

## Effect of Freeze Dried Powdered Probiotics on Gingival Status and Plaque Inhibition in Patients with Chronic Periodontitis

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### ABSTRACT

The term Probiotics was coined by Lilly and Stillwell in 1965. In, 1991 Holocombh was the first person to do research on Bifidobacterium bifidum as probiotics species. In the past few years, probiotics have been investigated for periodontal health. Studies have shown that certain gut bacteria can exert beneficial effects in the oral cavity by inhibiting pathogenic species. Studies have revealed that probiotic *Lactobacillus strains* (*L. reuteri*, *L. salivarius*, *L. casei*, *L. acidophilus*) were useful in reducing gingival inflammation. The present study was to be conducted to evaluate the effects of freeze-dried powdered probiotics on gingival status and plaque inhibition in patients with chronic periodontitis.

### **KEYWORDS**

Probiotics; Chronic periodontitis; Gingivitis; Gingival index; Plaque index

### INTRODUCTION

At the start of 20<sup>th</sup> century, the concept of bacteriotherapy was introduced and during the same era, Russian Nobel prize winner and father of modern immunology, Elie Metchnikoff, a scientist at the Pasteur University, was the first to conceptualize “Probiotics”. Pharmabiotics and probiotics in current use or under development belong to 2 of 3 domains of life, Eukarya (eg: Yeasts) and Bacteria (eg: Lactobacilli) [1]. The concept of probiotics has opened a new horizon on the relationship between diet and oral health [2]. Numerous studies have shown that probiotic bacteria administered in any form are safe for human use to a large extent [2].

Properties of probiotics have made it a subject of interest for various fields such as biotechnology, microbiology, etc. In the early 1930's, in Japan, Minoru Shirota developed a fermented milk product called Yakult. In 1935 he started marketing it as probiotic yogurt like product made by fermenting a mixture of skimmed milk with a special strain of *Lactobacillus casei shirota* [2]. With increasing antibiotic resistance, in recent years, the use of such beneficial bacteria in improving health of the host has gained much popularity in the field of medical research [1,2].

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### ***Functions of Probiotics***

Production of antimicrobial substances [1,2].

- Organic acids.
- Hydrogen peroxide.
- Bacteriocins.
- Biosurfactants.

Binding in oral cavity [3]

- Compete with pathogens for adhesion sites.
- Involved in metabolism of substrates.

Co-immunomodulatory [4]

- Stimulate non specific immunity.
- Modulate humoral or cellular immune response.

Miscellaneous [4]

- Lowers serum cholesterol.
- Reduction in risk of colon cancer.
- Improves lactose tolerance.

### ***Enhancement of Epithelial Barrier***

The intestinal epithelium is in permanent contact with luminal contents and the variable, dynamic enteric flora. The intestinal barrier is a major defense mechanism used to maintain epithelial integrity and to protect the organism from the environment. Defenses of the intestinal barrier consist of the mucous layer, antimicrobial peptides, secretory IgA and the epithelial junction adhesion complex. Once this barrier function is disrupted, bacterial and food antigens can reach the submucosa and can induce inflammatory responses, which may result in intestinal disorders, such as inflammatory bowel disease. Consumption of non-pathogenic bacteria can contribute to intestinal barrier function, and probiotic bacteria have been extensively studied for their involvement in the maintenance of this barrier. Several studies have indicated that enhancing the expression of genes involved in tight junction signaling is a possible mechanism to reinforce intestinal barrier integrity [5].

### ***Increased Adhesion to Intestinal Mucosa***

Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is important for the interaction

between probiotic strains and the host. Lactic acid bacteria (LABs) display various surface determinants that are involved in their interaction with intestinal epithelial cells (IECs) and mucus. IECs secrete mucin, which is a complex glycoprotein mixture that is the principal component of mucus, thereby preventing the adhesion of pathogenic bacteria. The microbial adhesion process of LAB also includes passive forces, electrostatic interactions, hydrophobic interactions, steric forces, lipoteichoic acids and specific structures, such as external appendages covered by lectins [5].

### ***Competitive Exclusion of Pathogenic Micro Organisms***

The mechanisms used by one species of bacteria to exclude or reduce the growth of another species are varied, including the following mechanisms: creation of a hostile microecology, elimination of available bacterial receptor sites, production and secretion of antimicrobial substances and selective metabolites, and competitive depletion of essential nutrients. Specific adhesiveness properties due to the interaction between surface proteins and mucins may inhibit the colonization of pathogenic bacteria and are a result of antagonistic activity by some strains of probiotics against adhesion of gastrointestinal pathogens. Lactobacilli and bifidobacteria have been shown to inhibit a broad range of pathogens, including *E. coli*, *Salmonella*, *Helicobacter pylori*, *Listeria monocytogenes* and Rotavirus. Organic acids, in particular acetic acid and lactic acid, have a strong inhibitory effect against Gram-negative bacteria, and they have been considered the main antimicrobial compounds responsible for the inhibitory activity of probiotics against pathogens [5].

### ***Clinical Relevance of Probiotics in Oral Diseases***

#### ***Dental caries***

*Streptococcus Mutans* is the most common organism causing dental caries and efficacy of probiotics on dental caries has been studied worldwide using different strain species of Lactobacillus like *L. rhamnosus* and *L. casei*. Probiotics neutralize acidic conditions and thus they help

in management of caries [3]. In a study conducted by Nikawa et al. in 2004, it was shown that concentration of *S. mutans* was reduced up to 80% when yogurt containing *L. reuteri* was consumed for 2 weeks [6].

#### ***Candida species management***

Hatakka et al. were the first person to study the effect of probiotics on the prevalence of oral Candida. The study showed a reduction of *C. Albicans* after taking probiotics in cheese [7].

#### ***Periodontal disease***

Various studies worldwide reported that periodontal disease could also benefit from the oral probiotic intake. This may be due to probiotic species like Lactobacillus which have the property to inhibit the growth of periodonto pathogens [3]. According to a study by Volozhin et al. in 2004, probiotic strains when given in a concentration of 10<sup>8</sup> CFU/ml in periodontal dressing have shown to reduce the number of periodontal pathogens like Bacteroides species [8].

#### ***Probiotics: A marvellous factor***

The list of beneficial effects attributed to probiotic bacteria is extensive that includes alleviation of lactose intolerance symptoms; serum cholesterol reduction; anticancer effects alleviating constipation, relieving vaginitis to name but a few studies have shown that probiotic bacteria prevent putative preneoplastic lesions or tumors induced by carcinogens such as 1,2-dimethylhydrazine or azoxymethane. Probiotic bacteria have antiviral effects. The mechanism of probiotic bacteria is not known, laboratory tests show that the inactivation of viruses can occur by chemical biological substances, such as extracts from marine algae extracellular agents of bacteria. The use of probiotics as beneficial bacteria, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to use antibiotics. Some benefits linked to the administration of probiotics are as competitive exclusion of pathogenic bacteria as a

source of nutrients enzymatic contribution to digestion as direct uptake of dissolved organic material mediated by the bacteria. Probiotics also act as enhancement of the immune response against pathogenic microorganisms [9,10].

#### ***Safety of Probiotics***

The main areas for probiotics use are prevention and prophylaxis or treatment of GIT disorders. The growing interest in healthy functional food underlies the basis of the expanding probiotic use. To fulfil public requirements of general health improvement and immune system strength, there is a demand for the development of new and more active strains of so-called friendly bacteria [9]. The principal idea seems to be “do more good than harm” [10,11]. Therefore, specific work should be done for the assessment of potential probiotic pathogenicity, including the dose or duration of use. When administered, the probiotics are alive and capable of producing toxins or possible infection in the body [11]. It should be remembered that the introduction of exogenous microorganisms might be related to health hazards. Several genus, species, or even strains of microorganisms are used as probiotics; their safety is strain-specific and depends on specific microbe properties. An important source of probiotic safety data is case reports. These kinds of reports detail epidemiological investigation and represent events that mostly happened in hospitalized, critically ill patients. Another important tool contributing to the knowledge of probiotic safety is data derived from meta-analysis. The idea of meta-analysis expanded scientific potential. Meta-analysis gives the possibility of insight into different studies and summarizes its results. The conclusions and safety profile cannot be generalized. Specific strain and target population should always be considered.

The aim of the present study was to evaluate the effects of freeze-dried powdered probiotics on gingival status and plaque inhibition in patients with chronic periodontitis.

## **MATERIALS AND METHOD**

### ***Source of Data***

Patients visiting the Department of Periodontics, of the institution and satisfying the inclusion and exclusion criteria were selected for the study.

### ***Sample Size***

This study had a sample size of 10 patients diagnosed with chronic generalized periodontitis.

### ***Inclusion Criteria***

- Systemically healthy patients.
- Age group 25 years - 55 years.
- Patients diagnosed with moderate to severe chronic periodontitis.

### ***Exclusion Criteria***

- Smokers.
- Medically compromised patients.
- Patients on antibiotic therapy in 6 months prior to the study.

Baseline Turesky Gilmore Glickman plaque index (PI) and gingival index (GI) were assessed for all the patients. The plaque index was assessed by a disclosing agent Alpha plac (two tone dye) using cotton tips. After thorough scaling and root planning, freeze dried powdered probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* (Prowel, Alkem Laboratories) was prescribed to the patients. All subjects were instructed to mix the powder in 30 ml of water and swish once daily for 3 minutes, for a week. Turesky Gilmore Glickman plaque index (PI) and gingival index (GI) were assessed for all the patients after 7 days.

## **RESULTS**

For the calculation of results Wilcoxon signed rank test was used and a p value of  $\leq 0.05$  was considered statistically significant. It is evident by the outcomes of the present study that there was a short-term improvement in

the mean GI and PI scores during subsequent week. Results of PI and GI are elaborated in Table 1.

	<b>Baseline</b>	<b>1 Week</b>	<b>p Value</b>
Plaque Index	1.52	1.45	<0.018
Gingival Index	2.9	2.79	<0.016

**Table 1:** Comparison of plaque index and gingival index at baseline and after one week.

## **DISCUSSION**

In our study we used symbiotic in the form of mouth wash, and it was given to 10 patients after scaling and root planning, which had beneficial effect on oral health of the patients. Gross et al reported that probiotic mouth rinses containing an active ingredient nisin showed bactericidal activity against a wide range of Gram-positive bacteria [12]. Similar results have been achieved in our study also by using Prowell synbiotic powder as a mouth wash. Calgar et al. concluded that probiotics have a beneficial effect on oral health when administered in the form of lozenges and chewing gums [13].

Wider application of probiotics in general health can increase its demand<sup>16</sup>. Genetically modified lactic acid bacteria have been proposed as a vehicle to deliver vaccines in the gastro-intestinal tract. In field of oral immunology probiotics are being used as passive local immunization vehicles against dental caries [14]. Recently by means of systemic immunization with a multivalent vaccine, *L. rhamnosus* GG was chosen as the vehicle to harbor IgG because of its widely known health benefits in humans and animals. Such advancement will lead to the development of a new generation of probiotics, the action of which could be selected for defined disease-associated deviations in gut microbiota [14]. This may also facilitate the potential use of genetically modified probiotics bacteria for pharmaceutical uses. Various processing advances, such as microencapsulation and bacterial coating and addition of prebiotic compounds used as growth factors for probiotic organisms, will provide a means to optimize the delivery and survival of strains [14]. For all these valid reasons, the use of probiotics has become an emerging subject in the field of dentistry at

present. Probiotics combined with prebiotics are innovative and revolutionary method in the treatment of dental diseases.

### **CONCLUSION**

The use of probiotics is an interesting emerging and not to be neglected field in general and oral health care. Probiotics have made their way into oral health care and are more likely to be our friend than one enemy. Despite our rapidly increasing knowledge of pathogen-host

interactions, the role of beneficial bacteria in preventing the emergence of pathogen species and oral health remains obscure. There is a great need to elucidate the role of oral beneficial microbiota, to identify beneficial bacteria and to conduct proper large-scale studies on the usefulness of probiotics to maintain and improve oral health.

### **CONFLICT OF INTEREST**

The authors attest there is no conflict of interest in the creation of this article.

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