Evaluation of efficacy of probiotic (BIFILAC) on Porphyromonas gingivalis: In vitro study

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ABSTRACT

Background: Periodontitis is inflammation of the periodontium and causes destruction of the connective tissue attachment of the teeth and alveolar bone. Porphyromonas gingivalis is the primary pathogen for the destructive periodontal diseases. The aim of the study is to evaluate the efficacy of probiotic on P. gingivalis.

Materials and Methods: An in vitro study was done to analyze the effectiveness of probiotic BIFILAC on P. gingivalis was determined using disc diffusion method. The minimum inhibitory concentration for BIFILAC lozenges was also determined using microdilution method. Results: In disc diffusion method, the antibacterial activity of BIFILAC was analyzed using various concentrations such as 2.5, 5, 10, and 20 µg/ml, of which 20 µg/ml was proved to have a maximum inhibitory zone of 22 mm. In microdilution method, concentration ranging from 7.25 to 100 µg/ml was used and 25 µg/ml was found to have the minimum inhibitory effect on P. gingivalis. Conclusion: The present in vitro study confirms that probiotic BIFILAC has an antimicrobial effect against P. gingivalis. Thus, proving that BIFILAC probiotic can be used as an adjunctive therapeutic modality in periodontitis.

KEY WORDS: Bacterial replacement therapy, periodontitis, Porphyromonas gingivalis, probiotics

Dental plaque a biofilm consisting of microorganisms is considered as an important etiological factor initiation and progression of periodontal diseases. There are thousands of species that are isolated in the oral cavity, of which Porphyromonas gingivalis, Gram-negative, rod-shaped, anaerobic, nonmotile species is a primary pathogen for causation of periodontal disease. It has been shown in an in vitro study that P. gingivalis can invade human gingival fibroblasts and gingival epithelial cells in high numbers and can survive in the presence of considerable concentrations of antibiotics. Collagen degradation observed in chronic periodontal disease results in part from the collagenase enzymes of this species. The inflammatory process can be slow down by means of mechanical and chemical plaque control. There has been a major shift in treatment paradigm, and the treatment modality is now focused on nonspecific approach to a specific approach.

Bacteriotherapy in the form of probiotics is the natural way to maintain health and to protect the oral tissues from diseases. Probiotics are “living organisms” which when administered in sufficient quantity has got the beneficial effect on the health of the host. They play a vital role in halting, altering, or delaying periodontal diseases. In the field of periodontics, probiotics have got a greater potential in plaque control, altering the bacterial colonization, and in improving the clinical parameters such as reduction in gingival bleeding, pocket depth, and clinical attachment loss. The probiotics inhibit the pathogen adhesion, colonization, and inhibit the growth of pathogen by the production of various substances which ultimately inhibit the biofilm formation. It has got numerous effects on a host such as inhibition of collagenases, inflammatory associated molecules, cytoprotective proteins in host cell surfaces and cytokine-induced apoptosis. It also modulates host immune response and proinflammatory pathways induced by pathogens.

Lactobacillus and Bifidobacterium are most commonly used genera in probiotics. This in vitro study aims at...
determining the combined efficacy of probiotic formulation on *P. gingivalis*.

**Materials and Methods**

**Probiotic product**

The study product (BIFILAC-lozenges) contains *Lactobacillus sporogenes* 100 million, *Streptococcus faecalis* T-110 Joint Parliamentary Committee (JPC) 60 million, *Clostridium butyricum* TO-A 4 million, and *Bacillus mesentericus* TO-A JPC 2 million. The combination of probiotic strains has been used that acts synergistically and enhances the possibility for permanent installation.

**Preparation of test organism**

**Preparation of bacterial suspension**

The average number of viable, Gram-negative *P. gingivalis* organisms per ml of the stock suspensions was determined using the surface viable counting technique. About \((10^8-10^9)\) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constantly so that suspensions with very close viable counts would be obtained.

**Antibacterial activity screening**

**Disc diffusion method**

The periodontopathic bacterial strains *P. gingivalis* ATCC33277 obtained from NCIM, Pune, was grown in half-strength brain heart infusion (BHI) broth (HiMedia Laboratories, USA) supplemented with 5 mg/mL yeast, 5 µg/mL hemin, and 1 µg/mL vitamin K1 (BHI-HK). The bacteria are grown at 37°C anaerobically (85% N₂, 10% H₂, and 5% CO₂). The disc diffusion method⁴ was used to screen the antimicrobial activity. The antimicrobial activity was determined using half-strength BHI agar in which 5% defibrinated sheep blood was supplemented, and the optical density (OD) of the bacterial inocula was adjusted to 0.1 at 600 nm (0.5 McFarland standard). The bacterial inoculum suspension (100 µL) was swabbed uniformly on a blood agar plate, and the plate was allowed to dry for 5 min. Various concentrations of extracts (2.5, 5, 10, and 20 µg/mL) were loaded at 20 µL onto a 6 mm sterile disc (50, 100, and 200 µg/disc, respectively). The disc loaded with extracts was placed on the surface of the medium, the compound loaded was allowed to diffuse for 5 min, and the plates were incubated at 37°C for 48 h. At the end of the incubation, the inhibition zones formed around the loaded disc were measured with a transparent ruler in millimeter units. This experimental study was performed in triplicate.

**Determination of the minimal inhibitory concentrations**

Minimal inhibitory concentration (MIC) was determined with 96-well plate microdilution method.⁵ Briefly, the bacterial strain was grown for 24 h anaerobically and inoculated into a final volume of 100 µL of new half-strength BHI broth containing 2-fold serial dilutions of samples. The final OD of the bacterial cells was adjusted to 0.1 at 600 nm in 100 µL of mixture. The mixture was cultured anaerobically at 37°C for 48 h and the bacterial growth was evaluated at an OD of 600 nm. The lowest concentration at which no growth (OD 600 nm ≤0.1) was observed was defined as MIC (µg/mL). The concentration of the sample used for MIC was between 7.25 and 100 µg/mL.

**Results**

This in vitro study examined the viability of *P. gingivalis* in the presence of probiotic (BIFILAC). Preliminary screening for antibiotic activity of BIFILAC on *P. gingivalis* is done using disc diffusion method. Various concentration of BIFILAC (2.5, 5, 10, and 20 µg/ml) was used to determine the antibacterial activity of which 20 µg/ml had higher inhibition zone of 22 mm as shown in Table 1 and Graph 1. The minimum inhibitory concentration was determined with the aid of microdilution method and the concentrations used ranged from 7.5 to 100 µg/ml. Of which the MIC of bifilac was 25 µg/ml as shown in Table 2.

**Discussion**

Probiotic technology represents a novel approach to maintain oral health by utilizing the natural beneficial bacteria.⁶ The use of probiotics for promoting general health was extensively studied over the past century after Elie Metchnikoff developed the hypothesis that lactic acid producing bacteria

| Table 1: Mean diameter inhibition zone (mm) of probiotic BIFILAC using disc diffusion method |
|-----------------------------------------------|---------------|
| BIFILAC concentration (µg/ml) | Mean diameter inhibition zone (mm) |
| 2.5 | 8 |
| 5 | 10 |
| 10 | 18 |
| 20 | 22 |

| Table 2: Minimal inhibitory concentration (µg/ml) of probiotic BIFILAC using microdilution method |
|-----------------------------------------------|---------------|
| Sample | Minimal inhibitory concentration (µg/ml) |
| BIFILAC | 25 |

**Graph 1: Minimum inhibitory zone**
in gastrointestinal tract could be beneficial for general health.[6] Probiotic species are thought to act through a variety of mechanisms including (i) exclusion and competition with potential pathogens for nutrients and epithelial cell adhesion, (ii) production of antimicrobial substances against periodontal pathogenic organisms, (iii) local and systemic immunomodulations, and (iv) enhancement of the mucosal barrier function. Immunomodulatory action of the probiotics regulates anti-inflammatory and proinflammatory cytokine production.[7] Probiotics lower the pH so that microorganisms cannot form plaque and calculus. They make an excellent maintenance product since they produce antioxidants and it prevents stain formation and plaque formation by neutralizing the free electrons that are needed for mineral formation.[8] In inflammatory conditions such as periodontitis, the pathogenic organisms play a vital role in disease progression.

P. gingivalis is a “Red complex” pathogen and a “keystone” bacterium in the onset of chronic periodontitis. Though P. gingivalis is found in low abundance in the oral cavity, it causes a microbial shift of the oral cavity, allowing for uncontrolled growth of the commensal bacterium.[9] P. gingivalis has many ways to evade host immune response, by using gingipain proteases, a capsular polysaccharide induction of host cell proliferation and the cleavage of chemokines responsible for neutrophil recruitment. We selected probiotic (BIFILAC) as the material of choice for our present study as it contains L. sporogenes, S. faecalis, C. butyricum, and B. mesentericus as the main constituents. Socransky et al. 1998[9] stated that Lactobacillus is one of the important probiotic strain that has been used and the inhibitory action against periodontal pathogens by the production of acid. Another study by Silva et al. 1987[10] found that Lactobacillus can produce different antimicrobial components including organic acids, low molecular weight antimicrobial substances, hydrogen peroxide, bacteriocins, and adhesion inhibitors. Very recently, Köll et al. 2008[11] characterized 22 strains of orally isolated lactobacilli with regard to antimicrobials activities on oral pathogens including pathogenic bacteria and tolerance to environmental stress in vitro.

The majority of probiotic strains including Lactobacillus salivarius were shown to suppress the growth of Aggregatibacter actinomycetemcomitans, P. gingivalis, and Prevotella intermedia suggesting a potential for oral lactobacilli to be used as probiotics for periodontal health. This is in accordance with our in vitro study in which the BIFILAC at a concentration of 20 μg/ml has shown maximum antibacterial activity and even at 7.5 μg/ml there is inhibition in growth of P. gingivalis. According to Matsuo et al., 2006,[12] the oral administration of probiotic tablets containing L. salivarius T12 711 (LS1) to healthy subjects significantly reduced the number of P. gingivalis in the saliva and subgingival plaque. In a study by Vivekananda et al., 2010,[13] the usage of Prodentis lozenges in chronic periodontitis patients had a reduction in a number of P. gingivalis, A. actinomycetemcomitans, and P. intermedia. Hence, it is evident that probiotics can be used as an adjunctive in addition to mechanical debridement during the maintenance phase. Further clinical trials are required to provide a strong evidence for the use of probiotics in periodontics.

**Conclusion**

The present in vitro study confirms that probiotic BIFILAC has an antimicrobial effect against P. gingivalis. Thus, proving that BIFILAC probiotic can be used as an adjunctive therapeutic modality in periodontitis.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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