

Probiotics and its multifaceted usage in dentistry

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Abstract

Background: Probiotics are micro-organisms, which confer benefits to the host's health. Many studies support the role of probiotics for improving gastrointestinal health. Several studies have been undertaken to prove its influence on oral health maintenance with the purpose of knowing the importance of using probiotics as preventive and therapeutic methods for oral infectious disease management.

Aim: The aim of this study was to design an indigenous probiotic solution and test its *in vitro* efficacy against *Streptococcus mutans*, the predominant bacteria implicated in dental caries.

Materials and Methods: 1. The aim of the present study was to design an indigenous probiotic solution and test its efficacy against *S. mutans*. 2. Proposed Indigenous Broth was formulated using apple pulp with honey and distilled water. 3. MRS broth (De Men, Rogosa and Sharpe agar): It is a selective culture media required for the growth of *Lactobacillus*. It was incubated at 35°C, pH 5–6.5 using 5% CO₂ for 24 h. It was used as a control to test the uptake of lactobacilli present in the probiotic capsules which consists of:

- *Lactobacillus acidophilus*: 0.5 billion.
- *Lactobacillus rhamnosus*: 0.5 billion.
- *Bifidobacterium bifidum*: 0.5 billion.
- *Bifidobacterium longum*: 0.5 billion.
- *Streptococcus thermophilus*: 0.25 billion.
- *Saccharomyces boulardii*: 0.25 billion.

Result: The proposed indigenous solution sustained the growth of probiotic bacteria and displayed inhibitory action against *S. mutans*.

Conclusion: The proposed solution is a viable medium of probiotics formulated from completely natural ingredients. Further studies need to be undertaken to prove its clinical efficacy.

Clinical Significance: Probiotics have been able to suppress the intraoral growth of *S. mutans* signifying that they can be used as an adjunct to routine dental care in prevention of dental caries. Probiotics can also be used in rural areas where advanced dental health-care services aren't readily available. They can also be prescribed to children and patients with physical disabilities and mental health disorders as a primary prevention against dental caries.

Introduction

Probiotics

The World Health Organization, 2001, has defined probiotics as living microorganisms which when administered in adequate amount in foods or as dietary supplements, confer benefits to the host's health.^[1] They were introduced by Elie Metchnikoff, Nobel Prize winner in Physiology and Medicine, 1908. Since

then, extensive literature refers to the use of probiotic strains (mainly Lactobacilli and Bifido bacteria), in the maintenance of balance of the human eco systems. Probiotic is being successfully used in prevention and treatment of various ailments including antibiotic associated diarrhea, bowel disease, acute pancreatitis, necrotizing enterocolitis, multiple organ dysfunction syndrome, allergy and immune response, ventilator associated pneumonia, and gastrointestinal disease. Studies have also indicated the

role of probiotics in prevention and treatment of periodontitis, oral cancer, dental caries, and oral diseases and infections. The most commonly used microorganisms as probiotic are from the *Lactobacillus* and *Bifidobacterium* genera, although, the fungus *Saccharomyces boulardii* is also considered as a probiotic.

With the ongoing evolution of food products market, ranges of probiotic containing food items have continued to grow. The most common products consisting of probiotic bacteria available in the market are dairy-based like cheese, buttermilk, milk powder, and yogurts, of which yogurt have the largest selling share.^[2,3] Some of the non-dairy products are also a source of probiotic bacteria such as soy products, fruit juices, and cereals. Few commercially available probiotic products are Yakult, Good Belly, Beyond Berry, DAN Active, PRE Probiotic Enhancer, KyoDophilus Powder, Cocobiotic, etc.

Mechanism of action of probiotic bacteria

Probiotic bacteria enhance the expression of genes responsible for maintaining intestinal barrier integrity. They also accelerate the repair of damaged mucosa and decrease apoptosis of intestinal mucosal cells. It has been seen that *Lactobacillus rhamnosus* GG is able to prevent cytokine-induced apoptosis in intestinal epithelial cell models by inhibiting tumor necrosis factor.^[5] Probiotics are also able to induce peptide production in human body cells which display antimicrobial activity against a wide variety of bacteria, fungi, and some viruses by production of antimicrobial substances. A part from this it has also been seen that strains of probiotic bacteria can compete efficiently with invading pathogens for binding sites to mucus layer and epithelial cells.^[6,7] They are thus efficiently able to modulate immune system of humans, conferring many health benefits.^[8] One another most important mechanism of action involves inhibition of quorum sensing signaling. Bacteria communicate with each other as well as with their surrounding environment through chemical signaling molecules called auto-inducers.^[9] This phenomenon is called “quorum signaling:” Cell-to-cell signal mechanisms which help bacteria to colonize. Probiotic bacteria efficiently interfere with this mechanism of disease causing bacteria.

Materials and Methods

The aim of the present study was to design an indigenous probiotic solution from homemade ingredients which could be a viable palatable alternative to direct consumption of probiotic tablets. The study was carried out from July 2018 to August 2018.

1. Name of the Capsule: Commercially available “Lee- biotic prebiotic and probiotic” capsules were dispensed into the proposed indigenous solution.

Strains of microorganisms present in the capsule were:

- *Lactobacillus acidophilus*: 0.5 billion
- *Lactobacillus rhamnosus*: 0.5 billion
- *Bifidobacterium bifidum*: 0.5 billion

- *Bifidobacterium longum*: 0.5 billion
- *Streptococcus thermophilus*: 0.25 billion
- *Saccharomyces boulardii*: 0.25 billion

2. Broth:

Proposed Indigenous Broth: Homemade broth containing apple extract (approximately 100 g) mixed with two tablespoons of honey and 100 ml of distilled water.

MRS Broth (de man, rogosa, and sharpe agar): It is a selective culture medium that allows the growth of *Lactobacillus*. The broth was incubated at 35°C and 5% CO₂ for 24 h.

3. Patient Selection: Decayed, Missing, and Filled Teeth index of 25, randomly selected patients from the OPD of SGT Dental College, Hospital and Research Institute was recorded of which ten patients were selected with highest values. Out of ten selected patients, seven were males and three were females. Mean age of patients selected was 44.1. Patients were informed about the reason for collecting the saliva samples verbally before initiating the procedure. Verbal consent was taken from each patient and their participation in the study was completely voluntary. Research and ethical committee of SGT Dental College, approved and gave ethical clearance in June, 2018.

4. Saliva collection: Unstimulated salivary sample was collected using Swab method. Swab method is also known as absorbent method and requires pre-weighed swab to be placed at the orifice of major salivary gland.^[10] Patients were asked to sit upright and rinse their mouth using normal distilled water. All the initial salivary secretions produced after rinsing the mouth were discarded by asking the patients to spit. The purpose of making the patient spit initially was to exclude any chance of collection of stimulated salivary samples. To collect the sample, sterile transport swabs were used to absorb the saliva. Samples were collected from the selected patients in the OPD of SGT Dental College, hospital, and research institute in morning hours around 11:00 AM. To collect the saliva samples, transport swabs were moved around the floor of the mouth (near the opening of submandibular salivary gland), and buccal mucosa (near the opening of parotid gland) adjacent to teeth affected by dental caries.

5. Culture sensitivity and Procedure:

- Under sterile conditions, the collected saliva samples were delivered to the Department of Microbiology, SGT University.
- *Streptococcus mutans* was isolated from these samples and incubated on Mitis salivarius agar plates under anaerobic conditions using gas packs and anaerobic jars. These agar plates were incubated for 48 h.
- Subsequently the broths, that is, MRS agar and homemade fruit juice broth were prepared. For every 5 ml of the broths, powder from a single probiotic capsule was added.
- Hence, four sections of broth were made:
 - Broth 1: MRS broth without powder from probiotic capsules (MRS).
 - Broth 2: MRS broth with powder from probiotic capsules (MRSC)

- Broth3: Homemade fruit juice broth without powder from probiotic capsules (FJ).
- Broth 4: Homemade fruit juice broth with powder from probiotic capsules (FJC).
- The above-mentioned, prepared broths, both with and without powder from capsule were incubated for 24 h under controlled conditions to allow the growth of *Lactobacillus* bacteria.
- Following the growth of *Lactobacillus* bacteria, plates were prepared using broth 1 and broth 3, applied on lawn cultures of *S. mutans* on Mitis Salivarius agar plates using disk diffusion method and plates were incubated. These culture plate was named using prefix Plate A for every patient (plate A1-A10).
- Other plates of Mitis salivarius agar, Plate B (plate B1-B10) was used to pipette material from all the four prepared broths and culture sensitivity was tested using disc infusion method.
- Similarly, Plate C (plate C1-C10) was set to pipette material from the all the four broths and tested using well diffusion method.
- Both well and disk diffusion methods were used simultaneously to avoid any chances of error.
- Plates were checked for appearance of inhibitory zones after 8 h, 24 h, 32 h, and 48 h.

Results

No positive results were found in any of the plate after 8 h. Most of the culture Plates B and C (1–10) showed zones of inhibition after 24 h and those in majority of Plates A (1–10) could be observed after 32 h. Radius of zone of inhibition increased in Plates C (1–10) after 48 h.

Zones of inhibition demonstrated in plates A1-A10 with culture of broths 1 and 3 signifies that when the indigenous broth is allowed to ferment, it acts as a natural reservoir for growth of probiotic bacteria that acted against *S. mutans*. It takes 32–48 h for the fruit juice broth to fully support the growth of probiotic bacteria, suggesting that home-made broth should be left for 32–48 h to ferment.

The culture Plates B and C demonstrating zone of inhibition against *S. mutans* when broths containing the probiotic bacteria (both from the capsule and without the content of capsule) were pipette onto them, signifies that the indigenous fruit broth also acts similarly to MRS broth as a selective medium for *Lactobacillus* species. Well diffusion method used for checking antimicrobial activity was more efficient than disc diffusion method [Tables 1-3].

Discussion

Probiotic has always been the area of interest among researchers. Many studies are ongoing and many have taken place to establish the fact that probiotics can effectively be used against

Table 1: Appearance of inhibitory zones after 24 h

Patient number	Plate A		Plate B		Plate C	
1	A1	NO	B1	YES	C1	YES
2	A2	NO	B2	NO	C2	NO
3	A3	YES	B3	NO	C3	YES
4	A4	NO	B4	NO	C4	YES
5	A5	NO	B5	YES	C5	YES
6	A6	YES	B6	YES	C6	NO
7	A7	NO	B7	NO	C7	YES
8	A8	YES	B8	YES	C8	YES
9	A9	NO	B9	YES	C9	YES
10	A10	NO	B10	YES	C10	YES

Table 2: Appearance of inhibitory zones after 32 h

Patient number	Plate A		Plate B		Plate C	
1	A1	YES	B1	YES	C1	YES
2	A2	YES	B2	YES	C2	YES
3	A3	YES	B3	NO	C3	YES
4	A4	NO	B4	YES	C4	YES
5	A5	YES	B5	YES	C5	YES
6	A6	YES	B6	YES	C6	YES
7	A7	YES	B7	YES	C7	YES
8	A8	YES	B8	YES	C8	YES
9	A9	NO	B9	YES	C9	YES
10	A10	YES	B10	YES	C10	YES

Table 3: Appearance of inhibitory zones after 48 h

Patient number	Plate A		Plate B		Plate C	
1	A1	YES	B1	YES	C1	YES
2	A2	YES	B2	YES	C2	YES
3	A3	YES	B3	YES	C3	YES
4	A4	YES	B4	YES	C4	YES
5	A5	YES	B5	YES	C5	YES
6	A6	YES	B6	YES	C6	YES
7	A7	YES	B7	YES	C7	YES
8	A8	YES	B8	YES	C8	YES
9	A9	NO	B9	YES	C9	YES
10	A10	YES	B10	YES	C10	YES

pathogens causing oral disease. Alamoudi *et al.*^[11] in his study on 178 children (age groups – 3–6) observed that children, when administered probiotic lozenges twice daily for 28 days had less incidence of dental caries and relatively less development of plaque than children who were not administered probiotics. Pahumunto *et al.* had almost similar observations in his study conducted on 124 children.^[12] His study group was fed on milk

containing *Lactobacillus paracasei* continuously for 3 months and later it was observed that the children had less level of *S. mutans* in their mouth due to the inhibitory action of *L. paracasei*.

Patients undergoing fixed orthodontic therapy are at a higher risk of developing periodontal problems and dental caries due to difficulty in maintaining oral hygiene and personal negligence. According to Contreras *et al.*, daily consumption of probiotics can be used in conjunction to orthodontic therapy to prevent development of such lesions.^[13] Cildir *et al.*^[14] in their study on patients with orthodontic appliances had similar observations. Patients who consumed fruit yogurt rich in Bifidobacterium had fewer levels of *S. mutans*.

Nikawa *et al.*^[15] mentions that yogurt rich in *Lactobacillus reuteri* has inhibitory effect on growth of *S. mutans*. Strains of *Lactobacillus* other than *L. reuteri* were unable to exert this inhibitory effect signifying that only certain groups of probiotic are active against *S. mutans*. Pujia *et al.*^[16] mention in their review article that *L. paracasei* increase the amount of salivary peptide HNP1-3 which is responsible for reduction in presence of dental caries.

Probiotic can also be adopted as one of the active ingredient of toothpaste, making its delivery easy among patients. Apart from this, its topical application can fasten the action of probiotics on *S. mutans*, increasing its efficacy. Bhupesh *et al.* incorporated LAB strain *L. acidophilus* JD11 in his own formulated toothpaste and with the help of his study he concluded that LAB isolates can remain viable in toothpaste formulation and can help overcome the side effects of germicidal used in toothpaste.^[17] *Lactobacillus* is capable of producing bacteriocin which enables it to compete with other commensals of the oral cavity. Incorporation of probiotics in other similar products can be beneficial to the public. Probiotic infused mouthwash can also become one handy option and can successfully reduce the activity of *S. mutans*.^[18]

Probiotic can be of special use in children to avoid dental caries. Näse *et al.* concluded from their study on 594 children that children when fed on milk rich in *L. rhamnosus* GG had less incidence of caries than those children who were fed on plain milk.^[19]

Mayanagi *et al.* states in their study that probiotic could serve as an adjunct to mechanical debridement in cases of periodontal disease.^[20] Furthermore, they can be administered on daily basis to avoid development of any periodontal pathogen in oral cavity. Shimazaki *et al.* evaluated the effect of lactic acid producing bacteria on periodontal health status of patients in Japan.^[21] They concluded that patients who consumed lactic acid foods had less probing depth and clinical attachment loss than people without the consumption of probiotics.

Production of volatile sulfur compounds by oral pathogens such as *Prevotella intermedia*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Fusobacterium nucleatum* is one of the major causes of halitosis. Malathi *et al.* mentions in their review article that gargling with a probiotic solution could reduce the production of sulfur compounds and aid in elimination of bad breath.^[22]

In the present study, the indigenous broth could sustain the probiotic bacteria and was found to be active against *S. mutans*,

hence could be a viable medium to deliver beneficial probiotic bacteria in a palatable, completely natural way. Further, it could be beneficial to population present in rural areas as the commercially marketed probiotic drinks, lozenges, etc., might not be readily available to them. Probiotics can also serve as a method of prevention of dental caries among children and mentally ill patients.

Second, it could be a useful and affordable alternative to the commercial probiotic solutions in community outreach programs to promote awareness about the multifaceted benefits of probiotics. The limitation of this study is that the longevity of the broth is questionable and further its clinical efficacy needs to be studied.

Conclusion

The indigenous broth is a viable alternative for administration of probiotic bacteria which is active against *S. mutans*, the bacteria predominantly implicated in dental caries.

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