Thermophilin 110 inhibits growth and biofilm formation of *Streptococcus mutans*

John A. Renye*, Dennis H. Steinberg

Dairy and Functional Foods Research Unit, Agricultural Research Service, USDA, 600 E. Mermaid Lane, Wyndmoor, PA 19038

ARTICLE INFO

Keywords:
Streptococci
Bacteriocins
Probiotics
Antimicrobials
Biofilms

ABSTRACT

Dental caries continues to occur in both children and adults worldwide resulting in significant economic burden, and consumers have expressed interest in natural products that can prevent these recurrent infections. In this study, *S. thermophilus* B59671, which produces thermophilin 110, was shown to inhibit the growth of *S. mutans* UA159. A thermophilin concentration ≥ 80 AU ml⁻¹ prevented the growth of *S. mutans* UA159 in batch culture, while ≥ 160 AU ml⁻¹ was required to prevent biofilm growth. Co-culturing *S. thermophilus* B59671 and *S. mutans* UA159 also resulted in impaired biofilm growth. Thermophilin 110 was also shown inhibit additional *S. mutans* strains and commensal oral streptococci at higher concentrations (640-1280 AU ml⁻¹). These results suggest that thermophilin 110 could be used as a natural antimicrobial in oral care products and support the need for additional studies to assess the probiotic potential of *S. thermophilus* B59671.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

* S. thermophilus B59671 naturally produces thermophilin 110, a bacteriocin that inhibits the growth of the oral pathogen *Streptococcus mutans*
* Thermophilin 110 was shown to prevent biofilm formation by *S. mutans* UA159
* Co-culturing *S. thermophilus* B59671 with *S. mutans* UA159 prevented biofilm formation.

1. Introduction

The oral microbiome is a diverse community where over 700 prokaryotic taxa have been identified through 16S rRNA sequencing, many of which remain unculturable [1]. Within a single individual the dental plaque may consist of several hundred microbial species [2], which exist in a relatively stable pattern of colonization [3]. However, the stability of this community is dependent on the diet, with frequent exposure to carbohydrates possibly leading to a dysbiotic microbiome with increased acid production [4]. The acidified biofilm results in the progressive loss of tooth enamel and dentine. Dental caries is considered the most widespread noncommunicable disease [5], with an estimated global economic impact of US$ 442 billion in 2010 [6]. Although public health efforts have focused on reducing the sugar intake in diets, dental caries remain a major concern globally, and the reduction of caries in all age groups continues to be an objective for the United States in Healthy People 2020 [7].

In humans, the bacteria most commonly associated with the development of dental caries have been mutans streptococci and lactobacilli [8]. *Streptococcus mutans* has long been considered to have central role in the development of dental caries due to its acidogenic nature and extracellular polysaccharides (EPS) production [9]. EPS production is dependent on the glucosyltransferase and fructosyltransferase enzymes which use sucrose as a substrate for the production of glucose and fructose homopolysaccharides respectively. The EPS assists in the adherence of *S. mutans* to the tooth surface and cell to cell interactions, which can increase plaque thickness and retain acid [10]. The role of sucrose in EPS and acid production has led to it being considered the most cariogenic carbohydrate.

The use of fluoride salts in drinking water, toothpastes and mouthwashes has been a primary strategy for preventing tooth decay due to its ability to inhibit the bacterial growth and prevent enamel demineralization. Fluoride also contributes to the remineralization of tooth surfaces; however, fluoride alone is not sufficient to prevent caries...
formation and is often combined with other antimicrobials [11, 12]. Chlorhexidine, triclosan and sodium dodecyl sulphate (SDS) are chemical agents which have been used in both toothpaste and mouthwash to prevent caries formation [11], but consumer concern over the safety of these components has resulted in the search for natural alternatives. Several plant extracts have been reported to inhibit the growth of mutants streptococci and/or prevent biofilm formation [13]; and xylitol has been successful as a sugar substitute since it cannot be metabolized by plaque bacteria and has bacteriostatic activity against S. mutans [14]. Antimicrobial peptides (AMP) with activity against oral pathogens have been identified from a variety of sources, including plants, animals and bacteria. However, the application of AMPs from plant and animal sources has been limited by concerns over production cost, and peptide stability [11].

AMPs produced by food grade and probiotic bacteria, called bacteriocins or bacteriocin-like substances (BLSIs), are gene encoded, and secreted from the producing cells in an active conformation. The generally regarded as safe (GRAS) designation of these bacteria allow for their use in food or non-food applications, which would reduce production costs associated with purifying active AMPs. Lacticin 3147 is two-peptide bacteriocin produced by Lactococcus lactis DPC3147 which has been shown to inhibit the growth and biofilm formation of S. mutans, suggesting the peptide could be used in applications to improve oral health [15]. Other studies have screened several LAB isolates from foods [16] or the oral cavity of healthy individuals [17] and identified novel bacteriocin producing strains with activity against oral pathogens. LAB also offer the advantage of serving as probiotics for improving oral health [18]. Specifically, Streptococcus salivarius strains K12 and M18 have been shown to produce broad spectrum salivaricins, and been used successfully as oral probiotics for preventing halitosis (oral malodor) and dental caries, respectively [19, 20].

Streptococcus thermophilus is a food-grade LAB that is commonly used as a starter culture for production of yogurt and cheese, and several strains have been reported to produce a broad-spectrum AMP encoded within the bacteriocin-like peptide (blp) gene cluster. The gene cluster was first characterized in strains LMG18311, LMD-9 and CNZR1066 [21]; however, only LMD-9 produced a broad-spectrum bacteriocin (thermophilin) following induction with a recombinant quorum sensing signal peptide [22]. The blp gene cluster has been characterized in additional strains, with strains ST109 and B59671 shown to naturally produce thermophilins 109 and 110, respectively [23, 24]. Broad-spectrum antimicrobial activity for multiple blp-encoded thermophilins has included Listeria monocytogenes and enterococci [25, 26]; and more recently it was shown that strain B59671 inhibited the growth of S. mutans UA159 in a well-diffusion assay [27]. Furthermore, another study reported that yogurt containing S. thermophilus and Lactobacillus bulgaricus reduced the survival rate of S. mutans in vitro; and suggested this may due to the presence of a heat-labile bacteriocin [28]. The current study aims to characterize the antimicrobial activity of thermophilin 110 against S. mutans to determine its potential for use in oral health applications.

2. Materials and Methods

2.1. Bacterial strains and bacteriocin preparation

S. thermophilus strains B59671, ST106, ST109, ST113 and LMD-9 were maintained in tryptone-yeast-extract-lactose (TYL) broth, and S. mutans (strains UA159, GS-5 and ATCC 25175), Streptococcus gordonii (strain DL-1); and Streptococcus sanguis (strains V736 and lac8) were maintained in Todd Hewitt broth (Difco Laboratories, Detroit, MI) at 37°C.

Thermophilin 110 was partially purified from 400 ml overnight culture of S. thermophilus B59671 as previously described [25]. Briefly, cell free supernatant (CFS) was mixed with 0.5 volume (200 ml) of chloroform and stirred vigorously for 45 min. The mixture was separated by centrifugation and both the aqueous and solvent phases were decanted and discarded. Sediment from the interface and wall deposits were dispersed in 15 ml of sterile water and centrifuged again to remove excess solvent. Trace amount of solvent was removed using a stream of air, and solid material was dispersed in 5 ml of water and stored overnight at 4°C. The sample was collected by centrifugation, the liquid phase was decanted and the remaining sediment was dispersed in 5 ml of water. Semi-quantitative analysis of the thermophilin sample determined by preparing serial two-fold dilutions of the thermophilin sample and using 50 μl of each dilution in a well-diffusion assay, with ST113 as the target bacterium. The arbitrary units (AU) ml⁻¹ were calculated as the reciprocal of the highest dilution multiplied by 20. The stock of thermophilin 110 was then diluted in water to obtain a concentration of 5,120 AU ml⁻¹.

2.2. Antimicrobial activity against streptococcal species

Inhibition of S. mutans UA159 growth was first demonstrated by both well diffusion and agar-agarse overlay assays [25]. Briefly, S. thermophilus NRRL-B59671 (USDA, Agricultural Research Service Culture Collection), ST106, ST109 and LMD-9 were grown overnight in TYY broth at 37°C. A synthesized 30-mer peptide, corresponding to the mature BlpC signaling peptide, was added to ST106 and ST109 cultures at 250 ng ml⁻¹ to induce expression of blp genes [29]. A 50 μl sample of CFS was loaded into precast wells within an agar medium seeded with S. thermophilus ST113 or S. mutans UA159 (0.5% v/v). Dishes were stored at 4°C overnight, and then incubated at 37°C and monitored for zones of inhibition. Well diffusion assays using 2-fold dilutions of thermophilin 110 were used to determine the concentration required to inhibit the growth of additional oral streptococci.

For overlay assays, thermophilin-producing cells were diluted and spread on TYY containing 0.5% agar and 0.7% agarose. Plates were incubated for 48 h at 37°C, after which the intact agar/agarose medium was removed and placed on top of TH agar inoculated with S. thermophilus UA159. Following overnight storage at 4°C, the plates were incubated at 37°C and monitored for inhibition zones corresponding to the location of S. thermophilus colonies.

Inhibition of S. mutans UA159 growth in broth medium was carried out using a Cytation 5 multi-mode plate reader (BioTeck, Winooski, VT). Prior to each experiment a stock of Todd Hewitt (TH) broth containing a 4-fold dilution of the thermophilin 110 stock was prepared, resulting in a final concentration of 1,280 AU/ml. Serial two-fold dilutions were then prepared in TH broth resulting the following concentrations: 640, 320, 160, 80, 40, 20, 10 and 5 AU/ml. A 96-well microtiter dish was loaded 200 μl of TH broth (control) and the TH + thermophilin 110 dilutions. Growth was monitored at 37°C for 24 h, and inhibition assays were repeated three times. Serial dilutions were also prepared for antimicrobials that have been used in oral care product for caries prevention, including: sodium fluoride (0.004, 0.008, 0.016, 0.031, 0.063, 0.125%) and triclosan (0.0003, 0.0006, 0.0013, 0.0025, 0.005, 0.01%). Growth inhibition of S. mutans UA159 in the presence of these antimicrobials was monitored by optical density at 37°C for 24 h.

2.3. Inhibition of biofilm formation

S. mutans biofilms were established in 96-well flat bottom dishes using diluted TH broth (50%) supplemented with 0.5% sucrose (THS) as the culture medium. An overnight culture of S. mutans UA159 was diluted 48-fold in 200 μl of THS and THS supplemented with dilutions of the thermophilin 110 stock solution (5-640 AU/ml). Biofilms were established for 48h at 37°C, and growth was assayed by crystal violet staining as previously described [30]. Briefly, the dish was inverted and stained as previously described [30] . Briefly, the dish was inverted and stained as previously described [30].
associated with adherent bacteria. Plates were dried and then treated with 200 µl of 30% acetic acid to solubilize the crystal violet. A 125 µl sample of the crystal violet/acetic acid solution was moved to a separate well in a new 96-well dish, and the optical density was measured at 590 nm and used to determine percent inhibition. Biofilm inhibition assays were repeated a minimum of three times for each condition tested.

Cell viability within biofilms exposed to thermophilin 110 was accessed. After 48 h the culture medium was removed and collected to determine the viability of planktonic cells, and then the adherent cells were washed twice with water. The biofilm was manually dispersed in 0.1% peptone water and plated on TH agar. S. mutans viability was reported as colony forming units (CFU) ml⁻¹. Cell viability was accessed from a minimum of three separate biofilms.

S. mutans UA159 was co-cultured with either S. thermophilus B59671 or S. thermophilus ST128 to determine the effect on biofilm formation. An overnight culture of S. mutans UA159 was diluted into fresh THB containing 0.5% sucrose to a final OD₅₆₀ of 0.02. The inoculated medium was transferred to a 96-well dish (200 µl per well). Wells were subsequently inoculated with S. thermophilus at a final OD₅₆₀ of 0.02 (1x), 0.05 (2.5x), 0.1 (5x) or 0.5 (25x). The 96 well dish was incubated at 37°C for 48 h, and then monitored for biofilm growth by crystal violet staining, as described above. Results are the average ± SD of three independent experiments.

3. Results and Discussion

S. thermophilus strains B59671, ST106, ST109 and LMD-9 have been shown produce broad-spectrum bacteriocins; however only CFS from B59671 was shown to inhibit the growth of S. mutans UA159 (Fig. 1A). In addition, localized inhibition zones could be observed on an overlay assay corresponding to the location of B59671 colonies, suggesting that the bacterium could prevent S. mutans from colonizing the same ecological niche (Fig. 1B). Although all four of these strains produce bacteriocins encoded within the blp gene cluster, it has been reported that the activity spectrum may require expression of multiple peptides [26]. In addition, the gene clusters for ST106, ST019 and LMD-9 contain blpD, which was reported to encode an essential peptide for the antimicrobial activity of LMD-9 [24, 26]. Strain B59671 is unique in that it does not possess a copy of blpD, but does contain blpU, which has been reported to potentially encode a bacteriocin in strain LMD-9. Although a copy of blpU is present within the gene clusters of ST106, ST109 and LMD-9, it is expressed more than 50-fold higher in S. thermophilus B59671 [27], which may be essential for the unique antimicrobial activity observed for this strain.

A partially purified preparation of thermophilin 110 was also tested for its ability to inhibit the growth of S. mutans UA159 in broth medium. Addition of the peptide preparation at concentrations ranging from 80 to 1,280 AU/ml prevented the growth of S. mutans; while concentrations between 10 and 40 AU/ml showed attenuated growth (Fig. 2). As CFS from an overnight culture of B59671 has been reported to contain between 320 AU/ml of thermophilin 110 [25], these results suggest that the amount of peptide naturally produced by the bacterium could suppress the growth of S. mutans. S. mutans UA159 growth was also inhibited by 0.063% sodium fluoride or 0.01% triclosan, with the final OD₅₆₀ reaching 0.213 and 0.188 respectively after 24 h (data not shown). These concentrations were similar to the amount of sodium fluoride (0.05%) [31] or triclosan (0.03%) [32] present within mouthwashes used for caries prevention; thus the comparable level of inhibition observed for ≥ 80 AU/ml of thermophilin 110 demonstrates its potential for oral care applications.

3.1. Thermophilin 110 inhibits S. mutans biofilm formation

Biofilm formation of S. mutans UA159 was monitored quantitatively by measuring the optical density of crystal violet recovered from solubilized cells (Figure 3). Thermophilin 110 at 20 and 40 AU ml⁻¹ reduced biofilm growth by an average of 29 and 48%; however, results varied considerably between experiments. Results were less variable when higher concentrations of thermophilin 110 were tested, with 80, 160 and 320 AU ml⁻¹ inhibiting biofilm growth by 79, 92 and 95%, respectively. Cell viability was assessed after removing biofilms from their adhered surface, and it was shown that control biofilms contained 6.89 ± 0.81 log CFU ml⁻¹. When grown in the presence of 80, 160 or 320 AU ml⁻¹ of thermophilin 110, viable counts were reduced to 6.63 ± 0.12, 6.06 ± 0.32 and 5.74 ± 0.28 log CFU ml⁻¹, respectively. Viability of planktonic bacteria was also assessed, with 7.85 ± 0.35 log CFU ml⁻¹ S. mutans cultured from untreated wells after 48 h. The inclusion of 160 or 320 AU ml⁻¹ of thermophilin 110 reduced the number of planktonic cells by approximately 2 logs (5.95 ± 0.30 and 5.80 ± 0.25 CFU ml⁻¹), suggesting that thermophilin 110 may kill planktonic cells and reduce the number of bacteria available to colonize a surface. Similar mechanisms of action have been proposed for other AMPs shown to inhibit the formation of biofilms by S. mutans [15, 33,34].

3.2. S. thermophilus B59671 prevents S. mutans biofilm formation

Concentrations of thermophilin 110 shown to prevent biofilm growth of S. mutans correlated to the amount naturally produced by S. thermophilus B59671 in batch culture (160-320 AU ml⁻¹). Hence the effectiveness of co-culturing S. thermophilus B59671 with S. mutans UA159 was tested for inhibiting biofilm growth (Fig. 4). Preliminary tests showed that S. thermophilus did not adhere when cultured alone in TH sucrose (data not shown); and when co-cultured with S. mutans UA159 at a 1:1 and 2.5:1 cell ratio, S. mutans’ biofilm growth was inhibited by 63% and 77% respectively. Increasing the amount of S. thermophilus in the co-culture did not lead to further reduction in biofilm growth. Co-culturing experiments were also performed using S. thermophilus ST128, which does not produce a bacteriocin. When ST128 was applied at a 1:1 and 2.5:1 cell ratio, S. mutans biofilm growth was reduced by 15% and 41% respectively. Increasing the number of S. thermophilus ST128 cells to a 5:1 cell ratio resulted in a 72% reduction in S. mutans UA159 biofilm growth. This result suggested that
S. thermophilus may prevent the growth of S. mutans’ biofilms in a bacteriocin-independent manner, but more studies are needed to determine the mechanism responsible for this growth inhibition. One possibility is that S. thermophilus is capable of degrading signaling peptides which facilitate cell-to-cell communication in S. mutans [36]. While disruption of quorum sensing has been shown to alter biofilm formation in S. mutans [37, 38], results from this study showed that production of thermophilin 110 by S. thermophilus B59671 enhanced its antagonistic activity, inhibiting S. mutans’ biofilm growth by >70% using less cells in the co-culture when compared to S. thermophilus ST128.

Previous studies have reported that S. thermophilus could inhibit the growth of Porphyromonas gingivalis, preventing the accumulation of volatile sulfur compounds responsible for malodour [35], and that S. thermophilus strain NCC1561 could adhere to saliva-coated hydroxyapatite beads and become incorporated within a mixed species biofilm mimicking the dental plaque [39]. These reports, in addition to the results from this study, suggest that S. thermophilus strains should be investigated further for their potential as oral probiotics. In addition, the concentration of thermophilin 110 naturally produced by S. thermophilus B59671 was shown to inhibit the growth of Streptococcus pyogenes [27], suggesting its use as a probiotic may be able to prevent infections of the pharynx, as was reported for the Streptococcus salivarius probiotic strains K12 and M18 [40].

Although the concentration of thermophilin 110 naturally produced by S. thermophilus B59671 was sufficient to inhibit planktonic and biofilm growth of S. mutans UA159, higher concentrations were required to inhibit the growth of S. mutans GS-5 and ATCC 25175 (640 and 1280 AU ml⁻¹ respectively). At these concentrations, thermophilin 110 was also observed to inhibit the growth of S. gordonii DL-1 and S. sanguis strains V736 and lac8 (Table 1). While S. gordonii and S. sanguis have both been reported to cause infective endocarditis [41, 42], they are commensal streptococci often associated with a healthy oral cavity due to their antagonistic activity towards mutans streptococci [36, 43]. These results suggest that further studies are needed to evaluate the potential for using concentrated levels of thermophilin 110 as an antimicrobial compound within oral care products.
Results from this study demonstrated that concentration of thermophilin 110 naturally produced by S. thermophilus inhibited planktonic and biofilm growth of S. mutans UA159; however, higher concentrations were required to inhibit strains GS-5 and ATCC 25175. While further investigation is required to demonstrate the potential for S. thermophilis B59671 as an oral probiotic, thermophilin 110 only inhibited health-associated commensal streptococci at elevated levels, suggesting it would not result in significant oral dysbiosis. Furthermore, a concentrated amount of thermophilin 110 may have potential as an antimicrobial for preventing the growth of oral streptococci for preventing caries formation.

| Table 1 |
|---------|
| Thermophilin activity against oral streptococci |

| Target strains | Concentration of thermophilin 110 (AU ml⁻¹) |
|----------------|--------------------------------------------|
| Streptococcus mutans GS-5 | 640 |
| Streptococcus mutans ATCC 25175 | 1280 |
| Streptococcus gordoni DI-1 | 1280 |
| Streptococcus sanguis lac8 | 640 |
| Streptococcus sanguis V736 | 640 |

* Sensitivity to thermophilin 110 was determined using a well-diffusion assay, with serial two-fold dilutions of the thermophilin 110 stock (5,120 AU/ml) prepared in sterile H₂O. Sensitivity was defined as the lowest dilution resulting in an inhibition zone around the well.

4. Conclusion

Results from this study demonstrated that concentration of thermophilin 110 naturally produced by S. thermophilus inhibited planktonic and biofilm growth of S. mutans UA159; however, higher concentrations were required to inhibit strains GS-5 and ATCC 25175. While further investigation is required to demonstrate the potential for S. thermophilis B59671 as an oral probiotic, thermophilin 110 only inhibited health-associated commensal streptococci at elevated levels, suggesting it would not result in significant oral dysbiosis. Furthermore, a concentrated amount of thermophilin 110 may have potential as an antimicrobial for preventing the growth of oral streptococci for preventing caries formation.

Declaration of Competing Interest

No conflict of interest to declare.

Acknowledgments

We thank A. White and J. Fisher (Penn State University) for technical assistance with antimicrobial assays performed in broth medium, and preparation of the figure reporting this data.

References

[1] T. Chen, W.H. Yu, J.J Oxana, V. Baranova, A. Lakshmanan, F.E. Dewhirst, The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information, Database (2010), https://doi.org/10.1093/database/baq013, 1 January 2010, bpq013.
[2] M. Kilian, I.L.C. Chapple, M. Hannig, P.D. Marsh, V. Meuric, A.M.L. Pedersen, M. Petti, G.A. Somkuti, J.A. Renye Jr, GA. Somkuti, Thermophilin 110 activity against oral streptococci, Biochemistry (2015) 88-96.
[3] S. Sookhe, M. Chulasiri, W. Prachyabrued, Lactic acid bacteria from healthy oral cavity of Thai volunteers: inhibition of oral pathogens, J. Appl. Microbiol. 90 (2001) 172-179.
[4] J. Bhushan, S. Chauca, Probiotics – Their role in prevention of dental caries, J. Oral Health Comm. Dent. 4 (2010) 78-82.
[5] J.A. Renye and D.H. Steinberg, Antimicrobial activity of Streptococcus salivarius K12 on bacteria involved in oral malodor, Arch. Oral Biol. 57 (2012) 1041-1047.
[6] J.F. Burton, B.K. Drummond, C.N. Chilcott, J.R. Tagg, W.M. Thomson, J.D.F. Hale, M.A. Wescombe, Influence of the probiotic Streptococcus salivarius strain M18 on indices of dental health in children: a randomized double-blind, placebo-controlled trial, J. Med. Microbiol. 62 (2013) 875-884.
[7] P. Hols, F. Hancy, L. Fontaine, B. Grossiord, D. Pruzin, N. Leblond-Bourget, B. Decaris, A. Bolotin, C. Delorme, S.D. Ehrlich, E. Guedon, V. Monnet, R. Renault, M. Kleerebezem, New insights in the molecular biology and physiology of Streptococcus thermophilus revealed by comparative genomics, FEMS Microbiol. Rev. 29 (2005) 435-463.
[8] L. Fontaine, C. Brouy, E. Guedon, A. Guillot, M. Ibrahim, B. Grossiord, P. Hols, Quorum-sensing regulation of the production of Blp bacteriocins in Streptococcus thermophilus, J. Bacteriol. 189 (2007) 7915-7923.
[9] J.A. Renye Jr, GA. Somkuti, Lipopoly saccharide regulated bacteriocin production in Streptococcus thermophilus, Biotechnol. Lett. 35 (2013) 407-412.
[10] G.A. Somkuti, J.A. Renye Jr, Effect of a Blp-based quorum-sensing induction peptide on bacteriocin production in Streptococcus thermophilus, J. Food Res. 4 (2015) 88-96.
[11] S.E. Gilbreh, G.A. Somkuti, Thermophilin 110: a bacteriocin of Thermophilus thermophilus ST110, Curr. Microbiol 51 (2005) 175-182.
[12] L. Fontaine, P. Hols, The inhibitory spectrum of thermophilin 9 from Thermophilus thermophilus LMD-9 depends on the production of multiple peptides and the activity of BltpG(Sc), a third-dinucleotide oxidase, Appl. Environ. Microbiol. 74 (2008) 1102-1110.
[13] J.A. Renye Jr, GA. Somkuti, Bacteriocin with novel activity, U.S. Patent 9,598,471, issued March 21, 2017.
[14] S. Petti, G. Tartini, A. Simonetti D’Arca, Antibacterial activity of yogurt against viridans streptococci in vitro, Arch. Oral. Biol. 53 (2008) 985-990.
[15] J.A. Renye Jr, GA. Somkuti, J.I. Garabal, D.H. Steinberg, Bacteriocin production by Streptococcus thermophilus in complex growth media, Biotechnol. Lett. 38 (2016) 1497-1504.
[16] J.H. Merritt, D.E. Kadouri, G.A. O'Toole, Growing and analyzing static biofilms, Curr. Protoc. Microbiol. Chapter 1 (2005), https://doi.org/10.1002/0470017729.mc01b100, Unit 18.3.
[17] N.A. Aminabadi, E. Balaei, F. Pouralibafi, The effect of 0.2% sodium fluoride mouthwash in prevention of dental caries according to the DMFT index, J. Dent. Res. Clin. Dent. Prospects. 1 (2007) 71-76.
[18] L.M. Weatherly, J.A. Gosse, Triclosan exposure, transformation, and human health effects, J. Toxicol. Environ. Health B. Crit. Rev. 20 (2017) 447-469.
[19] G.K. Wei, A.N. Campagna, L.A. Bobek, Effect of MUC7 peptides on the growth of bacteria and on Streptococcus mutans biofilm, J. Antimicrob. Chemother. 57 (2006) 1100-1109.
[20] Y. Liu, L. Wang, X. Zhou, S. Zhu, S. Zhang, H. Wu, Effect of the antimicrobial decapetide KSL on the growth of oral pathogens and Streptococcus mutans biofilm, Int. J. Antimicrob. Agents 37 (2011) 33-38.
[21] S.H. Lee, D.H. Baek, Effects of Streptococcus thermophilus on volatile sulfur compounds produced by Porphyromonas gingivalis, Arch. Oral. Biol. 59 (2014) 1205-1216.
[22] P.R. Kaspar, K. Lee, B. Richard, A.R. Walker, R.A. Burne, Direct interactions with commensal streptococci modify intercellular communication behaviors of Streptococcus mutans, ISME J 15 (2021) 473-488.
[23] Y.-H. Li, N. Tang, M.B. Aspiras, P.C.Y. Lau, J.H. Lee, R.P. Ellen, D.G. Cvitkovitch, Further studies on the growth inhibition of Streptococcus mutans OM2176 by syrto, Acta. Pathol. Microbiol. Immunol. Scand. 94 (1986) 97-102.
[24] G.A. Somkuti, P.M. O’Connor, P.D. Cotter, P.R. Ross, C. Hill, Impact of the broad spectrum antimicrobial peptide, lacticin 3147, on Streptococcus mutans growing in a biofilm and in human saliva, J. Appl. Microbiol. 111 (2011) 1515-1523.
[25] S. Sookhe, M. Chulasiri, W. Prachyabrued, Lactic acid bacteria from healthy oral cavity of Thai volunteers: inhibition of oral pathogens, J. Appl. Microbiol. 90 (2001) 172-179.
[26] J.A. Renye and D.H. Steinberg, Oral streptococci and their role in oral health, J. Med. Microbiol. 62 (2013) 875-884.