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ANTAGONISTIC ACTIVITY OF PROBIOTIC *BACILLUS MEGATERIUM* AGAINST *STREPTOCOCCUS MUTANS*

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ABSTRACT

As part of a screening program aimed at the discovery of new lead compounds for the treatment of oral diseases, probiotic *Bacillus megaterium* isolated from fish gut was studied for its anticaries activity. Our objective is to enumerate the antibacterial effect of *Bacillus megaterium* against the dental caries causing micro organism *Streptococcus mutans.* Agar well diffusion to determine Zone of inhibition and MIC value was assessed through broth 2-fold macro dilution method.Our study result showed that the fish gut bacteria possess potent inhibitory activity against potentially deleterious oral bacterial pathogen, *Streptococcus mutans*($P \leq 0.05$). Zone of inhibition was directly proportionate to probiotic concentration. At 2000µg of the probiotic concentration % inhibition of the oral pathogenic bacteria was more than 50%. This clearly indicates that MIC value was low against *Streptococcus mutans*. In conclusion the present investigation coincides with the earlier reports suggesting the potential use of probiotic *Bacillus megaterium* as an antimicrobial agent in treatment of Dental careis. However, detailed investigation of possible mechanism involved in anticaries activity must be probed through molecular studies.

KEYWORDS: ***Bacillus megaterium*, Probiotic, Dental Caries, *Streptococcus mutans,* oral pathogen**

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INTRODUCTION

Dental caries are commonly observed chronic bacterial disease prevalent mainly in children manifested as pain, inflammation, decayed teeth that are rarely fatal[1-2]. Dental infections are costly due to symptomatic treatment and non availability of preventive measures to control. The imbalances and accumulation of bacterial communities on tooth surfaces will result in plaque, decay and periodontal diseases due to the release of volatile sulphur compounds as a byproduct of microbial degradation of particles present in food[3-5]. Additionally, from primary caries, secondary caries may also occur due to micro leakage on the tooth surface [6]. *Streptococcus mutans* is the main etiological agent that has multiple mechanisms to colonize the tooth surface with high cariogenic potential[7-11]. As emphasized by Kiberstis and Roberts, the confronting problem for the biomedical researchers today is to analyze the factors involved in the disease process and to develop the strategy for diagnosis, prevention and therapy [12]. Dental caries cannot completely evade but can be managed by a multitude of interventions which includes maintenance of oral hygiene, diet and use of anticaries agent[13-17]. An effective treatment could be to promote colonization of caries inhibiting probiotic bacteria, a novel concept that needs further exploration. Use of probiotics to refurbish the oral non pathogenic bacteria has shown favourable results on the reduction of dental caries [18-20]. Probiotic bacteria, defined as "live microorganisms when administered in adequate amounts confer a health benefit on the host" plays a major role in the maintenance of oral health[21, 22]. Mechanisms of probiosis include alteration of intestinal microbial flora, inhibition of pathogens, immunomodulation, and instigation of cell proliferation and differentiation of the intestinal barrier. The mechanisms by which probiotics exert their effects largely involves alteration in pH of the gut, production of metabolites to antagonize the pathogens by competing for the receptor sites, nutrients and growth factors [23-26.] Probiotic bacteria that are known to have valuable effects in humans are mostly strain dependent[27-28] . *Lactobacillus* GG is a well documented probiotic bacteria that significantly minimizes the occurrence of nasal colonization with PPB[30-31](potentially pathogenic bacteria PPB(*Streptococcus pneumoniae*, *Staphylococcus aureus*, hemolytic streptococci, and *Haemophilus influenzae* reduces oxidative enzyme activity, and stimulating immunologic memory[31,32]. The gut microflora that can inhibit the growth or kill pathogenic microorganisms have a distinct advantage [33]. Comelli et al (2002) evaluated the efficacy of 23 dairy bacterial strains that could prevent dental caries, reported that *Streptococcus thermophilus* and *Lactcoccus lactis* adhered to a biofilm like dental plaque[34]. In vitro and invivo yoghurt with live bacteria showed selective anti-mutans activity with the bactericidal effect. Following the consumption of probiotics obtained from intestinal microbiota, development of oral tolerance and immunity mediated through immune response such as phagocytosis, modulation of the induced responses of CD4,, ICAM-1 production of Ig A by B cells, increase in IL-10 levels has been observed[,48]. *Bacillus megaterium ,* secretes secondary metabolites such as amino acids, enzymes and antibiotics forms a barrier that protect host from invading pathogens[49,50]. Hence, the focal theme of this study was to assess the antagonistic potential of probiotic fish gut bacterium *Bacillus megaterium* against caries causing microorganism *Streptococcus mutans.*

MATERIALS AND METHODS

Probiotic bacteria *Bacillus megaterium* was isolated from *Labeo rohita* fish gut[44]. The chemicals and media were procured from Merck and Himedia, India. In a 250 ml Erlenmeyer flasks 100 ml of nutrient broth containing bacterial culture were left 48 hours of incubation at 35 ± 1ºC in a shaker at 125 cycles/min. Then, it was centrifuged for 15 minutes, at 10,000 g, and supernatants were filtered (0.22 µm). The filtrates were lyophilized and different concentrations were used further for antimicrobial assays. Pathogenic bacteria *Streptococcus mutans*(MTCC 890) was obtained from Microbial Type Culture Collection (MTCC, Chandigarh, India)

Antibacterial activity

One cm diameter wells were punched in each plate before adding 200 µl aliquots of different concentration of lyophilized 24 h bacteria wherein fourth well on each plate inoculated with 200 µl sterile growth medium was negative control. The clear inhibitory zone formed around the wells indicates antibacterial activity. Each test was repeated three times and the mean of diameter of the inhibition zones (mm) confers antibacterial activity. Additionally, Chloramphenicol 1 g^{-1} final concentration served as a positive control.

Evaluation of minimum inhibitory concentration

MIC value was assessed through broth 2-fold macro dilution method. Briefly, the stock solution (5000 µg/mL) of the *Bacillus megaterium* was prepared by dissolving 5 mg of the culture in 1 ml distilled water. Various concentrations (1000, 2000, 3000, 4000 and 5000 µg /ml) were prepared with distilled water using the stock solution. The tubes containing *Streptococcus mutans* were incubated with the varying concentration of CFS aerobically at 30°C for 24 h. After incubation, the tube which shows no growth was considered as the MIC value of the microorganism[35]. The experiment was repeated three times using Cholramphenicol as a standard drug.

STATISTICAL ANALYSIS

The statistical analysis were carried out using SPSS software and expressed as mean ± SD.

RESULTS AND DISCUSSION

The results indicate that that there is gradual decrease in growth of pathogenic bacteria causing dental caries. There is simultaneous increase in antibacterial activity with increased concentration of probiotic($P \leq 0.05$). The highest diameter of inhibitory zone (23mm) was observed with probiotic when compared to standard drug (20mm) figure 1. Minimum inhibitory concentration

of probiotic required is lower than the standard antibiotic figure 2. There exists immense search for microorganisms that can produce antimicrobial compounds useful for many applications. The most promising compounds display low minimum inhibitory concentrations and produced cost-effectively. Recent researchers have revealed that the ability to inhibit other bacteria is most commonly found in bacteria isolated from aquaculture environments. It is often asserted that a novel probiotic organism targeted toward its utilization in the gastrointestinal tract conditions must be obtained from the gastrointestinal tract of the animal species [36]. Hence the probiotic bacteria were isolated from the gut of *Labeo rohita*. Probiotic bacteria are used in prophylaxis and therapy of broad range of human diseases and syndromes. Previous investigations revealed that intake of products containing probiotic lactobacilli could reduce the streptococci in saliva[37- 39]. Children in a day care centre in the 3–4-year-old age group given *Lactobacillus* GG for 7 months had significantly lower incidence of dental caries and a decrease in oral count of *Streptococcus mutans* after the treatment. Similarly, *L. rhamnosus* GG[40,41] and *L.reuteri*[42], *Lactobacillus paracasei,*[27], *Lactobacillus plantarum*[28], and *Lactobacillus rhamnosus*[29] completely prevented the growth of all mutans streptococci tested. Daily consumption of probiotic drink containing lactic acid bacteria reduces the deep penetration and loss of clinical attachment (gingiva to supporting bone), thereby periodontitis[43,44]. The present investigation coincides with the earlier reports suggesting the potential use of probiotic *Bacillus megaterium* as an antimicrobial agent[45, 51]. The main causative agent of dental caries is *Streptococcus mutans*. This study clearly illustrates the antagonistic effect against cariogenic agent *Streptococcus mutans* thereby prevention of dental caries. The possible effects of probiotics include stimulation of resistant IgA2 antibodies, hence; constitute a clinically important practical preventive therapy[46- 47].

Figure 1
Zone of inhibition effect of Probiotic *Bacillus megaterium* against *Streptococcus mutans.*

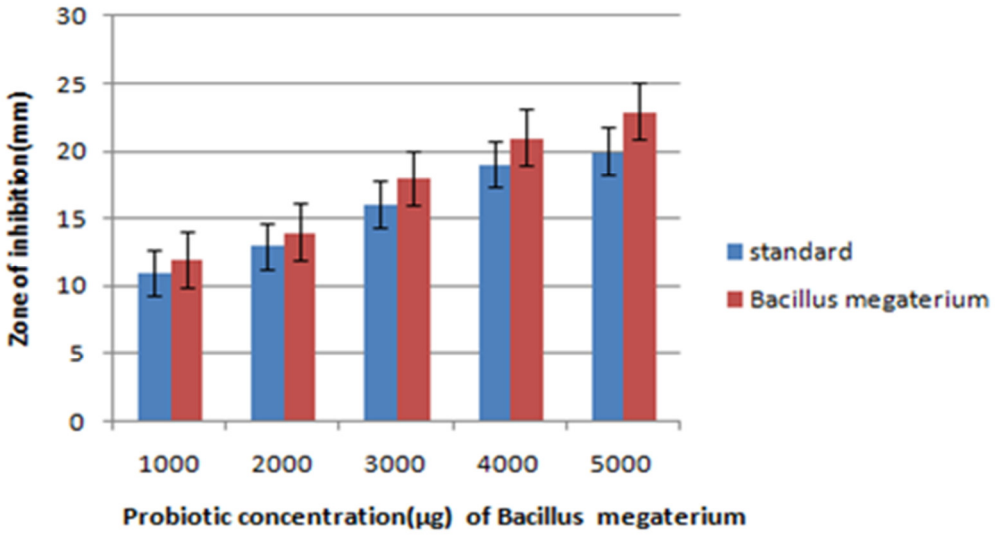
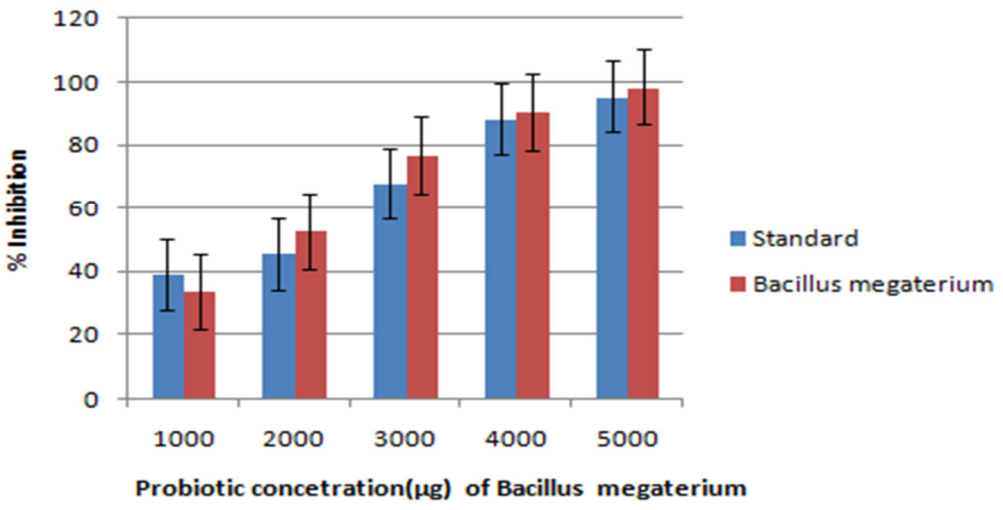


Figure 2
Minimum Inhibitory studies of Probiotic *Bacillus megaterium* against *Streptococcus mutans.*



CONCLUSION

Results of this study indicate that the potential of this probiotic to produce antimicrobial activity against oral microbe *Streptococcus mutans* is great and must be better explored. Further studies need to be undertaken to evaluate the significance of the antimicrobial activity and extraction of active principle. However, detailed investigation of possible mechanism involved in anticaries activity must be probed through molecular studies.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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