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Original Research Article

Comparative evaluation of efficacy of various probiotics on *Streptococcus* species

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ABSTRACT

Background: Probiotics which when administered in adequate amounts confer a health benefit on the host. The role of probiotics is the replacement of pathogenic species with non-pathogenic species. Dairy food like cheese, curd and milk are considered as useful vehicles to carry probiotic bacteria. Aim of the study was to compare and evaluate the efficacy of various probiotics against different Streptococcus species.
Methods: Three probiotic products viz probiotic milk, probiotic yogurt and probiotic capsules were used. *Streptococcus* species i.e. *S. mutans*, *S. sanguinis* and *S. sobrinus* were isolated from the saliva of children with moderate to high caries. 0.2 ml of each probiotic product was transferred to the blood agar plates coated with *Streptococcus* species.
Results: The zone of inhibition was observed in all the test groups, for all the *Streptococcus* species against all the probiotics, highest for *S. mutans* against probiotic milk. The growth of *S. mutans*, *S. sanguinis* and *S. sobrinus* was inhibited by *L. casei shirota* present in probiotic milk (Yakult), *Lactobacilli acidophilus* present in probiotic yogurt (Actiplus nestle) followed by *Lactobacilli rhamnosus* present in pre and probiotic capsule (Inlife).
Conclusions: The use of probiotic products that are readily available and cost effective like milk and yogurt can be inculcated in general population especially in children as a preventive tool for dental caries.

Keywords: Probiotics, *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus sobrinus*

INTRODUCTION

Probiotics are defined as live microorganisms, which when administered in adequate amounts confer a health benefit on the host.[1] [Food and Agriculture Organization (FAO) and World Health Organization (WHO), 2002]

The concept of probiotic was given by Elie Metchnikoff, that the bacteria in fermented products compete with microbes that are injurious to host and are hence, beneficial to health.[2] The role of probiotics is the replacement of pathogenic species with non-pathogenic species.

The most common types of microbes used are *Lactobacilli* and *Bifidobacteria*.[3] *Lactobacilli* species such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus GG*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus reuter*, *Lactobacillus paracasei* and Bifidobacterium species such as *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium* animalis strain DN-173 010 and others *Streptococcus salivarius*, *Weissella cibaria*.[4] Such bacteria that are health promoting are added to different commercial dairy products like milk, cheese, yoghurt, lozenges, tablets, mouth rinse, capsule, chewing gums and fruit drinks. Probiotic bacteria in the oral

environment acts by competition at binding sites, production of antimicrobial substances and activation and regulation of the immune response.[5]

Protective factors have been of increasing interest over the last several decades as dentistry has shifted from treating the existing disease to preventing future disease.[6] The use of health-promoting bacteria for curative purposes is one of the strongest emerging topics in medical as well as dental field. The expanding research and medicine for preventive therapy has led to the introduction of various phytochemicals to limit the virulence of Streptococcus species.[7] Hence, this present study was conducted to compare and evaluate the efficacy of various probiotics on Streptococcus Species (i.e. *Streptococcus mutans, Streptococcus sanguinis* and *Streptococcus sobrinus*).

Aim

To compare and evaluate the efficacy of various probiotics against different *Streptococcus* species.

Objectives

- To evaluate the effect of probiotic capsules, probiotic milk and probiotic yogurt on *Streptococcus mutans, Streptococcus sanguinis, Streptococcus sobrinus*.
- To evaluate/assess the role of probiotics as a preventive tool.

METHODS

Armamentarium

- Pre and probiotic capsule (Inlife) - *Lactobacilli rhamnosus*
- Probiotic milk (Yakult) - *Lactobacilli casei Shirota*
- Probiotic yogurt (Actiplus nestle) - *Lactobacilli acidophilus*
- Gloves and mask
- Sterile containers
- Petri dishes
- Blood Agar Culture media
- Nutrient broth

Methodology

Ethical clearance was obtained for the study from the Local Ethical Committee at Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur, India. Informed consent was obtained from the parents. 10 children of age group 4-8 years belonging to moderate to high caries risk group i.e. with an average caries score of 3 and more were selected. 3-5 ml of unstimulated saliva was collected in a sterile container by using Kochmans methods over 8-10 min. Sterile saliva container were then labeled and placed in an upright position in a cool storage device using dry ice. Within 30 minutes of sample collection, the containers were transported to the Microbiology laboratory for further processing. The blood agar plates were coated with the saliva samples and incubated at 37^0C under anaerobic conditions for 24 hours. After 24 hours, based on the colony morphology and staining characteristics, the bacteria were identified as *S. mutans, S. sanguinis*, and *S. sobrinus* under 100 X magnifications. The respective bacterial colony was then transferred to 2 ml of nutrient broth and incubated for 24 hours and thus pure culture was obtained. 4 wells of 3 mm depth and 10 mm diameter were made in blood agar plates. To avoid the bias all 3 probiotic products were taken in liquid form. Probiotic capsule containing 0.6 billion lactobacilli species was diluted in 5 ml of distilled water. Pure broth culture of each bacterium was spread by carpet culture method on three agar plates using a loop of diameter 5 mm. 0.2 ml of each probiotic product was transferred into the well of each plate using sterile droppers. One empty well was kept as control. Plates were again incubated at 37^0 C under anaerobic condition for 24 hours.

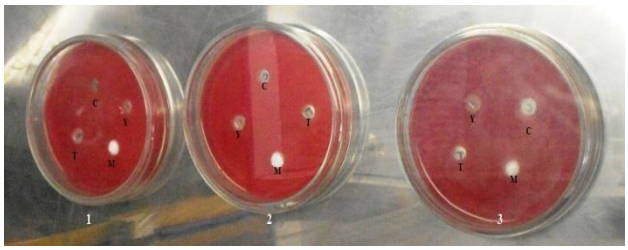

Figure 1: Blood agar plates with well (1: *S.mutans*, 2: *S. sobrinus*, 3: *S. sanguinis*).
M- milk, Y- yogurt, T- capsule, C- control.

The efficacy of this probiotic product was tested based upon zone of inhibition at 24 hours, 48 hours and 72 hours.

Statistical analysis

The statistical package for social sciences version 16 was used to determine the mean and standard deviation. Paired t test was used with confidence level of 95%, hence a p value less than or equal to 0.05 indicated a statistically significant difference.

RESULTS

After 24 hours, a zone of inhibition was observed in all the test groups, the highest for *S. mutans* against Probiotic milk. The growth of *S. mutans, S. sanguinis* and *S. sobrinus* was inhibited by the *Lactobacilli* species namely *L. casei Shirota* present in probiotic milk (Yakult), *L. acidophilus* present in probiotic yogurt (Actiplus Nestle) followed by *L. rhamnosus* present in pre and probiotic capsule (Inlife).

Table 1: Zone of inhibition after 24 hours, 48 hours and 72 hours.

	Milk	Yogurt	Capsule	Control
S. mutans				
24 hours	4.5 mm	1 mm	0.5 mm	0 mm
48 hours	5.5 mm	1 mm	1 mm	0 mm
72 hours	5.5 mm	1 mm	1 mm	0 mm
S. sanguinis				
24 hours	4 mm	1 mm	0.5 mm	0 mm
48 hours	4.5 mm	1 mm	0.5 mm	0 mm
72 hours	4.5 mm	1 mm	0.5 mm	0 mm
S. sobrinus				
24 hours	4 mm	1 mm	0 mm	0 mm
48 hours	4.5 mm	1 mm	0 mm	0 mm
72 hours	4.5 mm	1 mm	0 mm	0 mm
Statistical values	p=0.05	p=0.423	p=0.423	-

After 48 hours, the zone of inhibition had increased for all the three test materials against the three organisms. The largest zone of inhibition was for *Streptococcus mutans* and probiotic milk. The similar results were observed after 72 hours.

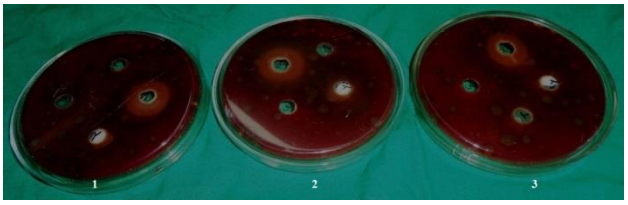

Figure 2: Blood agar plates with zone of inhibition after 24 hours (1: *S. mutans* 2: *S. sobrinus* 3: *S. sanguinis*).

A zone of inhibition was observed in all the test groups, the highest for *S. mutans* against probiotic milk after 24 hours and 72 hours was statistically significant (p=0.05), whereas zone of inhibition was seen in probiotic yogurt and probiotic capsule but was not statistically significant (p=0.423).

DISCUSSION

Dental caries is most common and debilitating oral disease and currently studies are focusing on finding preventive measures. Pediatric dentistry is now emphasizing more towards identifying the factors and to decrease the initial process of enamel demineralization and formation of dental caries. The etiology of dental caries is multifactorial process but it has direct correlation with Streptococcus species especially Streptococcus mutans.[8] The interaction of *S. sanguinus* with *S. mutans* is a significant factor associated with the caries status in children, suggesting the relative levels of these two microorganisms play an important role in caries development (Ge et al).[9] *S. mutans* and *S. sobrinus* together have a significantly higher incidence of dental caries than *S. mutans* alone (Oda et al).[10] Pathogenic microorganisms could be displaced by probiotic bacteria. Thus, the use of probiotic products could be exploited for the prevention of enamel demineralization (Bhalla et al).[11] Enamel demineralization can be prevented by increasing the calcium content of dental plaque which can be effectively done by consuming dairy products (Poureslami et al).[12] As observed in the current study that the zone of inhibition remained similar as after 48 hours. Saavedra et al stated that the long-term consumption of probiotic bacteria has not been shown to otherwise affect the health of children.[13]

Hence, it has been proven that all the probiotic products viz probiotic milk, probiotic yogurt and probiotic capsules are effective against all the three *Streptococcus* species with *S. mutans* is being highly sensitive to *S. sanguinis* and *S. Sobrinus*. Similarly probiotic milk is highly effective when compared to probiotic yogurt and probiotic capsule. The probiotic capsule was least effective of all the probiotic used.

CONCLUSION

Probiotic milk and probiotic yogurt are commercially and readily available daily consumable products and they are cost effective. So the use of these products can be inculcated in general population. Plain milk and yogurt can be replaced by probiotic milk and probiotic yogurt especially in children that will aid in prevention of most common dental disease in children i.e. “Dental Caries”. In the present study probiotic milk demonstrated highest zone of inhibition for *Streptococcus mutans*, *Streptococcus sanguinis* and *Streptococcus sobrinus*. Further studies are required to evaluate the in vivo effect of probiotics as preventive tool against dental caries.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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