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In-Vitro Comparison of the Antibacterial Activity of Probiotic Yogurt, Cheese, and Dough Against Two Common Oral Micro-Organisms

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Abstract

Background and objectives: There are several types of micro-organisms existing in the oral cavity that can lead to tooth decay. Probiotics are effective in reducing the level of these species. The aim of this study is a laboratory comparison of the antibacterial activity of three types of probiotic products on two common oral micro-organisms.

Materials and methods: In this experimental study, the minimum inhibitory concentration (MIC), as well as the bacterial inhibition zone diameter of probiotic dough, yogurt, and cheese containing *Lactobacillus acidophilus* and *Bifidobacterium*, against the micro-organisms were measured Following normality testing, group comparisons were performed using Kruskal-Wallis and Mann-Whitney U tests. Statistical significance was defined as $P < 0.05$.

Results: The results suggested a significant difference in the MIC of *Enterococcus faecalis* and *Streptococcus mutans*. The MIC of probiotic yogurt and cheese was significantly higher than that of the non-probiotic ones. Moreover, the level of MIC in the non-probiotic dough was significantly higher than that in the probiotic version.

Conclusion: The results of this *in-vitro* study suggest that probiotic dough, unlike yogurt and cheese, was more effective than non-probiotic types in reducing oral *Enterococcus faecalis* and *Streptococcus mutans*.

Keywords: Probiotic, *Streptococcus mutans*, *Enterococcus faecalis*, Minimum inhibitory concentration.

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1. Introduction

There are over 700 bacterial species living in the oral cavity, almost half of which are found in subgingival biofilms in both healthy and infected areas (1). *Streptococcus mutans* is a bacterium that initiates the demineralization of the enamel and consequently, tooth decay (2). The ability to grow in an acidic environment is the most important pathogenic feature of *Streptococcus mutans*; therefore, maintaining a normal flora and preventing the formation of acidic pH in the oral are critical preventive measures against tooth decay (3,4).

The Global Oral Health Data Bank reveals that the prevalence of dental caries ranges from 49% to 83%, affecting people of all ages (5). Nowadays, various methods have been suggested to prevent dental caries by reducing this bacterium, including the use of antibacterial substances such as chlorhexidine and the consumption of synthetic sugars (6). Moreover, probiotic products have also been introduced as a preventive method to reduce the amount of salivary *Streptococcus mutans* and subsequently dental plaque (7). According to the WHO and the FDA, probiotics are living micro-organisms that can be beneficial for their host when consumed in sufficient quantities (8).

Lactobacillus and *Bifidobacteria* species are widely used as probiotics. These nutritional supplements use various mechanisms to create unfavorable conditions for the growth of harmful micro-organisms, thereby reducing the risk of infection (7). Reduction of inflammation and infection, increasing mineral absorption, inhibiting the growth of harmful bacteria, and fighting against *Candida* and eczema are some of the advantages of probiotics (9-11).

As regards the fact that probiotics can reduce the growth of pathogenic strains, as well as the destructive effects of demineralization, they can be a promising option for preventing oral diseases such as periodontitis and stomatitis (12).

Given the high prevalence of caries and periapical lesions, as well as the lack of information on the effects of probiotics on the oral cavity, this study aims to compare the effects of three types of probiotic products (yogurt, cheese, and dough) on *Streptococcus mutans* and *Enterococcus faecalis*, which play important roles in caries development and periapical lesions.

2. Materials and Methods

The study was designed using agar disc diffusion method and micro broth dilution. Probiotic and non-probiotic yogurt, cheese, and dough were purchased (Kalleh Dairy, Tehran, Iran) with matching expiration dates. A single package of each product type was used for all experiments. Products were homogenized prior to testing. For determination of minimum inhibitory concentration (MIC), each dilution series was prepared in triplicate (three independent wells per microplate row). Inhibition zone diameters were measured in triplicate on separate agar plates for each concentration. Thus, all reported values and statistical analyses are based on n=3 technical replicates from a single product package per product type.

Since the cheese samples had a hard texture and were not easily mixed with the culture medium, 10 g of sterile cheese was transferred to a pre-sterilized mortar and homogenized with 90 ml of water containing 2% sodium citrate. 96-well microplates were used to determine the MIC of probiotic dough, yogurt, and cheese containing *Lactobacillus acidophilus* and *Bifidobacterium*, against the microorganisms. For each type of bacterium, one microplate row for probiotic cheese, one for yogurt, and one for dough was considered.

First, 100 µl of Tryptic Soy Broth (TSB) was poured into all microplate wells, and then, 100 µl of each probiotic product was separately homogenized and added to the first well of each row. The combined material and the culture medium were mixed in the first well, using a sampler, and 100 µl of them were transferred to the second well. The contents of the second well were also mixed and transferred to the third well. This cycle was continued up to the tenth well, and the last 100 µl was discarded. Well number 11 was considered as the negative control (free of bacteria and containing probiotic products), while well number 12 was considered as the positive control (free of probiotic products and containing bacteria).

In the next step, the standard strain of *Streptococcus mutans* (ATCC35668) and the standard strain of *Enterococcus faecalis* (ATCC29212) were prepared with turbidity equivalent to half McFarland and diluted 100 times with physiological serum. Then, 100 µl of bacterial suspension was added to all wells except the negative control, and after mixing, the microplate was sealed with Parafilm and incubated for 24 hours at 37 °C.

Since the microplate of *Streptococcus mutans* required more CO_2, it was placed in a candle jar. It should also be noted that *Streptococcus mutans* was incubated for 48 hours, and 50% of sheep-defibrillated blood was added to the TSB medium. After incubation, the first well with no bacterial growth was reported as the MIC. MIC values are expressed in g per 100 mL of product homogenate.

Data were reported as mean ± standard deviation (SD). The normality of data distribution was assessed using the Shapiro–Wilk test. Since the data were not normally distributed, non-parametric tests were employed for group comparisons. The Kruskal–Wallis test compared MIC values across probiotic products. Probiotic vs. non probiotic comparisons were also made using Mann–Whitney U tests. Inhibition zone differences were evaluated with Mann–Whitney U tests. All statistical analyses were performed using SPSS v.22 (IBM, USA), and a $P < 0.05$ was considered statistically significant.

3. Results

Table 1 presents the MIC values as mean ± standard deviation. Lower MIC values indicate greater antibacterial activity. For both *Streptococcus mutans* and *Enterococcus faecalis*, probiotic yogurt and probiotic cheese showed significantly higher MIC values, indicating lower antibacterial activity compared to their non-probiotic counterparts ($P < 0.001$ for all comparisons). In contrast, probiotic dough exhibited significantly lower MIC values than non-probiotic dough for both bacterial strains ($P = 0.002$).

Kruskal–Wallis test revealed significant differences in MIC among probiotic yogurt, cheese, and dough for both *S. mutans* and *E. faecalis* ($P < 0.001$). Moreover, probiotic dough had significantly lower MIC values than both probiotic yogurt ($P < 0.001$) and probiotic cheese ($P < 0.001$). No significant difference was observed between probiotic yogurt and probiotic cheese ($P > 0.05$).

Inhibition zone diameters were measured at two concentrations (20 g/100 mL and 10 g/100 mL). Across all products and concentrations, inhibition zones were significantly smaller than those produced by the vancomycin positive control ($P < 0.001$ for all). No significant differences were observed between probiotic and non-probiotic product types at the respective concentrations (Table 2).

Overall, probiotic dough demonstrated superior antibacterial activity (lower MIC) against both oral pathogens compared to its non-probiotic counterpart and to probiotic yogurt and cheese, while no differences were detected in inhibition zone diameters.

Table 1. Minimum inhibitory concentration (MIC) of probiotic and non-probiotic products against *Streptococcus mutans* and *Enterococcus faecalis*.

Variables	*Streptococcus mutans*			*Enterococcus faecalis*		
	MIC (g/100 mL)	Range	P-value*	MIC (g/100 mL)	Range	P-value*
Probiotic yogurt	15.1 ± 0.88	(14–17)	<0.001	15.4 ± 0.84	(14–17)	<0.001
Non-probiotic yogurt	10.4 ± 0.84	(9–12)		10.5 ± 0.85	(9–12)	
Probiotic cheese	15.5 ± 0.7	(15–17)	<0.001	15.7 ± 0.68	(15–17)	<0.001
Non-probiotic cheese	10.8 ± 0.79	(10–12)		10.7 ± 0.95	(10–13)	
Probiotic dough	6.25 ± 0.38	(6–7)	0.002	25 ± 0.8	(24–26.2)	0.002
Non-probiotic dough	12.5 ± 0.3	(11.9–12.8)		50 ± 1.59	(48–51.9)	
P-value**	<0.001			<0.001		

* Mann–Whitney U test
** Kruskal–Wallis

Table 2. Comparison of inhibition zone diameters (mean ± SD) at tested concentrations.

Product		Concentration (g/100 mL)	Inhibition zone (mm)	P-value *
Yogurt	Probiotic	20	12.2 ± 1.1	0.517
	Non-probiotic	20	12.17 ± 1.17	
	Probiotic	10	5.31 ± 0.37	0.631
	Non-probiotic	10	4.46 ± 0.52	
Cheese	Probiotic	20	12.4 ± 1.1	0.428
	Non-probiotic	20	10.3 ± 0.6	
	Probiotic	10	5.17 ± 0.76	0.631
	Non-probiotic	10	4.2 ± 0.31	
Dough	Probiotic	20	10.17 ± 1.47	0.061
	Non-probiotic	20	12 ± 1.41	
	Probiotic	10	8.17 ± 1.17	0.611
	Non-probiotic	10	8 ± 0.89	

* Mann–Whitney U test

4. Discussion

The primary aim of this *in-vitro* study was to compare the antibacterial activity of three probiotic products (yogurt, cheese, and dough containing *Lactobacillus acidophilus* and *Bifidobacterium*) against two common oral pathogens (*Streptococcus mutans* and *Enterococcus faecalis*). Findings revealed that probiotic yogurt and cheese exhibited higher MICs compared to non-probiotic versions for both bacterial strains, whereas probiotic dough demonstrated lower MICs than non-probiotic dough. No significant differences were observed in inhibition zone diameters between probiotic and non-probiotic products, though all probiotic products differed significantly from the vancomycin control.

Bacteriotherapy or consumption of probiotic products is used in healthcare areas considering its beneficial effects. decreased levels of oral *Streptococcus mutans*, which is one of the most important factors associated with dental caries, have been reported following the use of these products (13).

The present *in-vitro* investigation suggested that, despite probiotic yogurt and cheese, probiotic dough was significantly more effective in reducing oral micro-organisms compared to the non-probiotic type. Moreover, there was a statistically significant difference between the MICs of dough with yogurt and cheese, but no significant difference was observed between yogurt and cheese. In addition, no significant difference was observed in the inhibition zone diameter between the two product types, which may have been due to the small sample size.

Using probiotics is a new technique for preventing dental caries. In a review conducted by Amargianitakis et al, using dairy products was suggested as a suitable source of probiotics in patients with dental caries. However, other substances such as probiotic tablets, capsules, and cakes have been recommended for those allergic to dairy (14).

In line with our study, Cogulu et al. showed that consuming 3 weeks of kefir dough can reduce the saturation of *Streptococcus mutans* (15). Moreover, Jabbareh et al. suggested that kefir had antibacterial properties against oral micro-organisms (16). In addition, it was represented that probiotic species such as *Lactobacillus rhamnose*, *Lactobacillus plantarum*, *Lactobacillus retri*, and *Lactobacillus paracasei* have the maximum susceptibility to *Streptococcus mutans* (17,18). Since these micro-organisms are found in kefir, it can be expected that different types of kefirs have anti-*Streptococcus mutans* effects (19).

In a similar study conducted by Rayani et al, the effects of probiotic and non-probiotic dough on the oral micro-organisms were compared. They reported that probiotic dough is more effective in reducing oral *E. faecalis* and *S. mutans* and can be suggested as a preventive substance for oral diseases (20).

In a clinical trial conducted by Lesan et al, non-probiotic yogurt was compared with the probiotic version containing *Lactobacillus acidophilus* and *Bifidobacterium*. They found that the level of oral *Lactobacillus* was significantly reduced in the group consuming probiotic yogurt. However, the difference in the reduction of *S. mutans* was not statistically significant in the study and the control group (21).

Despite our study, Ahola et al. found that short-term use of cheese containing *Lacticaseibacillus rhamnosus* can reduce the level of oral *S. mutans* in youth (22). In line with their work, another study also suggested that probiotic cheese can decrease the oral number of salivary yeasts (23). Nevertheless, this controversy can be owing to the difference in the sample sizes and the type of bacteria assessed in each investigation. It is recommended that further studies be conducted to assess different types of oral micro-organisms.

This *in-vitro* study has several important limitations. First, experiments were conducted using only a single package of each product. Moreover, the findings may not generalize to clinical oral environments due to the absence of factors like saliva, pH variations, biofilms, and host immunity. Products were tested from a single brand, limiting generalizability to other formulations or strains. Future *in-vivo* studies with larger cohorts and a diverse range of products are needed to confirm the practical benefits for caries prevention.

5. Conclusion

The results of this *in-vitro* study demonstrate that probiotic dough exhibits stronger antibacterial activity against both *Streptococcus Mutans* and *Enterococcus faecalis* compared to probiotic yogurt and cheese. In direct comparisons with non-probiotic products, probiotic yogurt and cheese showed reduced effectiveness, whereas probiotic dough had significantly greater effectiveness for both bacterial strains. No significant differences were observed in inhibition zone diameters between probiotic and non-probiotic products across yogurt, cheese, and dough.

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Ethics

The study protocol was approved by the ethics committee of Hormozgan University of Medical Sciences (IR.HUMS.REC.1398.024).

Using artificial intelligence (AI)

It is declared by the authors of this manuscript that no generative artificial intelligence (AI) or AI-assisted technologies were used to generate content, ideas, or theories during the writing process of this work.

Author contribution

Arezoo Rayani: Methodology, Validation
Mohammad Ahanjan: Investigation, Methodology
Maryam Hosseini: Data curation
Melika Mollaei: Writing - Original draft, Writing – Review & editing
Maryam Zamanzadeh: Conceptualization, Methodology

Conflict of interest

The authors declare no conflict of interest.

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