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Competition between yogurt probiotics and periodontal pathogens *in vitro*

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Abstract

Objective. To investigate the competition between probiotics in bio-yogurt and periodontal pathogens *in vitro*. ***Material and methods.*** The antimicrobial activity of bio-yogurt was studied by agar diffusion assays, using eight species of putative periodontal pathogens and a 'protective bacteria' as indicator strains. Four probiotic bacterial species (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus,* and *Bifidobacterium*) were isolated from yogurt and used to rate the competitive exclusion between probiotics and periodontal pathogens. ***Results.*** Fresh yogurt inhibited all the periodontal pathogens included in this work, showing inhibition zones ranging from 9.3 (standard deviation 0.6) mm to 17.3 (standard deviation 1.7) mm, whereas heat-treated yogurt showed lower antimicrobial activity. In addition, neither fresh yogurt nor heat-treated yogurt inhibited the 'protective bacteria', *Streptococcus sanguinis.* The competition between yogurt probiotics and periodontal pathogens depended on the sequence of inoculation. When probiotics were inoculated first, *Bifidobacterium* inhibited *Porphyromonas gingivalis, Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans, Porphyromonas circumdentaria,* and *Prevotella nigrescens; L. acidophilus* inhibited *P. gingivalis, A. actinomycetemcomitans, P. circumdentaria, P. nigrescens,* and *Peptostreptococcus anaerobius; L. bulgaricus* inhibited *P. gingivalis, A. actinomycetemcomitans,* and *P. nigrescens;* and *S. thermophilus* inhibited *P. gingivalis, F. nucleatum,* and *P. nigrescens.* However, their antimicrobial properties were reduced when both species (probiotics and periodontal pathogens) were inoculated simultaneously. When periodontal pathogens were inoculated first, *Prevotella intermedia* inhibited *Bifidobacterium* and *S. thermophilus.* ***Conclusions.*** The results demonstrated that bio-yogurt and the probiotics that it contains are capable of inhibiting specific periodontal pathogens but have no effect on the periodontal protective bacteria.

Key Words: *Bifidobacterium, interaction*, Lactobacillus, *periodontal disease*

Introduction

The term 'probiotics' is defined by the Joint Food and Agriculture Organization/World Health Organization Working Group as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" [1]. Different organisms can be classified as probiotics, and the most common strains belong to the genera *Lactobacillus* and *Bifidobacterium* [2].

Probiotics have been extensively studied for their health-promoting effects. The main field of research has been focused on the gastrointestinal tract; however, in the past few years, probiotics have also been investigated from the oral health perspective. Generally, probiotics are delivered through dairy products (mainly fermented milks), and as food supplements in tablet form. Since these probiotic-containing products are consumed through the mouth, oral microbiota could be influenced by daily consumption. Petti et al. [3] reported that daily consumption of yogurt influences the human salivary microbiota. Cildir et al. [4] also reported that consumption of fruit yogurt containing probiotics affects the salivary microbiota of patients with fixed orthodontic appliances.

Early studies have shown that probiotics inhibit caries-causing *Streptococci,* and consumption of probiotics-containing food might reduce the incidence of caries, which is one of the most common infectious dental diseases. A randomized, double-blind, placebo-controlled intervention study [5] showed that long-term consumption of milk containing *Lactobacillus rhamnosus* GG (LGG) could reduce

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the risk of developing caries in children, especially in 3- to 4-year-olds. The results showed less dental caries and lower mutans *Streptococcus* counts in the LGG group. In a short-term intervention study, the consumption of cheese containing LGG and *L. rhamnosus* LC 705 was also found to diminish *S. mutans* counts in young adults [6]. Meurman et al. [7] reported that LGG inhibits the growth of *Streptococcus sobrinus in vitro.* Another *in vitro* study showed that yogurt with live bacteria possesses antimicrobial activity against *S. mutans* and *Streptococcus oralis* [8].

Chronic periodontitis, another common oral infectious disease, is largely associated with the imbalance of indigenous microbiota [9]. Certain species, predominantly Gram-negative anaerobic microorganisms, are considered to be pathogenic to dental-supporting tissues. *Porphyromonas gingivalis* has been implicated as a major etiologic agent in the development and progression of chronic periodontitis [10]. Other species contributing to gingivitis and periodontitis include *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum* and *Prevotella intermedia,* among others [11]. It was reported that *Lactobacillus reuteri* is effective in reducing both gingivitis and plaque in patients with moderate to severe gingivitis. A significant decrease in gingival bleeding and a reduction in gingivitis were observed in these patients after a 2-week intake of probiotic species [12]. Oral administration of a tablet containing *Lactobacillus salivarius* WB21 was also able to significantly decrease the plaque index and the pocket-probing depth in smokers [13]. In a clinical trial, *L. salivarius* TI 2711 (LS 1) successfully reduced the number of black-pigmented anaerobic rods in the saliva [14]. Although it was reported that probiotics possess antimicrobial activity against periodontal pathogens such as *P. gingivalis* and *P. intermedia in vitro* [14,15], it is still not clear whether the activity of yogurt probiotics against periodontitis is due to the antimicrobial activity of yogurt alone or the probiotics contained in it.

This study investigates whether bio-yogurt and probiotics isolated from yogurt have antimicrobial activity against periodontal pathogens *in vitro.* Our hypothesis was that the improvement in periodontal condition after yogurt consumption might be attributed to the direct and selective antimicrobial activity of probiotics against periodontal pathogens.

Material and methods

Bacterial strains

The tested periodontal pathogens (obtained from the State Key Laboratory of Oral Diseases, Sichuan University, Chengdu, China) were *Fusobacterium nucleatum* ATCC 25586, *Porphyromonas gingivalis* ATCC 33277, *Aggregatibacter actinomycetemcomitans* ATCC 29523, *Prevotella intermedia* ATCC 25611, *Prevotella nigrescens* ATCC 33563, *Peptostreptococcus anaerobius* ATCC 27337, *Bacteroids fragilis* ATCC 25285, and *Porphyromonas circumdentaria* NCTC 12469. *Streptococcus sanguinis* ATCC 10556, which is thought to play a beneficial role in periodontal health, was also included.

The tested strains were subcultured in tubes containing 10 ml of brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI) supplemented with 1% (w/v) hemin and 1% (w/v) vitamin K1, and incubated at 37°C anaerobically (90% N_2, 5% H_2, 5% CO_2).

Yogurt

Commercial yogurt (Bright, Chengdu, China) was purchased from a local supermarket. According to the information provided by the manufacturer, the fresh yogurt contained probiotics (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus,* and *Bifidobacterium*). For each test, the level of viable probiotics was assessed by plating yogurt and its dilutions (10–1–10–7) onto a series of selective medium agar plates, as described previously by Tabasco et al. [16]. *S. thermophilus* was grown on M-17 agar containing 1% lactose (M17-lactose) and incubated at 37°C for 24 h anaerobically. *L. bulgaricus* and *L. acidophilus* were grown on De Man, Rogosa, Sharpe (MRS) agar enriched with 0.2% Tween 80 and supplemented with 1% fructose, 0.8% casein acid hydrolysate, and 0.05% cysteine. The plates were incubated at 37°C for 72 h anaerobically. *Bifidobacterium* was grown on MRS agar supplemented with 1% raffinose, 0.05% LiCl, and 0.05% cysteine. Plates were also incubated at 37°C for 72 h anaerobically. The microorganisms were identified presumptively by Gram staining. *S. thermophilus* levels between 3.7 and 9.3×10^7 colony-forming units (cfu)/ml, *L. bulgaricus* levels between 1.1 and 2.9×10^6 cfu/ml, *L. acidophilus* levels between 0.7 and 2.8×10^6 cfu/ml, and *Bifidobacterium* levels between 0.3 and 1.4×10^5 cfu/ml were confirmed.

Inhibitory effects of bio-yogurt on indicator strains

The antimicrobial activity of bio-yogurt was evaluated by agar diffusion assays. Aliquots (50 µl) of the yogurt were placed in 7-mm wells previously cut on BHI agar plates seeded ($\approx10^8$ cfu/ml) with the indicator bacteria [17]. Chlorhexidine (0.2% w/v) was used as a positive control. The plates were kept at 4°C for 2 h to allow diffusion of the agents through the agar. The agar plates were incubated at 37°C for 72 h anaerobically. After 72 h, the diameter (in millimeters) of the cleared

zone was measured and recorded. Each strain was tested three times with two replicates.

To test whether the inhibitory effect of the yogurt was exclusively due to viable probiotics, yogurt heat-treated for 30 min at 70°C was also included. Heat treatment in order to reduce the proportion of viable microorganisms to <10 cfu/ml, as confirmed by plating undiluted and 10^{-1} and 10^{-2} dilutions of heat-treated yogurt on selective medium agar plates, was described above [8].

Isolation and identification of yogurt microorganisms

The selective methods described above were used to isolate yogurt probiotics. Colonies with different morphology (at least two to three colonies from each morphologic type) were isolated, provisionally identified and incubated for 24–48 h. A series of medium agar plates were repeatedly streaked to purify the culture. At each step of purification, the colony, as well as the cell morphology of an isolate, was checked.

Identification was based on the morphology of colonies, Gram staining, and polymerase chain reaction (PCR) results. DNA was isolated using a ColumnMate Bacteria gDNA Isolation Mini Kit (Watson Ltd, Shanghai, China) and frozen at –20°C for later analysis. PCRs were performed with thin-walled tubes and a DNA Engine Dyad Thermal cycler (Bio-Rad Ltd, Hercules, CA). All primers used in this study are listed in Table I. One μl of DNA template was added to a reaction mixture (50 μl final volume) containing 20 nmol of each primer, 40 nmol of deoxynucleoside triphosphates, and 1.25 U of TaKaRa Ex Taq polymerase (Takara Ltd, Dalian, China). Primers used for *L. bulgaricus* identification were DEL-F and DEL-R. Cycling conditions for these primers were 10 cycles for 20 s at 94°C, 75 s at 65°C, and 40 s at 72°C, followed by 35 cycles for 20 s at 94°C, 50 s at 55°C, and 30 s at 72°C. A final elongation for 3 min at 72°C was applied. Primers for *S. thermophilus* were THER-F and THER-R. The conditions of this PCR were 35 cycles for 20 s at 94°C, 60 s at 58°C, and 30 s at 72°C, followed by 3 min of elongation at 72°C [18]. Primers for *L. acidophilus* were LACFOR and LACREV. The amplification conditions were 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, 59°C for 20 s, 72°C for 20 s, and a final extension at 72°C for 7 min [19]. Primers for *Bifidobacterium* were lm26 and Lm3r. The conditions of this PCR were 35 cycles for 1 min at 94°C, 3 min at 57°C, and 4 min at 72°C [20].

The results of PCR amplification were examined by electrophoresis in a 1% agarose gel with DL2000 DNA Marker (Takara Ltd) as molecular weight marker. DNA was stained with ethidium bromide and visualized under short-wavelength ultraviolet light.

Pure strains of the four probiotic bacteria (*L. bulgaricus*, *S. thermophilus*, *L. acidophilus*, and *Bifidobacterium*) were isolated from the commercial yogurt and subcultured in tubes containing 10 ml of BHI broth supplemented with hemin and vitamin K1, and incubated at 37°C for 10–14 h anaerobically.

Competition assays on plates between probiotics and indicator strains

Competition assays on medium agar plates between yogurt probiotics and indicator strains, a protocol described previously, was performed with some modifications [21]. Briefly, 8 μl (10^8 cfu/ml) of an overnight culture of each species in BHI broth was inoculated onto a BHI agar plate as the early colonizers. After overnight incubation, 8 μl (10^8 cfu/ml) of the competing species was inoculated beside the early colonizer as the later colonizers. In a separate experiment, both species were inoculated simultaneously beside each other. The plates were incubated overnight at 37°C anaerobically. Growth inhibition was observed through the presence of a proximal zone of inhibition at the intersection of each colony. The experiment was done three times with similar results.

Table I. PCR primers used in this study for the identification of *L. bulgaricus*, *S. thermophilus*, *L. acidophilus*, and *Bifidobacterium*.

Species	Name	Sequence 5′→3′	Product (bp)
L. bulgaricus	DEL-F	AATTCCGTCAACTCCTCATC	715
	DEL-R	TGATCCGCTGCTTCATTTCA	
S. thermophilus	THER-F	CACTATGCTCAGAATACA	968
	THER-R	CGAACAGCATTGATGTTA	
L. acidophilus	LACFOR	TCTTGACATCTAGRGCAATC	280
	LACREV	GATTCGCTTGCCTTCGCAGG	
Bifidobacterium	lm26	GATTCTGGCTCAGGATGAACG	1.35k
	Lm3r	CGGGTGCTICCCACTTTCATG	

Statistics

Statistical analyses were performed using the SPSS program (version 17.0; SPSS Inc, Chicago, IL). Mean differences were established using the Wilcoxon signed-rank test. The differences between means were considered significant at $P < 0.05$.

Results

Antimicrobial activity of yogurt

Fresh yogurt was observed to inhibit all the periodontal pathogens used in this work, while heat-treated yogurt failed to inhibit *F. nucleatum* and *P. gingivalis* (Table II). For the same indicator strain, heat-treated yogurt showed a significantly smaller inhibition zone than fresh yogurt ($P < 0.05$). The inhibition zone of fresh yogurt against the beneficial strain, *S. sanguinis*, was the smallest of all inhibition zones of fresh yogurt against the investigated indicator strains.

Competition between probiotics and indicator strains

A simple competition assay was performed to test the antagonistic interactions between probiotics and the indicator strains. Three separate tests were conducted: (i) probiotics were inoculated first and allowed to grow overnight (as early colonizers) before the indicator strains were inoculated beside them (as late colonizers); (ii) the same procedure except that the indicator strains were inoculated first; and (iii) both species were inoculated at the same time [21].

As shown in Figures 1–5, competitive exclusion between probiotics and indicator strains occurred depending on the sequence of inoculation.

As the early colonizer, *Bifidobacterium* inhibited *P. gingivalis, F. nucleatum, A. actinomycetemcomitans, P. circumdentaria*, and *P. nigrescens*. When inoculated as late colonizer, *Bifidobacterium* no longer inhibited any periodontal pathogens; instead, *P. intermedia* inhibited *Bifidobacterium*. When inoculated simultaneously, *Bifidobacterium* and the periodontal pathogens did not inhibit each other. Figure 1 shows the interactions between *Bifidobacterium* and *F. nucleatum* and between *Bifidobacterium* and *P. circumdentaria*.

As the early colonizer, *L. acidophilus* inhibited *P. gingivalis, A. actinomycetemcomitans, P. circumdentaria, P. nigrescens,* and *P. anaerobius*. When inoculated as late colonizer, *L. acidophilus* no longer inhibited any periodontal pathogens. When inoculated simultaneously, *L. acidophilus* and the periodontal pathogens also exhibited no inhibiting activity against each other. Figure 2 shows the interactions between *L. acidophilus* and *P. gingivalis,* and between *L. acidophilus* and *P. anaerobius*.

As the early colonizer, *L. bulgaricus* inhibited *P. gingivalis, A. actinomycetemcomitans*, and *P. nigrescens*. When inoculated as late colonizer, *L. bulgaricus* no longer inhibited any periodontal pathogens. When inoculated simultaneously, *L. bulgaricus* and the periodontal pathogens also showed no evidence of inhibition against each other. Figure 3 shows the interactions between *L. bulgaricus* and *P. nigrescens,* and between *L. bulgaricus* and *A. actinomycetemcomitans*.

As the early colonizer, *S. thermophilus* inhibited *P. gingivalis, F. nucleatum,* and *P. nigrescens*. When inoculated as late colonizer, *S. thermophilus* no longer inhibited any periodontal pathogens; however, *P. intermedia* inhibited *S. thermophilus*. When inoculated simultaneously, *S. thermophilus* inhibited *P. nigrescens*. Figure 4 shows the interactions between *S. thermophilus* and *F. nucleatum,* and between *S. thermophilus* and *P. intermedia*.

S. sanguinis, as the 'protective bacteria', inhibited all four yogurt probiotics as the early colonizer. However, this competitive exclusion was reduced when both species (protective bacteria and yogurt probiotics)

Table II. Antimicrobial activities of fresh yogurt, heat-treated yogurt, and 0.2% chlorhexidine against oral bacteria *in vitro*.

	Inhibition zone (mm); mean[a] (SD)		
Strain	Yogurt	Heat-treated yogurt[b]	0.2% Chlorhexidine
F. nucleatum, ATCC 25586	11.4 (0.9)	(—)	20.5 (0.7)
P. gingivalis, ATCC 33277	10.6 (1.2)	(—)	20.5 (1.3)
A. actinomycetemcomitans, ATCC 29523	9.3 (0.6)	7.9 (0.3)	17.3 (0.4)
P. intermedia, ATCC 25611	11.5 (1.4)	7.9 (1.3)	18.4 (1.7)
P. nigrescens, ATCC 33563	13.7 (2.6)	9.9 (2.5)	20.0 (1.8)
P. anaerobius, ATCC 27337	17.3 (1.7)	11.2 (1.2)	28.3 (0.7)
B. fragilis, ATCC 25285	11.4 (0.7)	9.4 (0.7)	17.2 (0.4)
P. circumdentaria, NCTC 12469	11.2 (1.4)	8.3 (0.8)	21.9 (1.8)
S. sanguinis, ATCC 10556	7.9 (1.1)	(—)	16.7 (1.3)

[a]Mean = average of three-independent experiments with duplicate samples.
[b](—) = no inhibition zone.

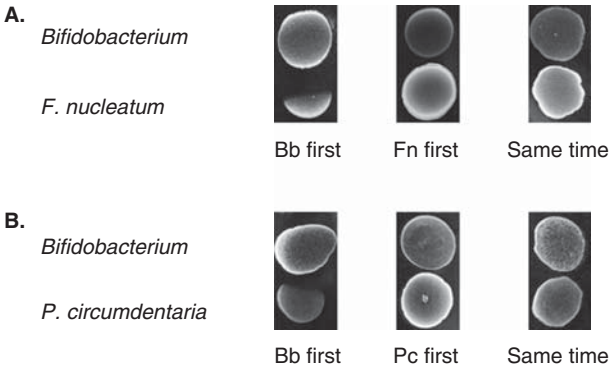

Figure 1. Competition assays on agar BHI plate. (A) Competition assays between *Bifidobacterium* and *F. nucleatum*. Left, *Bifidobacterium* (Bb) was inoculated first; middle, *F. nucleatum* (Fn) was inoculated first; right, Bb and Fn were inoculated at the same time. (B) Competition assays between *Bifidobacterium* and *P. circumdentaria*. Left, Bb was inoculated first; middle, *P. circumdentaria* (Pc) was inoculated first; right, Bb and Pc were inoculated at the same time.

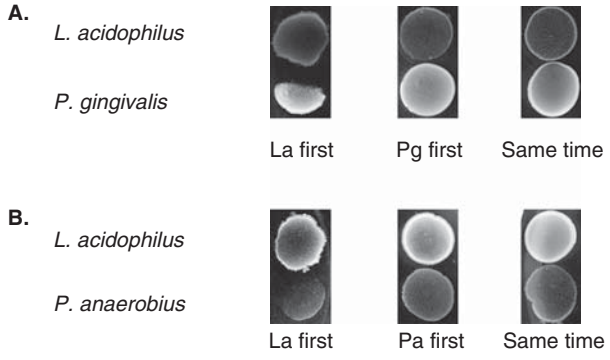

Figure 2. (A) Competition assays between *L. acidophilus* and *P. gingivalis*. Left, *L. acidophilus* (La) was inoculated first; middle, *P. gingivalis* (Pg) was inoculated first; right, La and Pg were inoculated at the same time. (B) Competition assays between *L. acidophilus* and *P. anaerobius*. Left, La was inoculated first; middle, *P. anaerobius* (Pa) was inoculated first; right, La and Pa were inoculated at the same time.

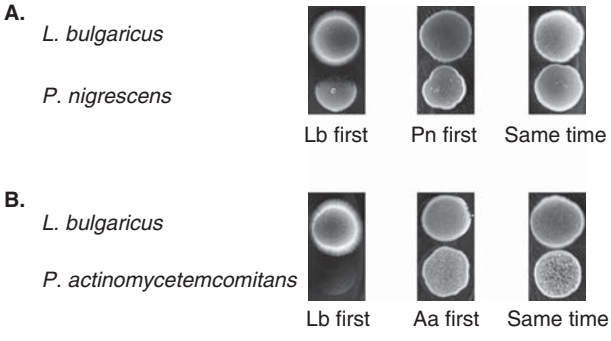

Figure 3. (A) Competition assays between *L. bulgaricus* and *P. nigrescens*. Left, *L. bulgaricus* (Lb) was inoculated first; middle, *P. nigrescens* (Pn) was inoculated first; right, Lb and Pn were inoculated at the same time. (B) Competition assays between *L. bulgaricus* and *A. actinomycetemcomitans*. Left, Lb was inoculated first; middle, *A. actinomycetemcomitans* (Aa) was inoculated first; right, Lb and Aa were inoculated at the same time.

were inoculated at the same time. Similar results were found with *S. sanguinis* as the late colonizer. Figure 5 shows the interactions between *S. sanguinis* and *L. bulgaricus,* and between *S. sanguinis* and *Bifidobacterium*.

Discussion

Since the inhibition activity of bio-yogurt could result from low pH, inhibitory substances, or live microorganisms [22], and since heat-treated yogurt has almost the same pH as fresh yogurt and contains negligible viable microorganisms compared with fresh yogurt [8], fresh yogurt was compared to heat-treated yogurt using agar diffusion assays to evaluate their inhibition activity.

The final results suggest that fresh yogurt inhibited all eight periodontal pathogens used in this study, while heat-treated yogurt inhibited them selectively. For the same indicator strain, heat-treated yogurt showed a lower antimicrobial activity than fresh yogurt. These results corroborate the conclusion of Petti et al. [8], who found that yogurt without viable microorganisms did not have antimicrobial activity similar to that of probiotics-containing yogurt. It is possible to speculate that at least part of the inhibition activity of bio-yogurt observed *in vitro* is due to the direct activity of live bacteria and/or their bacteriocins on the viability of the indicator strains, and not only to low pH [23]. The differences in antibacterial activity between the untreated and heat-treated yogurts, on the other hand, could not be fully attributed to live yogurt bacteria because heat treatment can also inactivate certain yogurt bacteriocins.

Pure strains of four probiotics were isolated from fresh yogurt according to the information provided by the manufacturer. Previous studies have demonstrated a so-called 'competitive exclusion' between two bacterial species depending on the sequence of inoculation [21,24]. Therefore, adopting the procedures illustrated by Kreth et al. [21], the interaction between periodontal bacteria and yogurt probiotics using different inoculating sequences was investigated.

In this study, competition assays on the medium agar plates demonstrated a competitive exclusion between yogurt probiotics and the periodontal pathogens depending on the sequence of inoculation. This competitive exclusion turned out to be a result of the production of inhibitory substances by the probiotics or the competition for nutrients.

As shown above, the growth-inhibiting ability of yogurt probiotics against each periodontal pathogen differs. Kõll-Klais et al. [14] observed that *L. acidophilus* and *L. bulgaricus* have antimicrobial activity against *A. actinomycetemcomitans* and *P. gingivalis,* but not against *P. intermedia*. Their result is consistent with the results of this study.

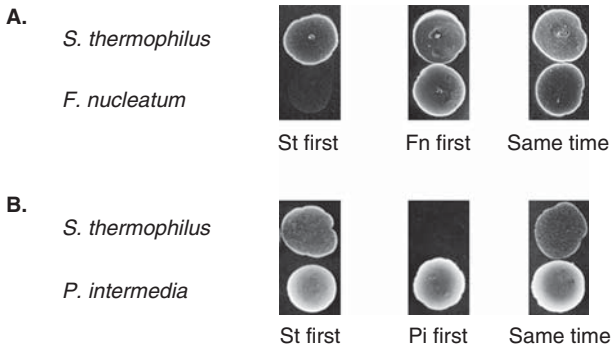

Figure 4. (A) Competition assays between *S. thermophilus* and *F. nucleatum*. Left, *S. thermophilus* (St) was inoculated first; middle, *F. nucleatum* (Fn) was inoculated first; right, St and Fn were inoculated at the same time. (B) Competition assays between *S. thermophilus* and *P. intermedia*. Left, St was inoculated first; middle, *P. intermedia* (Pi) was inoculated first; right, St and Pi were inoculated at the same time.

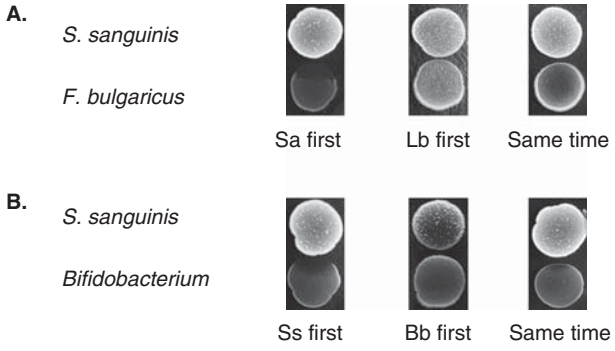

Figure 5. (A) Competition assays between *S. sanguinis* and *L. bulgaricus*. Left, *S. sanguinis* (Ss) was inoculated first; middle, *L. bulgaricus* (Lb) was inoculated first; right, Ss and Lb were inoculated at the same time. (B) Competition assays between *S. sanguinis* and *Bifidobacterium*. Left, Ss was inoculated first; middle, *Bifidobacterium* (Bb) was inoculated first; right, Ss and Bb were inoculated at the same time.

A species-specific antimicrobial activity was also observed by Stamatova et al. [25]. Their results showed that *L. bulgaricus* strains are more active against streptococcal species and *A. actinomycetemcomitans,* whereas *L. rhamnosus* strains show distinct inhibitory activity against *P. gingivalis* and *F. nucleatum*. Ishikawa et al. [15] also reported that daily intake of *L. salivarius* isolated from healthy humans leads to a decreased in black-pigmented anaerobic rods and that, in an *in vitro* system, *L. salivarius* completely kill *P. gingivalis* within 24 h when these bacteria are cultured together.

Antimicrobial substances produced by probiotics have a broad spectrum of activity, supporting the results of our study. Probiotics can produce lactic acid, hydrogen peroxide, and bacteriocins or bacteriocin-like substances that may act alone or in concert in inhibiting pathogens [26,27]. Very few studies, however, have investigated the competition between *Bifidobacterium*/*S. thermophilus* and periodontal pathogens *in vitro*. Caglar et al. [28,29] reported that short-term consumption of yogurt containing *Bifidobacterium* DN-173 010 could reduce the salivary levels of mutans *Streptococci*. Hojo et al. [30] surveyed the distribution of salivary *Bifidobacterium* species in periodontal patients and healthy subjects and observed that some *Bifidobacterium* species were specifically prevalent in young healthy subjects compared to patients with periodontitis, suggesting that *Bifidobacterium* might be beneficial to periodontal health. We observed that *Bifidobacterium* inhibited most of the periodontal pathogens used in our study, providing evidence that supports these clinical observations.

Earlier studies on the antimicrobial activity of *S. thermophilus* focused on the gastrointestinal tract [31]. In our study, *S. thermophilus* showed selective antimicrobial activity against periodontal pathogens: these new findings suggest a need for further studies. Similar to their better-known actions in the gastrointestinal tract, probiotics also exert their various effects in the oral cavity. The mechanism of action of probiotics in the mouth is expected to be similar to that observed in gastrointestinal indications.

The health-promoting activity of probiotic bacteria has been widely noted in previous studies and the high antioxidative ability of *Lactobacilli* has been proven [32]. Recently, one study revealed a novel immuno-stimulating aspect of *L. acidophilus* and *L. gasseri,* which induce significant chemotaxis of macrophages [33]. Hence, the presence of *Lactobacilli* with antimicrobial activity, as well as good antioxidative and immuno-stimulating properties, could be one of the factors regulating the presence and number of periodontal pathogens.

According to the 'ecological plaque hypothesis', the lack of so-called 'protective bacteria' plays an important role in periodontitis [9]. 'Protective bacteria' are microbial species that occupy a niche by sheltering pathogenic organisms or inhibiting certain pathogens through metabolic antagonism or by directly inactivating them [34]. In previous studies, *S. sanguinis* was considered a benign, or even a beneficial, bacterium with regard to periodontal diseases [35,36]. In this study, neither fresh nor heat-treated yogurt showed antimicrobial activity against *S. sanguinis* ($P > 0.05$). Also, no probiotics isolated from bio-yogurt showed antimicrobial activity against it. When inoculated first, *S. sanguinis* suppressed the growth of all four yogurt microorganisms investigated. This antimicrobial ability may be due to the production of hydrogen peroxide and sanguicin [37].

This selective antimicrobial activity of bio-yogurt *in vitro* may help to explain the results of clinical studies which reported that regular intake of probiotics-containing foods has a beneficial effect on periodontal disease [38]. The results of this study

also indicate that at least part of the inhibition activity of bio-yogurt can be attributed to live bacteria. However, none of the four yogurt probiotics showed antimicrobial activity against *P. intermedia* and *B. fragilis*. This is contrary to the results of the agar diffusion assays, which showed the antimicrobial activity of fresh bio-yogurt against *P. intermedia* and *B. fragilis*. We speculate that this observed antimicrobial activity shown in the assays may be due to the synergistic action between the present yogurt bacteria, and further investigations are thus needed.

In conclusion, yogurt possesses antimicrobial activity against periodontal pathogens but has no effect on *S. sanguinis in vitro*. This suggests that the reduction of gingivitis and periodontitis may be attributed to the direct and selective antimicrobial activity of probiotics against periodontal pathogens. Our results also suggest that regular consumption of probiotics-containing yogurt may provide favorable environmental conditions for periodontal health maintenance. Considering the sequence-dependent inhibition, the effect of consuming yogurt after tooth cleaning should be further investigated

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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