Turkish Journal of Agriculture - Food Science and Technology, 9(2): 339-345, 2021
DOI: https://doi.org/10.24925/turjaf.v9i2.339-345.3954

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Inhibitory Effect of Probiotics Lactobacillus Supernatants Against Streptococcus Mutans and Preventing Biofilm Formation

Tuğba Demir[1,a,*], Hakan Demir[2,b]

[1]*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, 58140 Sivas, Turkey*
[2]*Department of Prosthodontics, Faculty of Dentistry, Sivas Cumhuriyet University, 58140 Sivas, Turkey*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 05/10/2020
Accepted : 15/10/2020

Keywords:
Streptococcus mutans
Probiotic cultures
Biofilm activity
Oral health
Antimicrobial Activity

ABSTRACT

Probiotic microorganisms can release bioactive substances that can inhibit the growth and biofilm formation of pathogenic microorganisms such as *Streptococcus mutans*. Dental caries is a multi-factorial chronic infection disease, which starts with bacterial biofilm formation caused mainly by *S. mutans*. This study investigated the characteristics of *Lactobacillus spp*. strains as an oral probiotic. Twelve *Lactobacillus spp*. species obtained from fermented milk and dairy products were identified. Antimicrobial activity was determined with the Minimum Inhibition Concentration against *S. mutans* as an oral pathogen. Biofilm formation capabilities of the identified *Lactobacillus* strains in supernatant and culture media were determined. In addition, their ability to α-amylase tolerance and pH values (24h-48h) were determined. *L.plantarum* showed the highest antimicrobial activity compare other *Lactobacillus* strains. Also, *L. plantarum* inhibited biofilm formation.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 9(2): 339-345, 2021

Probiyotik Lactobacillus Süpernatantlarının Streptococcus Mutans'a Karşı İnhibitör Etkisi ve Biyofilm Oluşumunu Önleme

MAKALE BİLGİSİ

Araştırma Makalesi

Geliş : 05/10/2020
Kabul : 15/10/2020

Anahtar Kelimeler:
Streptococcus mutans
Probiyotik Kültürler
Biyofilm Aktivitesi
Ağız Sağlığı
Antimikrobiyal aktivite

ÖZ

Probiyotik mikroorganizmaların ürettiği biyoaktif maddeler, *Streptococcus mutans* gibi patojenik mikroorganizmaların büyümesini ve biyofilm oluşumunu engellemeye yardımcı olabilirler. Diş çürükleri, esas olarak *S. mutans*'ın neden olduğu bakteriyel biyofilm oluşumuyla başlayan, çok faktörlü kronik bir enfeksiyon hastalığıdır. Bu çalışmada oral probibiyotik olarak *Lactobacillus spp.* suşlarının karakteristik özellikleri araştırılmıştır. On iki *Lactobacillus spp.* türü fermente süt ve süt ürünlerinden elde edilmiştir. *S. mutans*'a karşı antimikrobiyal aktivite, Minimum İnhibisyon Konsantrasyonu ile belirlenmiştir. Tespit edilen *Lactobacillus* suşlarının süpernatant ve kültür ortamında biyofilm oluşturma yetenekleri araştırılmış, ayrıca α-amilaz toleransı ve pH değişim (24h-48h) kabiliyetleri incelenmiştir. *L. plantarum*, diğer *Lactobacillus* suşlarına kıyasla en yüksek antimikrobiyal aktiviteyi göstermiş, ayrıca *L. plantarum*, biyofilm oluşumunu inhibe etmiştir.

[a] *tugba@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-5195-9372* | [b] *hdemir@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-1769-1667*

Introduction

Probiotics are included in the literature as living microorganisms that can provide a health benefit to the host by balancing microorganisms (Meurman, 2005). Studies conducted in the field of characterization of probiotic bacteria have shown their anti-cancer, anti-oxidant, anti-bacterial and anti- fungal effects (Lee and Kim, 2014; Li et al., 2019). Probiotics can be found in different functional and traditional foods (yoghurt, types of cheese and fermented foods, etc.) (Lodi et al., 2010; Taha et al., 2017). Lactic acid bacteria are the most common group to obtain probiotics. *Lactobacillus spp*. and *Bifidobacterium* spp are the main ones treated intestinal dys-function and dys-biosis. (Meurman and Stamatova, 2007).

Probiotic bacteria aid in periodontitis by stabilizing the oral microbial-flora. *Lactobacillus spp* suppress growth of periodontal pathogens and inhibited them. In recent studies showed application of advantageous bacteria, as an asistant to inhibit re-colonization of periodonto pathogens and in overall pocket depth reduction. Probiotic microorganisms can adhere to dental tissues as part of the biofilm. These microorganisms with functional properties can compete with the growth of cariogenic bacteria or periodontal pathogens such as *S. mutans*. (Balakrishnan et al., 2000; Penala et al., 2016; Higuchi et al., 2019).

Dental caries and periodontal diseases are infectious diseases and public health problems (Coqueiro et al., 2018). *Streptococcus mutans* is one of the primary pathogens that resposible for tooth decay and oral cavity formation. *S. mutans* is among the bacteria that is early colonizer oral cavity, and responsible for formation of the biofilm in the oral cavity (Ahmed et al., 2014). *S. mutans* is also one of the cariogenic bacteria, and plays important role in the pathogenesis of dental caries (Balakrishnan et al., 2000).

The oral cavity is one of entrance door of the body, and is home to many microorganisms. It is stated in the literature that there are more than 700 microorganism species in the oral flora. This variety of hosts, consisting of gram-negative and gram-positive microorganisms, is in equilibrium with the body defense system. This balance could turn in favor of opportunistic microorganisms as a result of nutritional habits, saliva pH changes, and changes in body health. (Cutler and Jotwani, 2006, Wu et al., 2018). If acidic pH increases, the incidence of tooth decay increases, and as saliva ph becomes basic, the incidence of dental calculus and periodontal ailments increase (Palmer, 2014).

In dentistry, recent researches support that Lactobacillus spp. culture strains might play a role as antogonistic agents on cariogenic bacteria species inhibiting *S.mutans* level in saliva or pellicle (de Souza Rodrigues et al., 2020). The most commonly used species in oral probiotic preparations are *Lactobacillus bulgaricus, L. acidophilus, L. casei, L. helveticus, L. lactis, L. salivarius, L. plantarum, Streptococcus thermophilus, Enterococcus faecium, E. faecalis, Bifidobacterium* and *Saccharomyces boulardii*.

The major objective of this research is to determine the effect of *Lactobacillus spp* strains for preventing on tooth decaying. For this purpose, *Lactobacillus spp.* derived from fermented dairy product of the probiotic was investigated on the viability and in the virulence factors of *S. mutans* growth in supernatant culture medium, and determined their antimicrobial, biofilm and amylase tolerances.

Materials and Methods

Bacterial Strains and Sample Preparation

Lactobacillus spp. were isolated using Lactobacillus selective agar (Merck KGaA, Germany) from milk products. The selected isolates were identified according to their morphological and biochemical properties. Strains were propagated and maintained in MRS (Man-Rogosa-Sharpe) broth, at 37°C. Then, strains were cultured in MRS broth at 37°C (24h). They centrifuged for on min at 4°C, 1000 rpm to obtain the supernatant part, which were filtered and used as analysis samples. In addition, *Streptococcus mutans* (ATCC 25175) was incubated for 24h, 37°C and 5 %CO_2 in Brain Heart Infusion (BHI) Broth.

Antimicrobial Effect of Lactobacillus Spp. Aganist S. Mutans

The Miniumum Inhibitory Concentration (MIC) of samples were measured aganist *S. mutans* using 96-well-plates (Lim et al., 2020). *Lactobacillus spp.* (Filter-sterilized) supernatants were serial diluted using BHI broth (ranging from 100%-0,78%). The diluted samples were added into each well (10^6 CFU/mL). The lowest sample concentration (*Lactobacillus spp*) that inhibited max (99%) of the inoculums were considered as the minimum inhibition concentration. Ampicillin standard antibiotic kit was used as control group in MIC experiments. (500 µg/mL- 7.8 µg/mL).

Biofilm Formation of The Culture Supernatant of Lactobacillus Spp Against S. Mutans

The effect of the *Lactobacillus spp* supernatants on biofilm formation was evaluated as Stepanovic *et al* (Stepanović et al., 2000). 100µL aliquot of the supernatant was added to 100 µL BHI containing 10^6 CFU/mL of *S. mutans* each, and control sample was prepared by adding 100 µL MRS medium (instead of supernatant). Each sample (200 µL) was transferred to a 96 well plate following incubation (37°C, 24h). In order to assess the extent of biofilm formation in each microplate, the culture medium was discarded, and the plate was washed with 200 µL of PBS. Adherent biofilm cells were stained with 200 µL of 0.1% crystal violet for 10 min at room temperature. The plates were rinsed with distilled water, and then the adhered dye was dissolved with acid-alcohol solutions. The absorbance was measured as 540 nm, and biofilm inhibition amount was calculated. Three replicates were prepared for each sample.

A-amylase Tolerance

The pH of culture supernatant was measured after incubation for 24 h and 48 h. We also compared the growth characteristics of each *Lactobacillus spp*. strains α-amylase tolerances were assessed as a measure of resistance to oral enzymatic stress.

Table 1. Screening of twelve *Lactobacillus spp*. bacterial strains

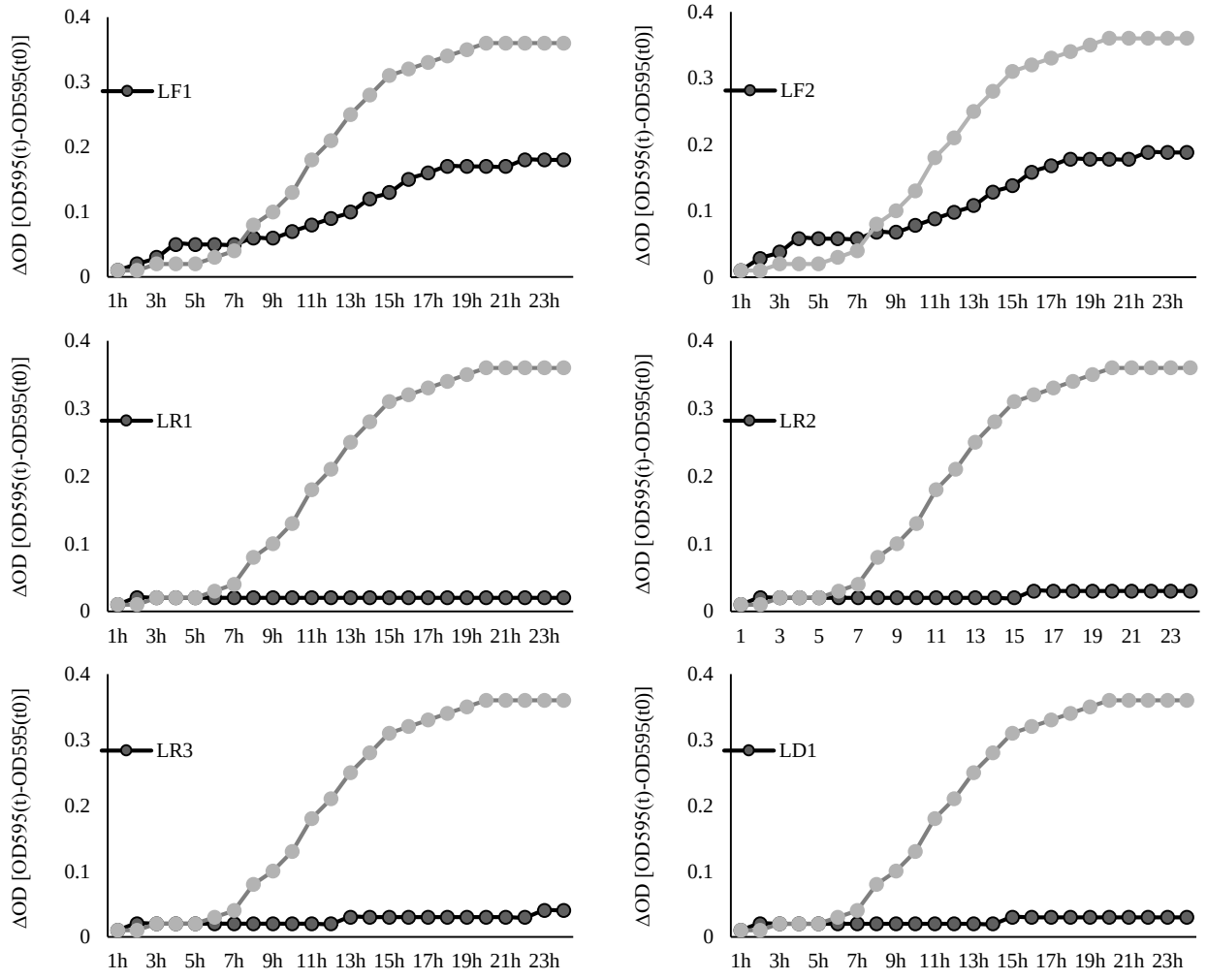
	Strain	Species[1]	%	α-amylase tolerance[2]	pH 24h 48h	Researches/ Results
1	LF1	L. fermentum	99.9	+++	6.024.13	Antagonistic activity on the growth of S. mutans, (Strahinic et al., 2007)
2	LF2	L. fermentum	99.9	++	6.104.15	
3	LR1	L. rhamnosus	99.9	+++	6.034.12	L. rhamnosous reduced S. mutans associated caries risk and initial caries development, (Cagetti et al., 2013)
4	LR2	L. rhamnosus	99.9	++	6.014.05	
5	LR3	L. rhamnosus	99.9	+++	5.954.03	
6	LD1	L. delbrueckii	99.9	+++	6.034.12	Inhibition of biofilm formation (Lim et al., 2020)
7	LD2	L. delbrueckii	99.9	++	6.103.88	
8	LP1	L. plantarum	99.9	+++	6.123.92	Hampers S. mutans growth and biofilm formation in vitro (Vuotto et al., 2014)
9	LP2	L. plantarum	99.9	+++	6.133.87	
10	LP3	L. plantarum	99.9	+++	6.053.95	
11	LA1	L. acidophilus	99.9	++	6.023.83	Showed marked salivary pH elevation and reduction of salivary S. mutans. (SrivaStava et al., 2016)
12	LA2	L. acidophilus	99.9	++	6.043.91	

[1]16S rRNA gene sequence identy %; [2]pH 6.8 1000U/mL of enzyme, 37°C,4h.

Table 2. Antimicrobial activity of the *Lactobacillus spp*. aganist *S.mutans*

Activity (S.mutans)	Minimum Inhibitory Concentration (%)											
	LF1	LF2	LR1	LR2	LR3	LD1	LD2	LP1	LP2	LP3	LA1	LA2
S.mutans*+BHI broth	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
S.mutans+supernatant (24h)	<50^{b}	<50^{b}	<12.5^{a}	<12.5^{a}	<12.5^{a}	<12.5^{a}	<12.5^{a}	<12.5^{a}	<12.5^{a}	<12.5^{a}	>50^{c}	>50^{c}
S.mutans+supernatant (48h)	>50^{c}	>50^{c}	<25^{b}	<25^{b}	<25^{b}	<25^{b}	<25^{b}	<12.5^{a}	<12.5^{a}	<12.5^{a}	ND	ND
Ampicillin (µg/mL)	<0.78	<0.78	<0.78	<0.78	<0.78	<0.78	<0.78	<0.78	<0.78	<0.78	<0.78	<0.78

Ampicillin was used as control and it was serially diluted from 100 µg/mL to 0.78 µg/mL. a, b, c values with different letters in the same row are significantly different (P<0.05), ND: not detected. *; 106 cfu/mL.



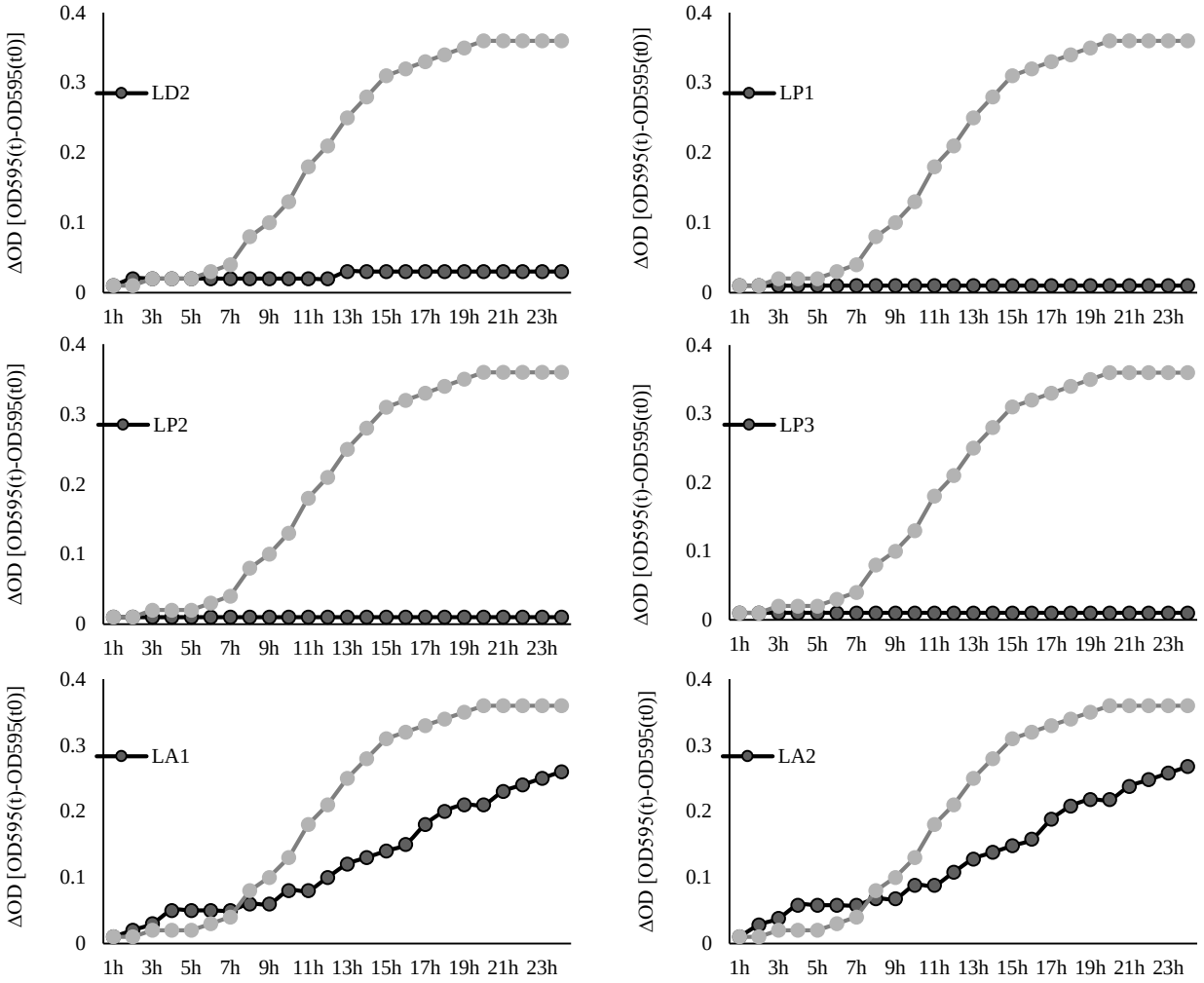


Figure 1. Growth of *S. mutans* in BHI mixed with the spent culture supernatant from each *Lactobacillus spp*. strains

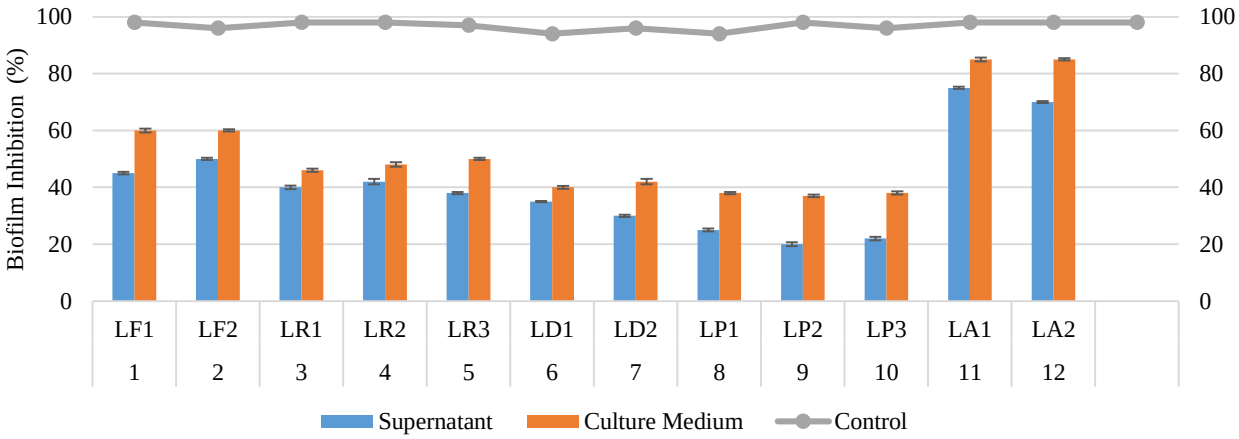


Figure 2. Inhibitory effect of the culture medium and supernatant of *Lactobacillus spp* strains on *S. mutans* biofilm formation (P<0.05).

For this experiment, the pH value of the BHI broth was adjusted to 6.8, and broth was supplemented α-amylase (1000 IU/mL) (A0521; Sigma Aldrich, USA). All strains were incubated (4h, 37°C), the samples were diluted in 0.05M buffer solution (sodium phosphate). α-amylase tolerance was determined by comparing final count with the initial count. The all experiments were performed three times

Statistical Analysis

All the tested sample data are reported as the mean and standard deviation. One way analysis of varience (ANOVA) with SPSS statistical software (SPSS version 19.0 software, SPSS; Chicago, IL, USA) was used to determine the significant differences. All the mean values were used for the Duncan's multiple tests to perform posthoc verification (P<0.05).

Result and Discussion

The antimicrobial activity of the *Lactobacillus spp.* supernatants of the selected strains against *S. mutans* was interpreted by measuring the optical densities at 595 nm (Optical Density$_{595nm}$). We selected 12 strains of *Lactobacillus spp.* that were previously isolated form the milk and milk products, including 12 strains of *L. fermentum* (LF1, LF2), 3 strains of *L. rhamnosus* (LR1, LR2, LR3), 2 strains of *L. delbrucki* (LD1, LD2), 3 strains of *L. plantarum* (LP1, LP2, LP3) and 2 strains of *L. acidophilus* (LA1, LA2). All of the strains were screened antimicrobial activity against *S. mutans* using cultures and MIC for that purpose we analyzed indirect effect of the *Lactobacillus spp.* using only the culture filtrate that was obtained then its growth in BHI broth for 12h and 24 h (*S. mutans* + Lactobacillus supernatant interection group).

All 12 *Lactobacillus* strain analysis showed antimicrobial activity, only the *L. acidophilus* strains (LA1, LA2) had no inhibitory effects on *S. mutans* (Figure 1) after 24h in culture. Based on the results, it could be said that the eight strains had the highest antimicrobial activity, i.e., *S. mutans* LP1, LP2, LP3, LD1, LD2, LR1, LR2 and LR3 (Figure 1). These strains reduced *S. mutans* growth by more than 87.5% after 24h culture (Table 2). The virulance of *S. mutans* might be due to their ability to survive in acidic pH, and productability of biofilm (Krzyściak, et al., 2014; Simón-Soro and Mira, 2015).

The antimicrobial activities of *Lactobacillus spp.* were investigated against *S. mutans* (Table 1). Ampicillin standard was used as control and its MICs were <0.78% for *S mutans*. The highest MIC values of LP1, LP2, LP3 were 12.5%, 12.5%, and 12.5% against *S. mutans* at 24h. The lowest MIC values of LA1, LA2, were 50% and 50% against *S. mutans* (Table 2).

LD and LR strains showed acceptable and sensitive antimicrobial activity against *S. mutans*. These effects could be attributable on the antibacterial substances produced including organic acids, bacteriocin and biosurfactants (Lin et al., 2017). The most of oral *Lactobacillus spp.* can inhibit the growth of pathogens causing periodontitis and caries *in vitro* (Kõll-Klais et al., 2005).

Rossoni et al. (Rossoni et al., 2018) compared the effects of twenty-two strains of *Lactobacillus* that were isolated from oral cavities of caries-free subjects. All of the *Lactobacillus spp.* showed antimicrobial activity against *S. mutans*. *L. paracasei* 25.4, *L. fermentum* 20.4, *L. paracasei* 20.3 and *L. paracasei* 11.6 observed the highest antimicrobial activity against *S. mutans*. These strains reduced *S. mutans* growth by more than 86% after 24 h in culture.

Eight *Lactobacillus spp.* strains showed different bactericidal activities in the time-kill assay. Strains LP1, LP2 and LP3 from traditionally fermented milk products showed stronger antibacterial activity compared with other *Lactobacillus spp.* (LA1, LA2) strains against *S. mutans* after incubation for 24h. The reason for this exclusion might be closely correlate to race, living environment, health status and food intake of the host.

A large number of extracellular polysaccharides synthesized by *S. mutans* are important to the complex tridimensional structure of a dental plaque (Bowen and Koo, 2011). According to a report by Nobbs and colleagues, the formation of an early biofilm of *S. mutans* can be observed at 24 h (24 h: maturation of early-stage biofilm and 48 h: maturation of the later-stage biofilm (Nobbs et al., 2009).

In this study, we added the *L. plantarum* strains fermentation supernatant at these two time points (24 h and 48 h) to mediate the formation of the *S. mutans* biofilm. The ability of a probiotic to colonize the oral cavity is key to its function, and this ability is strain-specific. *Lactobacillus* family may effectively prevent biofilm formation, perhaps because *L. plantarum* (5D-3) had the advantage of pre-colonization of the oral cavity (Zhang et al., 2020).

The all strains that completely inhibited the growth of *S. mutans* was further investigated for effects on biofilm inhibition formation. Among to twelve strains showing antibacterial activity, LP1 exhibited the strongest antibiofilm formation activity. (Figure 2). Different studies have shown that the *L rhamnosus* and *L. paracasei* origin comsumption could reduce *S. mutans* biofilm formation (Chuang et al., 2011).

In our study, all *Lactobacillus spp.* strains prevented biofilm formation by inhibition at different levels. When our study findings are evaluated, supernatant cultures ability to inhibit biofilm formation parallels the antimicrobial activity findings. Our results related to the antimicrobial activity and biofilm formation inhibition levels of *Lactobacillus spp*. strains on *S. mutans* are close to those of previous studies. Reports have emphasized the importance of biofilm formation in the development of dental caries or oral cavity microflora (Klein, et al., 2015; Salli et al., 2017). Recently, researchers are taking more interest in the use of probiotics to maintain the oral health (Rossoni et al., 2018; de Souza Rodrigues et al., 2020; Lim et al., 2020).

While both the containing culture medium and supernatant of *Lactobacillus spp*. could inhibit the *S. mutans* biofilm formation, the supernatant ingredients samples showed higher inhibitory effects (Figure 2). *L. plantarum* inhibited the *S. mutans* biofilm formation by over 80%, at the same volume, and showing it as the strongest inhibitor agents. *L. acidophilus* could inhibit the *S. mutans* biofilm formation at low rates (<30%). These biofilm mechanisms heve been announced to be due to the coaggregation with *Lactobacillus spp*. resulting in physical interference and induction of exopolisaccharides production (Wu et al., 2015; Ahn et al., 2018).

Lee and Kim found that Lactobacillus rhamnosus LGG suppressed *S. mutans* biofilm forma- tion by reducing glucan production and antimicrobial activity (Lee and Kim, 2014).

A mature dental plaque biofilm is a three-dimensional micro-ecological environment comprising various bacteria embedded in a matrix mainly composed of water-insoluble polysaccharides with a certain thickness (Featherstone, 2004).

As shown in Table 1, the initial pH values also showed the α-amylase tolerance ability. The initial pH of the cultures was 6.90. After that pH value decreased to 5.95-6.13 then 24 h of incubations. The end of the incubation 45 h cultures pH of the strains was significantly lower, which ranged from 3.83-4.15 ($P<0.05$). Culture strains LF1, LR1, LR3, LD1, LP1, LP2 and LP3 showed the highest α-amylase resistance, with survivalibilty >90%.

Conclusion

Within the limitations of this *in vitro* study, the following conclusions were drawn.

- Probiotics showed different levels of antimicrobial and antibiofilm activity on oral flora.
- Probiotic bacteria contained in traditional fermented milk products *S. mutans* inhibition was found at significant levels. Especially *L. plantarum* is thought to be a potent oral probiotic for oral and dental health by preventing biofilm formation.
- The identification of these *Lactobacillus* spp. strains, which naturally inhabit the oral cavity and show antimicrobial activity against *S. mutans*, contributes to the development of new probiotic agents to prevent dental caries.

References

Ahmed A, Dachang W, Lei Z, Jianjun L, Juanjuan Q, Yi X. 2014. Effect of Lactobacillus species on Streptococcus mutans biofilm formation. Pakistan Journal of Pharmaceutical Sciences, 27(5):1523-1528

Ahn KB, Baik JE, Park OJ, Yun CH, Han SH. 2018. Lactobacillus plantarum lipoteichoic acid inhibits biofilm formation of Streptococcus mutans. PLoS One, 13(2): e0192694.

Balakrishnan M, Simmonds RS, Tagg JR. 2000. Dental caries is a preventable infectious disease. Australian Dental Journal, 45(4): 235-245.

Bowen WH, Koo H. 2011. Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Research, 45(1): 69-86.

Cagetti MG, Mastroberardino S, Milia E, Cocco F, Lingström P, Campus G. 2013. The use of probiotic strains in caries prevention: a systematic review. Nutrients, 5(7): 2530-2550.

Chuang LC, Huang CS, Ou-Yang LW, Lin SY. 2011. Probiotic Lactobacillus paracasei effect on cariogenic bacterial flora. Clinical Oral Investigations, 15(4): 471-476.

Coqueiro AY, Bonvini A, Raizel R, Tirapegui J, Rogero MM. 2018. Probiotic supplementation in dental caries: is it possible to replace conventional treatment. Nutrire, 43(1): 6.

Cutler CW, Jotwani R. 2006. Dendritic cells at the oral mucosal interface. Journal of Dental Research, 85(8): 678-689.

De Souza Rodrigues JZ, Passos MR, de Macêdo Neres NS, Almeida RS, Pita LS, Santos IA, Lima BO. 2020. Antimicrobial activity of Lactobacillus fermentum TcUESC01 against Streptococcus mutans UA159. Microbial Pathogenesis, 142: 104063.

Featherstone JDB. 2004. The continuum of dental caries evidence for a dynamic disease process. Journal of Dental Research, 83(1): 39-42.

Higuchi T, Suzuki N, Nakaya S, Omagari S, Yoneda M, Hanioka T, Hirofuji T. 2019. Effects of Lactobacillus salivarius WB21 combined with green tea catechins on dental caries, periodontitis, and oral malodor. Archives of Oral Biology, 98: 243-247.

Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. 2015. Streptococcus mutans-derived extracellular matrix in cariogenic oral biofilms. Frontiers in Cellular and Infection Microbiology, 5(10): 1-8.

Kõll-Klais P, Mändar R, Leibur E, Marcotte H, Hammarström L, Mikelsaar M. 2005. Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. Oral Microbiology and Immunology, 20(6): 354-361.

Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. 2014. The virulence of Streptococcus mutans and the ability to form biofilms. European Journal of Clinical Microbiology & Infectious Diseases, 33(4): 499-515.

Lee SH, Kim YJ. 2014. A comparative study of the effect of probiotics on cariogenic biofilm model for preventing dental caries. Archives of Microbiology, 196(8): 601-609.

Lim SM, Lee NK, Paik HD. 2020. Antibacterial and anticavity activity of probiotic Lactobacillus plantarum 200661 isolated from fermented foods against Streptococcus mutans. LWT, 118: 108840.

Lin X, Chen X, Tu Y, Wang S, Chen H. 2017. Effect of probiotic lactobacilli on the growth of Streptococcus mutans and multispecies biofilms isolated from children with active caries. Medical science monitor: International Medical Journal of Experimental and Clinical Research, 23: 4175.

Lodi CS, Manarelli MM, Sassaki KT, Fraiz FC, Delbem ACB, Martinhon CCR. 2010. Evaluation of fermented milk containing probiotic on dental enamel and biofilm: in situ study. Archives of Oral Biology, 55(1): 29-33.

Meurman JH. 2005. Probiotics: do they have a role in oral medicine and dentistry? European Journal of Oral Sciences, 113(3): 188-196.

Meurman JH, Stamatova I. 2007. Probiotics: contributions to oral health. Oral Diseases, 13(5): 443-451.

Nobbs AH, Lamont RJ, Jenkinson HF. 2009. Streptococcus adherence and colonization. Microbiology and Molecular Biology Reviews, 73(3): 407-450.

Palmer Jr RJ. 2014. Composition and development of oral bacterial communities. Periodontology 2000, 64(1): 20-39.

Penala S, Kalakonda B, Pathakota KR, Jayakumar A, Koppolu P, Lakshmi BV, Mishra A. 2016. Efficacy of local use of probiotics as an adjunct to scaling and root planing in chronic periodontitis and halitosis: a randomized controlled trial. Journal of Research in Pharmacy Practice, 5(2): 86.

Rossoni RD, dos Santos Velloso M, de Barros PP, de Alvarenga JA, dos Santos JD, dos Santos Prado ACC, Junqueira JC. 2018. Inhibitory effect of probiotic Lactobacillus supernatants from the oral cavity on Streptococcus mutans biofilms. Microbial Pathogenesis, 123: 361-367.

Salli KM, Gürsoy UK, Söderling EM, Ouwehand AC. 2017. Effects of xylitol and sucrose mint products on Streptococcus mutans colonization in a dental simulator model. Current Microbiology, 74(10): 1153-1159.

Simón-Soro A, Mira A. 2015. Solving the etiology of dental caries. Trends in Microbiology, 23(2): 76-82.

SrivaStava S, Saha S, Minti KuMari SM. 2016. Effect of probiotic curd on salivary pH and Streptococcus mutans: a double blind parallel randomized controlled trial. Journal of Clinical and Diagnostic Research: JCDR, 10(2): ZC13.

Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. Journal of Microbiological Methods, 40(2): 175-179.

Strahinic I, Busarcevic M, Pavlica D, Milasin J, Golic N, Topisirovic L. 2007. Molecular and biochemical characterizations of human oral lactobacilli as putative probiotic candidates. Oral Microbiology and Immunology, 22(2): 111-117.

Taha S, El Abd M, De Gobba C, Abdel-Hamid M, Khalil E, Hassan D. 2017. Antioxidant and antibacterial activities of bioactive peptides in buffalo's yoghurt fermented with different starter cultures. Food Science and Biotechnology, 26(5): 1325-1332.

Vuotto C, Longo F, Donelli G. 2014. Probiotics to counteract biofilm-associated infections: promising and conflicting data. International Journal of Oral Science, 6(4): 189-194.

Wu CC, Lin CT, Wu CY, Peng WS, Lee MJ, Tsai YC. 2015. Inhibitory effect of Lactobacillus salivarius on Streptococcus mutans biofilm formation. Molecular Oral Microbiology, 30(1): 16-26.

Wu CY, Su TY, Wang MY, Yang SF, Mar K, Hung SL. 2018. Inhibitory effects of tea catechin epigallocatechin-3-gallate against biofilms formed from Streptococcus mutans and a probiotic Lactobacillus strain. Archives of Oral Biology, 94: 69-77.

Zhang Q, Qin S, Huang Y, Xu X, Zhao J, Zhang H, Chen W. 2020. Inhibitory and preventive effects of Lactobacillus plantarum FB-T9 on dental caries in rats. Journal of Oral Microbiology, 12(1): 1703883.