Biotechnology Reports 21 (2019) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Inhibition of *Streptococcus mutans* and *S. sobrinus* biofilms by liamocins from *Aureobasidium pullulans*☆

Timothy D. Leathers*, Joseph O. Rich, Kenneth M. Bischoff, Christopher D. Skory, Melinda S. Nunnally

Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 N. University St., Peoria, IL, 61604, USA

ARTICLE INFO

Article history:
Received 15 November 2018
Received in revised form 30 November 2018
Accepted 17 December 2018

Keywords:
Streptococcus mutans
Streptococcus sobrinus
Biofilm
Aureobasidium pullulans
Liamocins

ABSTRACT

The aim of this study was to determine if the novel anti-streptococcal inhibitors, liamocins, also inhibit biofilm formation by *S. mutans* and *S. sobrinus*. *S. mutans* strain ATCC 25175 and *S. sobrinus strain* ATCC 33478 were tested for biofilm formation in a rapid microtiter plate (MTP) assay and the effects of added liamocins were determined. This assay measures relative biofilm growth on pin lids. Results were verified in a biofilm flow cell assay, using hydroxyapatite-coated coupons to simulate dental material. Planktonic cultures of *S. mutans* and *S. sobrinus* were inhibited by 0.1 mg liamocins/ml. When liamocins were added after the adhesion phase in a rapid microtiter plate assay, *S. mutans* was inhibited 53% by 5 mg liamocins/ml, while *S. sobrinus* was more sensitive, showing 100% inhibition at 0.5 mg liamocins/ml. When liamocins were added during the adhesion phase, biofilms of *S. mutans* showed 78% inhibition at 3.0 mg liamocins/ml. In a biofilm flow cell assay, liamocins added after the adhesion phase at 0.5 mg liamocins/ml inhibited biofilms of *S. sobrinus*, and appeared to remove biofilms over time. Liamocins were shown for the first time to inhibit biofilm formation by *S. mutans* and *S. sobrinus*. Since liamocins are specific for *Streptococcus* spp., they are potential new inhibitors of oral streptococcal biofilms that should not affect normal oral microflora.

1. Introduction

Dental caries is associated with biofilm formation by oral bacteria, particularly *Streptococcus mutans* and *S. sobrinus* [1,2]. Dental plaque is a type of biofilm, which provides a matrix for various cariogenic bacteria [3,4]. Thus, control of streptococcal biofilms has become a major target for management of dental caries and periodontic diseases. Antibiofilm agents currently under study include cetylpyridinium chloride [33], carolacton [5], pentose sugars and xylitol [6,7], green tea extracts [8], red propolis [9], anti-fungal azoles [10], lemon essential oil [11,12], and clove essential oil [13].

Liamocins are novel inhibitors of *Streptococcus* species produced by the fungus *Aureobasidium pullulans* [14]. *A. pullulans* is a polymorphic ascomycete that is best known as the source of the commercial polysaccharide pullulan, used in water-soluble films for food packaging and fast dissolving oral films [15–17]. Nagata et al. [18] first reported the production of unusual heavier-than-water "oil" by *Aureobasidium* spp. A partial structure showing a polyol lipid was proposed by Kurosawa et al. [19]. Price et al. [20] first deduced the complete structure of these oils, subsequently named "liamocins." Liamocins are composed of a single polyol headgroup (usually mannitol), partially *O*-acylated with either three or four 3,5-dihydroxydecanoic ester groups, which may contain a single 3′-*O*-acetyl group [20]. Variations in the polyol headgroup and ratios of lipid tailgroups are possible, depending on the strain and culture conditions [21–23].

Liamocins are surface active [22,24,25] and inhibit mammalian cancer cells [22,26,27]. Bischoff et al. [14] discovered that liamocins have antibacterial activity with specificity for planktonic cultures of *Streptococcus* species, including *S. mutans*. However, liamocins have not previously been tested for inhibition of biofilms.

2. Materials and methods

2.1. Strains and culture conditions

Aureobasidium pullulans strain NRRL 50380 was from the ARS Culture Collection, Peoria, IL. Cultures were maintained on yeast

☆ Mention of any 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 US Department of Agriculture. USDA is an equal opportunity provider and employer.

* Corresponding author.
E-mail address: tim.leathers@ars.usda.gov (T.D. Leathers).

https://doi.org/10.1016/j.btre.2018.e00300

malt extract agar (YMA). For oil production, 50 ml cultures in 250 ml flasks were grown in 5% (w/v) sucrose, 0.06% (w/v) peptone, 0.04% (w/v) yeast extract, 0.5% (w/v) K_2HPO_4, 0.04% (w/v) $MgSO_4 \cdot 7H_2O$, and 0.1% (w/v) NaCl for 7 days at 28 °C with shaking at 150 rpm. Liamocins were extracted by the whole culture method of Manitchotpisit et al. [22], using 100 ml of methyl ethyl ketone (MEK), dried, and then resuspended in 10 ml of 1:1 MEK and DMSO.

Streptococcus mutans strain ATCC 25175 and *S. sobrinus strain* ATCC 33478 were from the American Type Culture Collection, Manassas, VA. Cultures were grown on a modified Todd-Hewitt broth (THB) (1.5% agar for plates) with 11 mM glucose for culture maintenance or 3 mM sucrose for biofilm production. Composition of modified THB per liter is 2% (w/v) trypticase peptone, 1% (w/v) beef extract powder, 0.2% (w/v) $NaHCO_3$, 0.2% (w/v) NaCl, and 0.04% (w/v) Na_2HPO_4, plus carbon source.

2.2. Assays of growth inhibition of planktonic cultures

Growth inhibition in liquid planktonic cultures was measured by growing *S. mutans* strain ATCC 25175 and *S. sobrinus* strain ATCC 33478 in 10 ml liquid cultures of THB-glucose containing liamocins at 0–5 mg/ml for 24 h at 37 °C aerobically. In preliminary experiments, it was determined that *S. sobrinus* required a higher level of aeration than *S. mutans* for comparable growth, so *S. sobrinus* cultures were shaken at 200 rpm while *S. mutans* cultures were not shaken. Test cultures were inoculated to 0.1 OD_{600} and incubated with or without potential inhibitors under the same conditions for 24 h. Maximal solvent concentrations were 1.0% (v/v). Culture growth was measured by optical density, verified by viable cell counts.

2.3. Assays of biofilm formation in 96-well plates

S. mutans strain ATCC 25175 and *S. sobrinus* strain ATCC 33478 were tested for production of biofilms using a rapid microtiter plate assay as modified from Rich et al. [28]. Inocula were grown in 10 ml liquid cultures of THB-glucose for 48 h at 37 °C with (for *S. sobrinus*) or without (for *S. mutans*) shaking at 200 rpm. For both strains, these saturated cultures contained $>10^7$ CFU/ml. Twenty-five μl of cells were added per well of a 96-well microtiter plate (Nunc, Denmark), containing 125 μl of THB-sucrose per well. A sterile 96-pin lid (Nunc) was added and plates were incubated for 48 h to correspond to the adhesion phase of a conventional biofilm reactor. Pin lids then were transferred to fresh plates (time point zero) and subsequently transferred every 48 h until the assay endpoint at 144 h. Again, preliminary studies indicated that *S. sobrinus* required a higher level of aeration than *S. mutans* for comparable growth, so plates containing *S. mutans* strain ATCC 25175 were incubated in an anaerobic chamber at 37 °C, while plates containing *S. sobrinus* strain ATCC 33478 were incubated under static, aerobic conditions at 37 °C. Stocks of liamocins in 1:1 DMSO/MEK were diluted into medium at final concentrations of 0.1 to 5 mg/ml in 1.0% (v/v) solvent. Alternatively, xylitol was added to aqueous medium at a final concentration of 5 mg/ml. Replicator lid pins were stained according to Rich et al. [28] with a 0.1% crystal violet solution for 30 min at room temperature, and destained with 95% ethanol. The amount of crystal violet staining, representing biofilm growth, was measured at 600 nm using a SpectraMax M5 microtiter plate reader (Molecular Devices, Sunnyvale, CA). Viability was tested by direct plating of culture dilutions onto THB plates without liamocins.

2.4. Assays of biofilm formation in biofilm flow cells

S. mutans strain ATCC 25175 and *S. sobrinus* strain ATCC 33478 were tested for production of biofilms in biofilm flow cells by a modification of methods previously described [29]. Preinocula were grown in 20 ml of THB medium in 50 ml flasks at 37 °C for 48 h with shaking at either 200 rpm (for *S. sobrinus*) or 25 rpm (for *S. mutans*). For both strains, these saturated cultures contained $>10^7$ CFU/ml. Biofilm cultures were grown in a BST FC 271 flow cell (BioSurface Technologies Corp, Bozeman, MT, USA) equipped with hydroxyapatite-coated stainless steel sampling coupons. Preinocula were diluted 1:25 into 500 ml of THB-sucrose and flow cells were inoculated with this dilution at a flow rate of 0.2 ml/min for 16 h. This corresponds to the static adhesion phase of a conventional biofilm reactor. For post-adhesion (continuous phase) biofilm growth, flow cells were provided with a continuous flow of fresh medium at 0.2 ml/min. Flow cells are constructed with parallel dual channels, one of which received medium with liamocins at 0.5 mg/ml. Flow cells were maintained at 37 °C on heated pads (Model TP-3E temperature therapy pads, Stryker Medical, Portage MI). Biofilm coupons were sampled as previously described [29] and assayed on THB solid medium.

3. Results and discussion

3.1. Effect of liamocins on planktonic cultures of S. mutans and S. sobrinus

Bischoff et al. [14] reported that liamocins possess antibacterial activity with specificity for a number of species of *Streptococcus*, including *S. mutans*. MICs for tested strains were less than 0.1 mg liamocins/ml [14]. In the current study, the effect of liamocins was assessed on planktonic cultures of *S. mutans* strain ATCC 25175 and *S. sobrinus* strain ATCC 33478 (Fig. 1). Cultures were inhibited by 0.1 mg liamocins/ml, and little or no growth was observed at 0.5 mg liamocins/ml. *S. sobrinus* appeared to be more sensitive than *S. mutans* to liamocins. Solvent controls had no effect on cultures (Fig. 1). Thus, liamocins inhibit the planktonic growth of *S. mutans* and *S. sobrinus*. Viability assays showed that control cultures of both species (without liamocins) contained $>10^7$ CFU/ml, while cultures containing 1 mg liamocins/ml contained less than 300 CFU/ml (the lower limit of detection for this assay). These results are consistent with previous studies that showed that Liamocins are bacteriocidal rather than bacteriostatic for Streptococcus species [14,34].

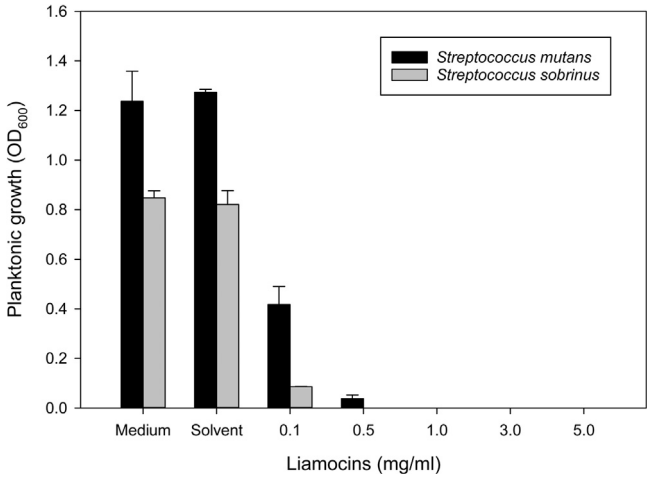

Fig. 1. Planktonic growth of *Streptococcus mutans* and *S. sobrinus* in the presence of liamocins at the indicated concentrations. Controls include growth medium with and without solvent at 1.0% (v/v).

3.2. Effect of liamocins on biofilm formation by S. mutans and S. sobrinus

Liamocins previously were reported to have no effect on biofilm formation by species of *Lactobacillus* [30]. Although liamocins inhibited planktonic cultures of *S. mutans* and *S. sobrinus* (Fig. 1), biofilms are generally known for their resistance to antibiotics [31]. Biofilm growth of *S. mutans* strain ATCC 25175 and *S. sobrinus* strain ATCC 33478 was assessed in a rapid microtiter plate assay modified from Rich et al. [28]. In the absence of added liamocins, strains showed crystal violet staining of about 0.8 OD_{600}, representing good biofilm growth [28]. When liamocins were added after the adhesion phase, biofilm growth of *S. mutans* was inhibited at liamocins concentrations of 0.5–5.0 mg/ml, showing 53% inhibition at 5 mg liamocins/ml (Fig. 2A). *S. sobrinus* was more sensitive, showing 72% inhibition at only 0.1 mg liamocins/ml, and 100% inhibition at 0.5 mg liamocins/ml (Fig. 2A). When liamocins were added during the adhesion phase, *S. mutans* biofilms were somewhat more sensitive, showing 78% inhibition at 3.0 mg liamocins/ml (Fig. 2B). These results compare favorably with those for xylitol inhibition of *S. sobrinus* biofilms. In our MTP assay, *S. sobrinus* was inhibited 75% by 5 mg xylitol/ml added after the adhesion phase, and 91% by xylitol added during the adhesion phase.

Thus, biofilms of *S. mutans* and *S. sobrinus* are inhibited by liamocins, particularly during the adhesion phase of biofilm formation. It has been suggested that liamocins may have application in mouthwashes against oral *Streptococcus* species [32]. Results suggest that liamocins might be most effective immediately after dental cleanings, before biofilms are established.

3.3. Biofilm formation in biofilm flow cells

S. mutans strain ATCC 25175 and *S. sobrinus* strain ATCC 33478 were cultured in commercial biofilm flow cells, using stainless steel coupons coated with hydroxyapatite to simulate dental material [33]. To confirm the differential effects of liamocins on *S. mutans* and *S. sobrinus*, 0.5 mg liamocins/ml was added after the initial adhesion phase. Under these conditions liamocins had little effect on *S. mutans* biofilms (Fig. 3A), but inhibited *S. sobrinus* biofilms at 4.5 h after the start of continuous flow phase (Fig. 3B). These results are consistent with those obtained from the rapid microtiter assays. Interestingly, liamocins appear to not only prevent growth of *S. sobrinus* biofilms, but actually to remove biofilms over time.

The mechanism of action for liamocins is thus far unknown, although it appears to kill cells by disrupting the cell membrane [14,34]. The basis for its selective action against species of *Streptococcus* is not understood. Interestingly, liamocins appear to be more active against *S. sobrinus* strain ATCC 33478 than against *S. mutans* strain ATCC 25175. Song et al. [35] found diversity among the genomes of strains in the mutans streptococci. Conrads et al. [36] concluded that *S. mutans* and *S. sobrinus* should be considered equally virulent with regard to dental caries, although they found that *S. sobrinus* has fewer two-component signaling systems and bacteriocin-related genes, and lacks central competence genes found in *S. mutans*. Further studies are needed to clarify the selectivity and mechanism of action of liamocins. In previously published studies on the inhibition of mammalian cancer cells, liamocins were generally inactive against control cell lines, usually

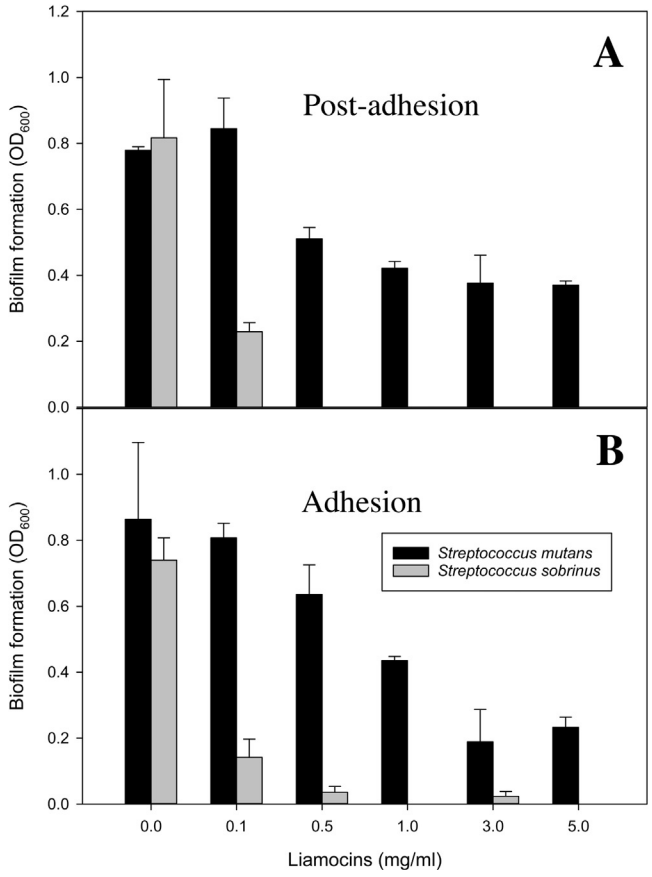

Fig. 2. Biofilm formation by *Streptococcus mutans* and *S. sobrinus*, determined by rapid microtiter plate bioassay [28]. Liamocins at the indicated concentrations were added (A) after the biofilm adhesion phase or (B) during the adhesion phase.

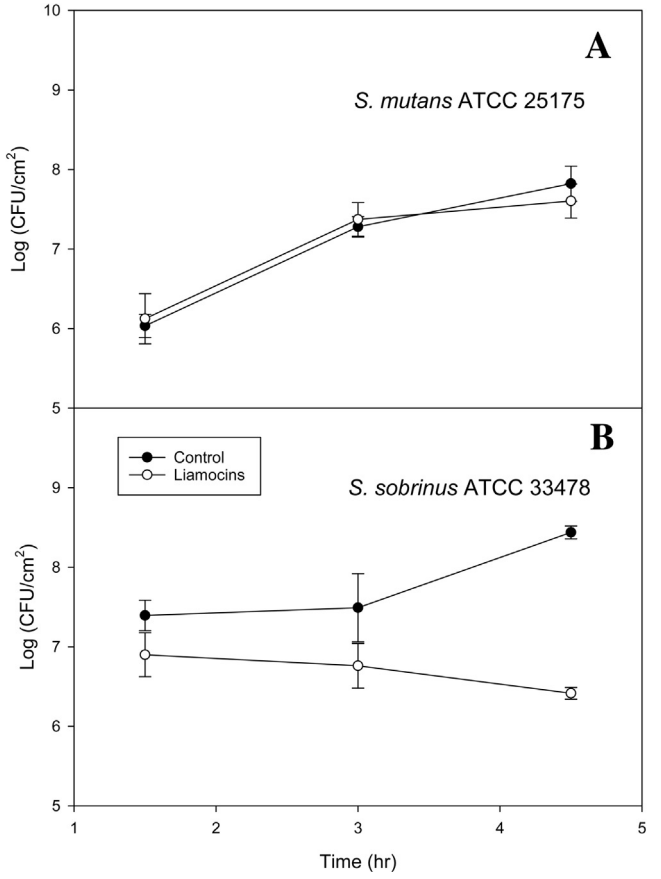

Fig. 3. Biofilm formation by *Streptococcus mutans* and *S. sobrinus*, determined by biofilm flow cell assay [29]. Liamocins at 0.5 mg/ml were added after the biofilm adhesion phase to (A) *S. mutans* or (B) *S. sobrinus*.

Vero monkey cells [22,26,27]. However, further testing would be necessary to determine the clinical safety and appropriate applications of liamocins. Using an optimized medium, liamocins have been produced at up to 22 g/l [37]. However, further research into formulations for clinical applications would be required.

In conclusion, liamocins were tested for the first time as potential inhibitors of biofilm formation by *S. mutans* and *S. sobrinus*. Biofilms were sensitive to liamocins in a rapid microtiter plate assay, particularly during the adhesion phase of biofilm formation. Biofilm flow cell assays using hydroxyapatite-coated coupons validated the rapid microtiter plate assay. Since liamocins specifically inhibit species of *Streptococcus*, they would be expected to reduce the development of plaque biofilms and thus dental caries, without disrupting the normal oral microflora.

Funding

None.

Conflicts of interest

The authors have no competing interests.

Ethical approval

Not required.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors dedicate this study to the memory of our friend and colleague, Dr. Ken Bischoff.

References

[1] J.M. Hardie, R.A. Wiley, The genus streptococcus – oral, in: M. Dworkin, S.J. Falkow (Eds.), The Prokaryotes: A Handbook on the Biology of Bacteria, Springer, New York, 2006, pp. 76–107.
[2] S. Hamada, H.E. Slade, Biology, immunology, and cariogenicity of *Streptococcus mutans*, Microbiol. Rev. 44 (2) (1980) 331–384.
[3] J. Nishimura, T. Saito, H. Yoneyama, L.L. Bai, K. Okumura, E. Isogai, Biofilm formation by *Streptococcus mutans* and related bacteria, Adv. Microbiol. 2 (2012) 208–215.
[4] M.D. Dinesh, M.S. Uma, S. Meenatchisundaram, V.M. Anjali, P.S. Athira, A. Carmel, Streptococcal dental caries – a short review, Int. J. Curr. Res. Acad. Rev. 4 (1) (2016) 160–170.
[5] C. Apel, A. Barg, A. Rheinberg, G. Conrads, I. Wagner-Doebler, Dental composite materials containing carolacton inhibit biofilm growth of *Streptococcus mutans*, Dent. Mater. 29 (2013) 1188–1199.
[6] P. Kurola, T. Tapiainen, J. Sevander, T. Kaijalainen, M. Leinonen, M. Uhari, A. Saukkoriipi, Effect of xylitol and other carbon sources on *Streptococcus pneumonia* biofilm formation and gene expression in vitro, APMIS 119 (2) (2011) 135–142.
[7] H.-J. Lee, S.C. Kim, J. Kim, A. Do, S.Y. Han, B.D. Lee, H.H. Lee, M.C. Lee, S.H. Lee, T. Oh, S. Park, S.-H. Hong, Synergistic inhibition of Streptococcal biofilm by ribose and xylitol, Arch. Oral Biol. 60 (2015) 304–312.
[8] C.-Y. Wu, T.-Y. Su, M.-Y. Wang, S.-F. Yang, K. Mar, S.-L. Hung, Inhibitory effects of tea catechin epigallocatechin-3-gallate against biofilms formed from *Streptococcus mutans* and a probiotic lactobacillus strain, Arch. Oral Biol. 94 (2018) 69–77.
[9] M.L. Martins, K.L.F. Leite, E.F. Pacheco-Filho, A.F.M. Pereira, M.R.V. Romanos, L. C. Maia, A. Fonseca-Goncalves, W.W.N. Padilha, Y.W. Cavalcanti, Efficacy of red propolis hydro-alcoholic extract in controlling *Streptococcus mutans* biofilm build-up and dental enamel demineralization, Arch. Oral Biol. 93 (2018) 56–65.
[10] W. Qiu, B. Ren, H. Dai, L. Zhang, Q. Zhang, X. Zhou, Y. Li, Clotrimazole and econazol inhibit *Streptococcus mutans* biofilm and virulence in vitro, Arch. Oral Biol. 73 (2017) 113–120.
[11] Y. Liu, X. Zhang, Y. Wang, F. Chen, Z. Yu, L. Wang, S. Chen, M. Guo, Effect of citrus lemon oil on growth and adherence of *Streptococcus mutans*, World J. Microbiol. Biotechnol. 29 (2013) 1161–1167.
[12] Y. Sun, S. Chen, C. Zhang, Y. Liu, L. Ma, X. Zhang, Effects of sub-minimum inhibitory concentrations of lemon essential oil on the acid tolerance and biofilm formation of *Streptococcus mutans*, Arch. Oral Biol. 87 (2018) 235–241.
[13] H.-J. Yoo, S.-K. Jwa, Inhibitory effects of β-caryophyllene on *Streptococcus mutans* biofilm, Arch. Oral Biol. 18 (2018) 42–46.
[14] K.M. Bischoff, T.D. Leathers, N.P.J. Price, P. Manitchotpisit, Liamocin oil from *Aureobasidium pullulan* has antibacterial activity with specificity for species of *Streptococcus*, J. Antibiot. 68 (2015) 642–645.
[15] T.D. Leathers, Pullulan, in: E.J. Vandamme, S. De Baets, A. Steinbüchel (Eds.), Biopolymers, Vol. 6, Polysaccharides II: Polysaccharides from Eukaryotes, Wiley-VCH, Weinheim, 2002, pp. 1–35.
[16] R.S. Singh, G.K. Saini, J.F. Kennedy, Pullulan: microbial sources, production and applications, Carbohydr. Polym. 73 (2008) 515–531.
[17] Y.S. Pathare, V.S. Hastak, A.N. Bajaj, Polymers used for fast disintegrating oral films: a review, Int. J. Pharm. Sci. Rev. Res. 21 (1) (2013) 169–178.
[18] N. Nagata, T. Nakahara, T. Tabuchi, Fermentative production of poly(β-L-malic acid), a polyelectrolytic biopolyester, by *Aureobasidium* sp, Biosci. Biotechnol. Biochem. 57 (1993) 638–642.
[19] T. Kurosawa, K. Sakai, T. Nakahara, Y. Oshima, T. Tabuchi, Extracellular accumulation of the polyol lipids, 3,5-dihydroxydecanoyl and 5-hydroxy-2-decenoyl esters of arabitol and mannitol, by *Aureobasidium* sp, Biosci. Biotechnol. Biochem. 58 (1994) 2057–2060.
[20] N.P.J. Price, P. Manitchotpisit, K.E. Vermillion, M.J. Bowman, T.D. Leathers, Structural characterization of novel extracellular liamocins (mannitol oils) produced by *Aureobasidium pullulans* strain NRRL 50380, Carbohydr. Res. 370 (2013) 24–32.
[21] T.D. Leathers, N.P.J. Price, K.M. Bischoff, P. Manitchotpisit, C.D. Skory, Production of novel types of antibacterial liamocins by diverse strains of *Aureobasidium pullulans* grown on different culture media, Biotechnol. Lett. 37 (2015) 2075–2081.
[22] P. Manitchotpisit, N.P.J. Price, T.D. Leathers, H. Punnapayak, Heavy oils produced by *Aureobasidium pullulans*, Biotechnol. Lett. 33 (2011) 1151–1157.
[23] N.P.J. Price, K.M. Bischoff, T.D. Leathers, A.A. Cosse, P. Manitchotpisit, Polyols, not sugars, determine the structural diversity of anti-streptococcal liamocins produced by *Aureobasidium pullulans* strain NRRL 50380, J. Antibiot. 70 (2017) 136–141.
[24] J.S. Kim, I.K. Lee, B.S. Yun, A novel biosurfactant produced by *Aureobasidium pullulans* L3-GPY from a tiger lily wild flower, *Lilium lancifolium* Thumb, PLoS One 10 (4) (2015) e0122917.
[25] D.P. Meneses, E.J. Gudina, F. Fernandes, L.R.B. Goncalves, L.R. Rodrigues, S. Rodrigues, The yeast-like fungus *Aurebasidium thailandense* LB01 produces a new biosurfactant using olive oil mill wastewater as an inducer, Microbiol. Res. 204 (2017) 40–47.
[26] H. Isoda, T. Nakahara, Antiproliferative effect of polyol lipids, 3,5-dihydroxydecanoyl and 5-hydroxy-2-decenoyl esters of arabitol and mannitol on lung cancer cell line A549, J. Ferment. Bioeng. 84 (1997) 403–406.
[27] P. Manitchotpisit, R. Watanapokasin, N.P.J. Price, K.M. Bischoff, M. Tayeh, S. Teeraworawit, S. Kriwong, T.D. Leathers, *Aureobasidium pullulans* as a source of liamocins (heavy oils) with anticancer activity, World J. Microbiol. Biotechnol. 30 (2014) 2199–2204.
[28] J.O. Rich, T.D. Leathers, M.S. Nunnally, K.M. Bischoff, Rapid evaluation of the antibiotic susceptibility of fuel ethanol contaminant biofilms, Bioresour. Technol. 102 (2011) 1124–1130.
[29] T.D. Leathers, K.M. Bischoff, Biofilm formation by strains of *Leuconostoc citreum* and *L. mesenteroides*, Biotechnol. Lett. 33 (2011) 517–523.
[30] T.D. Leathers, K.M. Bischoff, J.O. Rich, N.P.J. Price, P. Manitchotpisit, M.S. Nunnally, A.M. Anderson, Inhibitors of biofilm formation by biofuel fermentation contaminants, Bioresour. Technol. 169 (2014) 45–51.
[31] P.S. Stewart, P.K. Mukherjee, M.A. Ghannoum, Biofilm antimicrobial resistance, in: M. Ghannoum, G.A. O'Toole (Eds.), Microbial Biofilms, ASM Press, Washington, 2004, pp. 250–268.
[32] K.M. Bischoff, T.D. Leathers, N.P.J. Price, P. Manitchotpisit (2018). Oils having antibacterial activity. U.S. Patent 9,950,986.
[33] J. Latimer, J.L. Munday, K.M. Buzza, S. Forbes, P.K. Sreenivasan, A.J. McBain, Antibacterial and anti-biofilm activity of mouthrinses containing cetylpyridinium chloride and sodium fluoride, BMC Microbiol. 15 (2015) 169.
[34] K.M. Bischoff, S.L. Brockmeier, C.D. Skory, T.D. Leathers, N.P.J. Price, P. Manitchotpisit, J.O. Rich, Susceptibility of *Streptococcus suis* to liamocins from *Aureobasidium pullulans*, Biocatal. Agric. Biotechnol. 15 (2018) 291–294.
[35] L. Song, W. Wang, G. Conrads, A. Rheinberg, H. Sztajer, M. Reck, I. Wagner-Dobler, A.-P. Zeng, Genetic variability of mutans streptococci revealed by wide whole-genome sequencing, BMC Genom. 14 (2013) 430.
[36] G. Conrads, J.J. de Soet, L. Song, K. Henne, H. Sztajer, I. Wagner-Dobler, A.-P. Zeng, Comparing the cariogenic species *Streptococcus sobrinus* and *S. mutans* on whole genome level, J. Oral Microbiol. 6 (2014) 26189.
[37] T.D. Leathers, C.D. Skory, N.P.J. Price, M.S. Nunnally, Medium optimization for production of anti-streptococcal liamocins by *Aureobasidium pullulans*, Biocatal. Agric. Biotechnol. 13 (2018) 53–57.