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Brief report

Evaluation of the antibacterial and antibiofilm activity of probiotic bacteria against causative bacterial pathogens of dental caries

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Abstract

The most important factor in tooth decay and periodontal disease is the attachment of oral bacteria, especially streptococci, to different levels of the mouth and teeth. Therefore, by changing the microbial ecology in the mouth using probiotic producing bacteria, we can help prevent tooth decay and periodontal infections. This study aimed to evaluate the antibacterial and antibiofilm activities of probiotic producing *Lactobacillus* against several streptococci that cause tooth decay. Antimicrobial activity and minimal inhibitory concentration (MIC) of probiotic lactobacilli was determined by disk diffusion method and standard broth microdilution, respectively. Antibiofilm activity was assayed by a microtiter-plate screening method. The five isolates of *Lactobacillus* strains with probiotic properties include *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, and *Lactobacillus brevis* were tested against *Streptococcus mutans* and *Streptococcus sanguinis*. Most of tested *Lactobacillus* strain at concentrations above 125 µg/mL showed antibacterial properties. Also, examination of the MICs showed that probiotic bacteria had greater effects on *S. sanguinis*. While, the tested probiotic bacteria did not show a significant antibiofilm effect. Our results suggest that lactobacilli with potential probiotic properties can be effective used for eliminating oral streptococcal colonization.

Keywords: Probiotic, *Streptococcus mutans*, *Streptococcus sanguinis*, Antibiofilm, Antibacterial

1. Introduction

Oral infections are the most common and expensive forms of human infections. Dental caries and periodontal disease occur in more than half of the population [1]. Changing microbial ecology as a mechanism to prevent dental caries is an important issue. Also, the increase of bacterial resistance to antibiotics needs the looking for newer and better antimicrobials agents for the prevention and treatment of oral disease [2, 3].

Streptococcus mutans can convert sucrose into water-soluble glucan (WIG), which is one of the most important components of oral biofilm. [4]. In addition, they are important and potent pathogens of tooth decay that occur in dental plaque after adhesion to the tooth surface due to the accumulation of lactic acid,

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therefore, acid producers are considered to be likely to cause tooth decay [5]. Although streptococci such as *Streptococcus sanguinis* and *Streptococcus salivarius* have been reported to produce organic acid, their acid tolerance is weaker than that of mutant streptococci, limiting the contribution of these oral streptococci to caries [6]. The presence of *S. sanguinis* in the oral cavity may also be the result of moderate caries in children [7].

The use of probiotics as a new method to eliminate pathogenic bacteria in the oral cavity can be considered. The use of probiotics is a promising way to fight infections with the help of beneficial bacteria and replace them with pathogenic microorganisms [8]. Mutans streptococci are one of the most important cariogenic bacteria and various *in vitro* studies have shown that *Lactobacillus rhamnosus* and *Lactobacillus paracasei* can reduce the number of mutans streptococci significantly [9]. Different species of bacteria have been introduced as probiotics, the most common of which are *Lactobacillus* and *Bifidobacterium*. Today, the benefits of using probiotics in systemic diseases, especially gastrointestinal diseases, have been identified [10]. However, there are limited studies in this field and their role in maintaining oral health needs further investigation.

Probiotics can work through several mechanisms; Probiotic bacteria inhibit the colonization of pathogenic bacteria by colonizing the intestinal tract, binding to each other, using nutrients in the body before being consumed by pathogenic microorganisms, and producing toxins [11]. Other potential benefits of probiotics in host health include reduced susceptibility to infections, prevention and treatment of allergic manifestations, treatment of lactose intolerance, regulation of blood pressure and serum cholesterol, treatment of cardiovascular disease and cancer, it also eliminates the side effects of overuse of antibiotics (such as diarrhea) and especially the emergence of bacterial resistance [12].

Lactobacilli have no role in initiating the caries process and are mostly associated with dentin caries and are present at the site of carious lesion progression [13]. It has been shown that salivary streptococci are significantly reduced immediately after the end of daily use of the probiotic *Lactobacillus* [14]. However, in people with advanced dentin caries, consumption of probiotic products may lead to an increase in lactobacilli in the carious lesion and further progression of caries [15]. In this study, we aimed to evaluate the antibacterial and antibiofilm activity of probiotic bacteria against streptococci that cause tooth decay.

2. Materials and Methods

2.1 Bacterial strains

In a study conducted at Guilan University of Medical Sciences (ethics code IR.GUMS.REC.1399.343), 42 isolates of *Lactobacillus* which was previously isolated from human fecal samples were studied. Of these, the results of 5 strains which belonged to *Lactobacillus plantarum, L. rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, and *Lactobacillus brevis* species were more promising and can be considered as potential probiotics sources for functional products. Two strains of bacteria including *S. mutans* ATCC 35668, *S. sanguinis* ATCC10556, were used. The strains were recovered from stocks by cultured overnight at 37°C.

2.2 Determination of antimicrobial activity

To investigate the antibacterial effect of probiotic producing *Lactobacillus* strains, microbial susceptibility testing by the well-diffusion method was used. McFarland 0.5 standard concentration was obtained from the bacteria and cultured on Mueller Hinton agar enriched with 5% sheep blood using a swap. In the next step, wells with a diameter of 6 mm were created on this medium, and then supernatant without cells of *Lactobacillus* strains was added to each well (62.5 – 500 µg/mL). Finally, the plates were incubated for 24 hours at 37°C and then the diameter of the growth inhibition zone was measured [16].

2.3 Determining the minimal inhibitory concentration (MIC)

Probiotic bacteria MICs were determined by standard broth microdilution method based on the clinical and laboratory standards institute (CLSI) guidelines [17]. Muller Hinton was used to determine the MIC for the studied strains. To evaluate the inhibitory effects of probiotics on bacterial growth, each 96-well microplate was filled with a concentration range (15.6 to 500 µg/mL). After 24 hours of incubation at 37°C, the wells were examined for microbial growth and the lowest concentration at

which no growth was observed was considered as MIC.

2.4 Biofilm formation assay

Probiotic bacteria were added to the microtiter plate wells and incubated at 37°C for 48 h. After staining with crystal violet and adding ethanol, the absorbance was measured at 570 nm [18]. The tests were done at least three times.

2.5 Statistical Analyses

In this study, the obtained results were entered into SPSS software ver.25. To describe quantitative variables from the mean and standard deviation and for qualitative variables numbers and percentages were used.

3. Results

The diameter of the inhibition zone was measured by the well-diffusion method at different concentrations (Supplementary figure 1) that was estimated between 10 and 16 mm (Table 1). Most isolates have an inhibition zone at concentrations above 125 µg/mL.

The MICs values of the five *Lactobacillus* isolates against *S. sanguinis* and *S. mutans* bacteria were estimated at 62.5 to 250 µg/mL and 125 to 500 µg/mL, respectively. The detailed results of MIC of were shown in Table 2.

Probiotic bacteria could not significantly inhibit the formation of biofilms in the suspension tested at concentrations of 62.5 to 500 µg/mL (data not shown).

The minimum inhibitory concentration for tested bacteria was 125 µg/mL. At this concentration, the diameter of the inhibition zone was estimated to be approximately 10 mm.

Consistent with our results in a study conducted by Rayani et al. to compare the effect of probiotic and regular buttermilk on two common oral microorganisms, *S. mutans* and *Enterococcus faecalis*, thier results indicate a significant difference in the MIC of the two types of probiotic dough used against tested bacteria [19].

Our current results in a laboratory study indicate that probiotic lactobacilli affect the growth and inhibition of oral streptococci. This finding is in agreement with the results of a study that showed that consumption of yogurt or probiotic drinks can significantly reduce the level of *S. mutans* [20, 21]. The results of a study in Denmark showed that *L. rhamnosus* significantly reduced the risk of caries. Therefore, milk containing probiotic bacteria may have beneficial effects on children's dental health [22]. Several studies have shown that the amount of *S. mutans* in saliva decreases significantly immediately after the end of daily consumption of probiotic lactobacilli [23, 24].

Previously, a study conducted in Ahvaz (Southwestern Iran) to investigate the effect of consumption of probiotic yogurt containing *Bifidobacterium lactis* B12 on *S. mutans* and lactobacilli in students with early stages of tooth decay, contrary to the results of our study, in which the studied probiotics had little effect on the removal of bacterial biofilm, a significant decrease in biofilm was observed in the intervention group compared to the

Table 1. The diameter of the inhibition zone in different concentration of probiotic isolates

	S. sanguinis				***S. mutans***			
Bacteria	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
L. plantarum	14 mm	12 mm	0	0	12 mm	11 mm	0	0
L. rhamnosus	14	12	11	0	15	12	10	0
L. reuteri	15	12	10	0	14	12	10	0
L. fermentum	12	12	10	0	11	0	0	0
L. brevis	16	13	10	10	14	11	10	0

4. Discussion

In the present study, the antibacterial and anti-biofilm effects of native probiotic bacteria on caries-causing bacteria and dental plaque were measured.

control group [25]. Another study conducted in China examined the effect of probiotic lactobacilli on *S. mutans* and the development of biofilms in children with severe caries. Their results showed *Lactobacillus*

casei significantly reduced the number of *S. mutans*, *S. sanguinis,* and all bacteria in mixed biofilms compared to the control group [26]. As the main limitations of the present study, a narrow range of tested bacteria and the lack of toxicity evaluation should be acknowledged.

Table 2. MIC of probiotic bacteria on both *Streptococcus* strains

Bacteria	MIC	
	S. sanguinis	***S. mutans***
L. plantarum	250 µg/ml	250 µg/ml
L. rhamnosus	125 µg/ml	125 µg/ml
L. reuteri	250 µg/ml	250 µg/ml
L. fermentum	250 µg/ml	500 µg/ml
L. brevis	62.5 µg/ml	125 µg/ml

The results of the study showed that probiotic bacteria with potential antibacterial properties against bacteria that cause tooth decay can be used to prevent dental caries. Probiotics will be used as a new method in the future due to their cost-effectiveness and fewer side effects compared to other antibacterial compounds. However, our local probiotics did not show a significant effect on preventing biofilms formation.

Supplementary files

Supplementary file 1.

Author contribution

HS, MA, MH: conceived the study. HS, SHR, MA, GR, KD: participated in the design of the study and performed the statistical analysis. PJ, HS, SHR, EM, GR, TY, MH: interpreted the data. HS, MH, MA, SHR: obtained ethical clearance and permission for study. HS, MA, HS: Supervised data collectors. PJ, HS, SHR, EM, GR, KD, TY, MH: Drafting the article or revisiting it critically for important intellectual content. HS, MA, MH were project leaders and primary investigators of the study. All authors read and approved the final manuscript.

Conflict of Interests

There is no conflict of interest.

Ethical declarations

The study protocol was approved by the Guilan University of Medical Sciences. The ethical approval code is IR.GUMS.REC.1399.343.

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