decreased, as well as the variance and the standard deviation. Just in *Lactobacilli* total average was higher in post yogurt phase, but only 0.02, however, the variance, which is the squared average between the differences of the samples lower and higher values, has decreased. This implies that the samples values are closer to the average than more disperse, as well as the standard deviation which is the variance square root.

In the Figures, the most representative region is the largest area below the curves, where the curve presents its peak value. As the larger area is located more to the left side of the graphic, it implies that most volunteers have a low count of bacteria; as the curve moves to the right side of the graphic, it means that most volunteers have a higher count of bacteria. The higher and narrower the curve, the more concentration of individuals within the same range; the lower and wider the curve, the more disperse the samples (volunteers).

With the objective of evaluating whether introduction of the yogurt prepared with the new probiotic S. *salivarius* BIO5 could, in some way, change the oral microbiota of the volunteers studied, the parameter evaluated was the number of microorganisms considered transient in the oral cavity and normally resistant to antimicrobials related to hospital infections: *Pseudomonas*, *Staphylococcus*, Yeast and *Enterobacteriacea* and those of bacteria associated to the activity of tooth decay, like *Streptococcus* mutans and *Lactobacilllus*. The microorganisms considered transient install temporarily in the oral cavity and after some time disappear naturally, or not. The results were also analyzed and compared with the microorganisms that compose the normal microbiota of a healthy individual, the resident microorganisms, like the number of total anaerobic bacteria, resident in the gingival sulcus and *streptococci* total oral resident in the mucosae and dental surfaces.

It was observed that collection of the first two saliva samples pre yogurt presented a small variation not only in the number of each microorganism but also in the number of each microorganism between one collection and the other. The objective of making two collections was to decrease error of interpretation. The one week break between one collection and the other made it possible for us to be certain of the variation and obtaining of the average of its presence. The results of these collections, expressed in the variation of results, match the expected transient microorganisms, i.e., for *enterobacteria*, yeast, *pseudomonas*, *staphylococci*, *lactobacilli* and *streptococci* of the mutans group. The same did not occur with the oral *streptococci* and the anaerobic bacteria, which reside in the structures of the mucosae, of the teeth and those of the gingival sulcus, respectively, remaining practically unchanged.

Introducing a new probiotic through the yogurt, with a strain whose natural habitat is the mucous membranes of the oral cavity, especially the tongue, leads us to confirm its total reliability, guaranteeing that it will not generate any kind of lack of equilibrium and consequent damage to our oral health especially when children are the target. What we verified, was that not only it did not cause any damages to oral microbiota, but it decreased the number of transient bacteria, which are potentially pathogenic.

The presence of intestinal bacteria is rather common in children's oral cavity, especially with the youngest ones as they put their hands into their mouth more frequently and due to the contact they have with all types of objects, people, a variety of toys, earth, pets, etc. Out of the children studied, 86% were carriers of such bacteria in their oral cavity in the pre yogurt phase, evidencing the big prevalence of this microorganism in this habitat. In the post yogurt phase, we verified that 71% of the children presented lower *enterobacteriaceae* counts and this shows that despite the fact that the inoculated probiotics does not present any antagonistic effect through bacteriocins, just its colonization, routinely introduced, leads the introduced probiotic to occupy its natural niche, preventing or making it difficult the entrance and colonization of enteric microorganisms.

In the pre yogurt phase the highest concentration of volunteers presented a number of *Enterobacteriaceae* within the range between 3 and 7 CFU/ml of saliva (log 10) seeing that in the post yogurt phase the higher concentration of volunteers with *Enterobacteriaceae* decreased to a range between 2 and 6 CFU/ml of saliva (log 10). Additionally to the fact that pre and post yogurt total average of counts decreased by 0.5 (log 10), the variance decreased nearly by half, showing that if initially, in the pre yogurt phase, most volunteers presented *Enterobacteriaceae* in their oral cavity with CFU/ml between 10^3 and 10^7, in the post yogurt phase most volunteers presented CFU/ml of saliva between 10^2 and 10^6, clearly indicating that the values obtained after introduction of the new probiotics brought the benefit of the decrease of number of *Enterobacteriaceae* in the oral cavity, a microorganism which, despite the fact that it is common, it is not desirable in this environment [17].

In relation to the yeast we observed that differently from the *Enterobacteriaceae*, the presence of these microorganisms was less common, with 56% of the volunteers hosting this microorganism before introduction of the probiotics. Of these volunteers, 81% presented a decrease in the quantity of yeast in the post yogurt phase and in 14% no yeast was found during the three months of use. In no case an increase in the quantity of yeast was observed after introduction of the new probiotics, decreasing from within the range between 2.5 and 4.5 CFU/ml

of saliva to within the range between 2 and 4 CFU/ml of saliva, respectively.

We can detail that within the range between 2 and 3 CFU/ml of saliva, we have nearly four volunteers in the pre yogurt phase. Within the same range, in the post yogurt phase we have an increase to eight volunteers. Within the range between 4 and 5 CFU/ml of saliva, in the pre yogurt phase we have five volunteers and in the post yogurt phase just two. This shows that the highest concentration of volunteers in the post yogurt phase presents a lower quantity of yeast.

As regards *pseudomonas*, a Gram-negative opportunistic microorganism, *Pseudomonas aeruginosa* is the most studied species of all due to its high virulent potential and because it is responsible for several outbreaks of hospitals infections [18,19]. Our study with *pseudomonas* has shown that all the volunteers were carriers of this microorganism in numbers which varied from child to child, as it might be expected from a transient microorganism. Of the children under study, 65.7% presented a decrease in *pseudomonas* after taking the yogurt.

A change was observed in the average of the condition of this bacterium carrier from 6 CFU/ml to 5 CFU/ml after the children had taken the yogurt. Within the range of 6 CFU/ml of saliva in the pre yogurt phase, we had 13 children and in the post yogurt phase this number decreased to 9. We can detail that within the lowest range, which is that of 5 CFU/ml of saliva, we have nearly 6 children in the pre yogurt phase and in the same range, in the post yogurt phase, we have an increase to 10 children. The highest concentration of them had *pseudomonas* within the range between 5 and 7 CFU/ml of saliva (log 10), having this range changed into 4 and 6 CFU/ml of saliva (log 10), after the three months of routine intake of yogurt.

Bacteria of the *staphylococcus* are extremely common not only in the mouth but also in the nasal mucosa and skin, seeing that they might be considered resident microorganisms because of their constant presence in these habitats of the body. In the case of the children under this study, all hosted *Staphylococcus* ssp and 63% of them presented a decrease in the quantity of this microorganism in their saliva samples after taking the yogurt. It is noticed that the range of children with low number like that of 3 CFU/ml had 3 children, moving up to 7 children after taking the yogurt. As the average in the pre yogurt phase is closer to 5 CFU/ml, the increase in the number of children with a number which is more distant than the average shows a decrease. The same occurred with the children within the range of 6 CFU/ml which, from 6 moved down to one single child after the yogurt routine consumption.

Of the children studied, just 14% did not present *lactobacilli*, a sign that indicates low risk of tooth decay in these children, as the count of these microorganisms is one of the evaluation tests of this oral disease. Of the children who hosted this microorganism, 59% presented a *lactobacilli* decrease.

Count total average before and after consumption of the yogurt remains practically the same. However, it can be observed that variance has decreased, that is, although the average has remained the same, dispersion of the samples is lower after yogurt consumption, showing that there was a little standardization in *lactobacilli* count. The change that the variance indicates and that the graphic shows is that after yogurt consumption, fewer children presented zero count.

Before yogurt consumption, the highest concentration of children presented a quantity of *lactobacilli* within the range between 4 and 6 CFU/ml of saliva (log 10). After yogurt consumption, the highest concentration of children lowered to within the range between 3 and 5 CFU/ml of saliva (log 10).

We can detail that within the range of 3 CFU/ml of saliva, we have a variation from 2 to 9 children after yogurt consumption. Within the range of 6 CFU/ml of saliva, before yogurt consumption, we have 9 children and after yogurt consumption it has decreased to 3.

The anaerobic bacteria, typically present, especially in the gingival sulcus of an individual's health mouth, increase significantly when this region becomes ill with gingivitis and periodontal diseases.

In the children studied, it is clearly observed the existence of a pattern and equilibrium between the numbers found in all children, not only before yogurt consumption but also after it. What is observed is that after probiotics introduction, almost all of the children (90%) presented a slight decrease of anaerobic, not statistically significant though. Therefore, yogurt consumption for the period of three months did not lead to change in these microorganisms, considered typical dwellers and resident in the oral cavity.

The *streptococci* of the mutans group are considered the main agents of tooth decay due to its capacity of adhesion to the tooth enamel. The rates of this group of microorganisms indicate higher or lower possibility of tooth decay development. It is therefore an important group to be studied when oral microbiota should be investigated. Like the other transient bacteria, their presence in the mouth is highly variable. Unfortunately, in this study, it was only possible to collect saliva once before yogurt consumption. This prevented us from obtaining an average so that we could have some more efficient data of comparison. However, of all children studied, 78% present a decrease in the number of this microorganism.

Our studies are based on the fact that when dealing with the environment where nature, through evolving processes, selected certain microorganisms organizing a resident microbiota, we should respect this equilibrium reached through natural selection long-term mechanisms. When this natural equilibrium is broken up, generating lack of equilibrium in the health, we should use the same organisms of this natural microbiota to restore the equilibrium lost. Thus we would avoid using antibiotics, which could be used in cases of infection in tissues typically sterile. Today we use the term Probiotics to address these microorganisms. These "friendly" bacteria like the *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and several species of *Streptococci* are our body's first line of defense and act through a relation of antagonism against other microorganisms, pathogens to the host.

In 2005 in New Zealand, a product made with S. *salivarius* strain K12 (BLIS K12-Throat Gard) to be used orally in form of tablets

was launched. Each tablet contains 10^8 viable cells of S. *salivarius* and is useful to control halitosis and to prevent tonsillitis by GAS [2].

Power et al. formulated a powder preparation of the oral probiotic *Streptococcus salivarius* K12 to be given to children with otitis media [20]. The strain K12 colonized the oral cavity and extended beyond the nasopharynx or adenoid tissue.

Dierksen et al. compared the efficacies of salivaricin A (SalA)-producing *Streptococcus salivarius* strains 20P3 and 5 when given in milk to 219 children, using either 2-day or 9-day dosing regimens [19]. The drink contained a blend of freeze-dried cells, skim milk powder, and chocolate powder. The count of *S. salivarius* in each of the freshly reconstituted BLIS-milks were 3.10^7 CFU mL/ml.

In a previous study in which the objective was to study the control of tonsillitis, it was found that of the children with recurrent tonsillitis, who took the yogurt prepared with the S. *salivarius* BIO5 strain, about 90% had the infection cured [14].

Our proposal is to favor a suitable ecological equilibrium in the oral cavity by using yogurt, prepared with the strain of the probiotic *Streptococcus salivarius* subsp *salivarius* BIO5. Through routine consumption of the yogurt, being the *Streptococcus salivarius* species a typical inhabitant of the tongue, it would easily colonize this environment and would make it difficult for pathogenic transient microorganisms to invade the site, preventing the development of infectious diseases. The BIO5 strain produces bacteriocin against one of the main agents of bacterial tonsillitis, S. *pyogenes*.

Decrease in the transient bacteria studied, which was statistically evidenced, is already a benefit in itself as it keeps the equilibrium of the microbiota, which, because of the introduction of the probiotics residing in that habitat, adjusts and reproduces without causing any damages to the resident microbiota.

As yogurt is already a worldwide food accepted both by adults and mainly by children, we see this food as a great potential to prevent bacterial tonsillitis and to keep the microbiota equilibrium and oral health. Pediatricians, nutritionists, mothers and children also approve it for being a great food and its delicious taste and flavors proposed by the market. This yogurt combines the benefit of oral microbiota equilibrium and consequent protection of this habitat [21].

CONCLUSION

Our objective was to evaluate if routine introduction of the probiotic S. *salivarius* subsp *salivarius*-BIO5 strain via yogurt could change equilibrium of the normal microbiota of a group of students.

In short, we can conclude that, after routine use of the yogurt:

- 71% of the children presented a decrease of *enterobacteriaceae*
- 81% presented a decrease in the quantity of yeast
- 65% presented a decrease in the quantity of *pseudomonas*
- 63% presented a decrease in the number of *staphylococci*
- 59% presented a decrease in the number of *lactobacilli*
- 78% presented a decrease in the number of bacteria of the mutans group

Routine consumption of yogurt with the new probiotic S. *salivarius* BIO5 demonstrated it can contribute to a better oral health, for its help to decrease transient and potentially pathogenic microorganisms in this environment.

ACKNOWLEDGMENT

The authors would like to thank all the children and school staff where the research was conducted.

FUNDING

The authors acknowledge funding from (FINEP)-FINANCIADORA DE ESTUDOS E PROJETOS-n° 0854/08.

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