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Comparative evaluation of probiotic drink containing lactobacillus casei strain Shirota with plain curd on salivary *Streptococcus mutans* count

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Abstract
Probiotics are live microorganism that provide numerous health benefits without creating any hindrance to the immunity of the host organism. Probiotic bacteria's in the gut have worked wonders and aided the immunity against virulent bacterial species. A similar effect could be expected in the environment where the probiotics can be used to reduce the *S. Mutans* species that is known to cause caries in the oral cavity. With this aim the efficacy of probiotic drink Yakult was tested by analysing the reduction in the colony counts of *S. Mutans* against normal curd by using Mitis salivarius bacitracin agar.

Keywords: Microorganism, probiotic, oral, *Streptococcus mutans*

Introduction
Probiotics, or 'for life', are defined as live microorganisms which when administered in adequate amounts, confer a health benefit on the host [1]. The mode of action of probiotics are attributed to competition with pathogens for nutrients, production of various antimicrobials compounds, and also by activation and modulation of the immune system [2-3]. Consumption of probiotics in diet is increased by adding various probiotics strains in dairy products like cheese, curd, ice cream etc.

Probiotics are known for creating beneficiary bacterial environment in *GUT*, thus contributing towards general health, they are believed to be also involved in improving overall oral health and hygiene [2-4]. Probiotic bacteria reach the intestine alive unlike other bacterias present in yoghurt and compete with other microorganism, their survival from acid attack can be benefiacial for oral health as well. They can displace *Streptococcus mutans* which is most common causative organism of dental caries [6]. various studies have been performed and the most commonly used probiotic bacteria are lactobacillus and bifidobacterias [6]. In the present study Yakult probiotic health drink was used as it contains billion probiotic bacteria. The strain Lactobacillus casei shirota present in Yakult has been less explored for their benefits on oral health and reduction of *Streptococcus mutans* in oral cavity.

Aim and objectives
The aim of the study was to evaluate the effect of lactobacillus casie shirota on the salivary *Streptococcus mutans* count. Objective of this study was to compare the levels of salivary mutans streptococci before and after consumption of probiotic health drink-*YAKULT*.

Material and Methods
The study was carried out in Sri Aurobindo institute of medical sciences, Indore. Written informed consent was obtained from the participants prior to the study. 30 subjects of age group 18-19 years were chosen for the study.

Materials used for study (Figure 1)
1 Sterile bottles with markings to collect saliva.

2. Mitis salivarius bacitracin agar with potassium tellurite (Himedia, Mumbai, India)
3. Agar plates (Borosil, Ahmedabad, India)
4. Calibrated loop (Replicon Scientific, Haryana, India)
5. YAKULT probiotic health drink (Danone India pvt LMT.)
6. Plain curd (Sanchi, Indore, India)

Fig 1

Equipment used for study

1. Laminar air flow (Glowmax Engineers, Mumbai, India)
2. Incubator (Atico Medical pvt ltd, Ambala, India)

Method of the study

30 subjects were divided into two equal groups, group A (plain curd- Sanchi), and group B (given YAKULT health drink) comprising 15 individuals each, who were given 200 gm of respective drinks, once a day for 7 days. The individuals were refrained from having curd during other times of day. They were, however, encouraged to maintain good oral hygiene. No tooth brushing was allowed for at least 1 h after having their, respective, drinks.

Saliva sample collection

Unstimulated saliva samples were collected in a sterile container on day one, before consumption of probiotic drink or curd and this sample was treated as baseline value. Further samples were collected after one hour of consuming their drinks on day 1 and final samples were collected on day 7.

Microbiological study

Samples were inoculated on the Mitis Salivarius Bacitracin Agar. The plates were incubated at 37°C. Colony characteristics were studied after 72 h. *Streptococcus mutans* in saliva was determined by using a colony counter and the number of colony forming units was counted (figure 2).

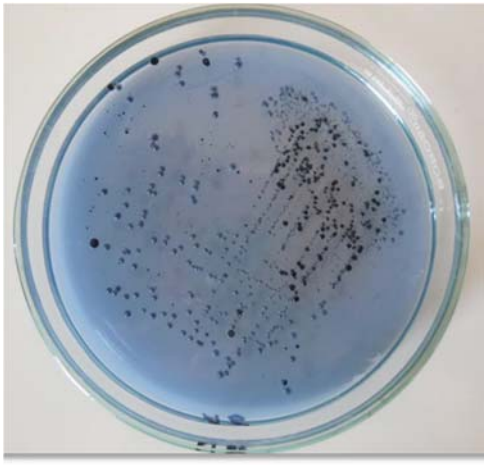


Fig 2: *Streptococcus mutans* colonies on Agar

Observations and Results

Table 1: Comparison of *Streptococcus mutans* colony count between Group A and Group B (N=30)

Time Interval	Group A (Mean ± SD)	Group B (Mean ± SD)	't' Value, Degree of Freedom	P Value
At baseline	253.87 ± 36.09	255.53 ± 37.78	0.12, df=28	0.903, NS
At 1 hour	249.47 ± 35.74	208.73 ± 33.80	3.21, df=28	0.003*
At 7th days	251.27 ± 36.45	140.60 ± 18.90	10.43, df=28	0.000*

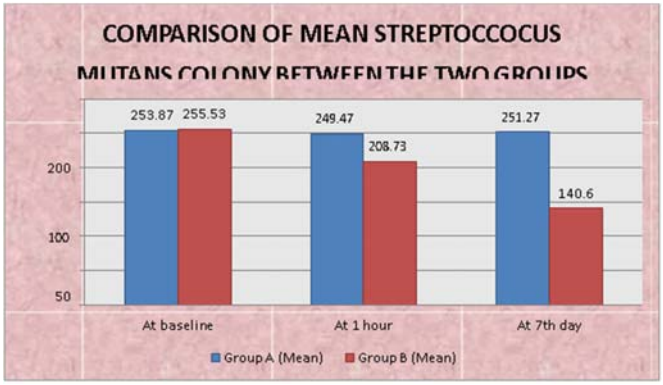
Unpaired 't' test. *P*<0.05, *Significant

The above table shows the comparison of mean *Streptococcus mutans* colony count between Group A and Group B.

In Group A, the mean *Streptococcus mutans* colony count at baseline was 253.87 ± 36.09, at 1 hour was 249.47 ± 35.74 and at 7th day was 251.27 ± 36.45.

In Group B, the mean *Streptococcus mutans* colony count at baseline was 255.53 ± 37.78, at 1 hour was 208.73 ± 33.80 and at 7th day was 140.60 ± 18.90.

The comparison of mean *Streptococcus mutans* colony counts between the two groups was done using Unpaired 't' test. The P value obtained at baseline was >0.05, which is statistically not significant. Thus, at baseline the colony count in both the groups were comparable.



Statistical Analysis

The comparison of mean *Streptococcus mutans* colony counts between the two groups at 1 hour and at 7th day was done using unpaired 't' test. The P value obtained was < 0.05, which is statistically significant. Thus, there was a significant difference in the colony counts of *Streptococcus mutans* between the two groups at 1 hour and at 7th day with lower counts in Group B in comparison to Group A.

The mean *streptococcus mutans* colony count within the group were compared using Paired 't' test and between the groups were compared using Unpaired 't' test. A P value of < 0.05 was taken as statistically significant. The statistical package Mini Tab Version 17.0 was used for analysis.

Discussion

Caries incidence is increasing in children and young adults due to consumption of refined carbohydrates and junk food. Probiotics can provide a simple and yet effective method in managing oral health due to lower cost involved and, almost, no change required in food habits. For better effects probiotic bacteria should adhere to the tooth structure and should be able to displace the *Streptococcus mutans* and it should produce low acid levels while present in oral biofilms [6]. Through this study we tried to analyse the effectiveness of probiotics in improving oral health by their ability to reduce population of mutant streptococci in oral cavity.

The study involved consumption of a probiotic drink (YAKULT) and plain curd (Sanchi) and determining count of mutant streptococci in saliva at different period after consumption, specifically after one hour of consumption on day 1 then after day 7 of continuous consumption of 200 ml drinks/curd once a day.

In this study, we found that there is significant reduction of mean mutans streptococci count ($P \leq 0.05$) after 7 days of consumption of probiotic health drink as compared to consumption of curd. There was, however, no significant reduction in mean count of mutant streptococci after 1 hour of consumption ($P > 0.05$). This could be attributed to lesser time period involved between introduction of probiotics, by means of consumption of probiotic drink and collection of sample, thus not providing enough time for probiotics to grow. The findings of this study are, partially, in line with previous reported studies carried out by Bhalla *et al.* [1], Singh *et al.* [4] and Jindal *et al.* [5] where they too found significant reduction ($P \leq 0.05$) of streptococci count on short term consumption of probiotics.

The mechanism of action of Probiotics may be production of anti-microbial substances such as, organic acids, hydrogen peroxide, carbon peroxide, biosurfactants in the oral cavity.

The probiotic bacteria are involved in the metabolism of substrates and they compete with pathogens for adhesion sites. These probiotics play a role in immunomodulation by stimulating non-specific immunity and by modulating humoral and cellular immune response. They also modify oral conditions by modification of oxidation-reduction potential and by modulating pH [6-7].

A good probiotic should be a strain, which is capable of exerting a beneficial effect on the host, e.g., increased growth or resistance to disease. It should be non-pathogenic and non-toxic. It should be present as viable cells, preferably in large numbers. It should be capable of surviving and metabolizing in the gut environment. It should be stable and capable of remaining viable for periods under storage and field conditions.

Lactobacilli are the most common probiotic bacteria associated with the human gastrointestinal tract; therefore, it may also play an important role in the ecophysiology of the oral microbiota [6]. Lactobacillus casei strain shirota used in this study has shown their benficiery effects on oral health by reduction of *Streptococcus mutans* count in oral cavity.

The population size under consideration, in this study, is very small. Furthermore, streptococci population, in oral cavity, is not the only causative factor in dental carries and streptococci count can only be treated as one of the endpoints in analysing prevalence of carries. The study has to be carried out on a larger population to determine the actual effectiveness of probiotics in managing dental health of patients.

Conclusion

Lactobacillus casei strain shirota has been found to be effective in reducing *Streptococcus mutans* count in saliva thus contributing in reduction of dental caries. This bacterio-therapy is also useful for other health benefits. Awareness should be created amongst dentists and other general practioners to advocate probiotics for better oral health status. More research is needed on large population for better understanding of these micro-organisms.

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