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Inhibitory Effects of Sucrose Fatty Acid Esters on Biofilm Formation by Food-borne Staphylococcus aureus

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Research Article

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ABSTRACT

Biofilm formation is a significant hazard in food industry. This study aims to investigate the ability of sucrose esters to inhibit biofilm formation of food-borne *Staphylococcus aureus*. Twelve *Staphylococcus aureus* strains were isolated, identified and used for biofilm formation ability assay in presence or absence of sucrose esters. Bacteria adhesion the hydrocarbons test (BATH) was employed to analysis cell surface hydrophobicity rates of sucrose-ester treatments and correlation between biofilm formation ability and hydrophobicity. Various levels of biofilm formation inhibitory of sucrose esters were observed among all the isolates. Cell surface hydrophobicity (CSH) of all sucrose esters at concentrations of 3 mg/mL and 4 mg/mL were reduced, suggesting a positive correlation between the biofilm-producing ability and the rates of CSH ($P<0.05$) as well as a significantly reduced CSH rates of sucrose esters during biofilm development ($P<0.05$). SE possesses great potential as an inhibitor for biofilm formation of food-borne pathogens.

INTRODUCTION

Staphylococcus aureus, as an important food-borne pathogen, is recognized as being responsible for outbreaks related to the consumption of fresh and processed food. Biofilm formation on the surfaces of food processing plants by *Staphylococcus aureus* interferes hard eliminating contamination [1-3]. Biofilms are communities of microorganisms that are encased in a self-synthesized extracellular polymeric matrix (EPS) and attaches to a biotic or abiotic surface during growth [4,5]. Bacterial biofilms impact human health significantly [6,7], including dental caries, infected implants and food contamination, due to their enhanced pathogenic capability relative to bacteria in solution by virtue of sessile behaviour, which increases its resistance to sanitizers and other antimicrobials [8].

It is necessary to clarify biofilm-related contamination that led to the removal of microbial biofilms from a contaminated site. Recent researches on biofilm mainly focus on the study of chemical inhibitors, which regulate the formation of pathogenic bacteria biofilm [9]. However, in food-processing environment, some of these anti-biofilm compounds are prohibited due to safety concerns. Recently, sucrose esters as being a natural constituent of a number of foods, was widely investigated in the past decades and are used in food, cosmetic and detergent industries. In addition, several types of synthetic SE have shown potent insecticidal activities and could be used as a new bacteriostatic agent with a brilliant commercial prospect. Monoglyceride of lauric acid (monolaurin) performs considerable antibacterial activity against *Listeria monocytogenes* and *Escherichia coli* O157:H7 [10,11].

Although widely studied, the fundamental mechanism of SE inhibiting bacteria has not been fully understood. We hypothesized that sucrose ester as a surfactant solution plays a role in cell surface hydrophobicity related to the biofilm formation. Thus, the aim of this study is to investigate the correlation between biofilm formation and surface hydrophobicity of *S. aureus* treated with sucrose ester, and assess the potential use of commercially SE compounds as an inhibitor for biofilm formation.

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MATERIALS AND METHODS

Reagents and Isolation of *S. aureus*: Three types of sucrose esters (SE), SE7 (hydrophilic-lipophilic balance 7) ,SE11(hydrophilic-lipophilic balance 11)and SE13(hydrophilic-lipophilic balance 13) were obtained from Zhejiang Deyer Chemicals Co., Ltd. (Jinhua, Zhejiang province, China), Tryptic Soy Broth (TSB), Baird - Parker agar, rabbit plasma, blood agar plates were purchased from Hope Bio-Technology Co., Ltd. (Qingdao, China).

S. aureus were isolated from 30 samples including expired beef (20 samples), bacon (5 samples), and frozen pork dumplings (5 samples), which were collected from the local grocery stores and small supermarkets in Guangzhou, China. 12 strains were isolated and identified as *S. aureus* according to National Food Safety Standards of Food Microbiology test *Staphylococcus aureus* inspection (GB 4789.10-2010) standard operation. All the samples were individually agitated into 10ml tubes containing 75 g/L sodium chloride broth at 37°C for 18 to 24 h, and then inoculated with Baird– Parker plate for 24 h at 37°C. Colonies forming a spot and clear zone on the plate were selected and purified with blood agar plate. After incubation, potential *S. aureus* were generated to Gram stain, cell morphology and plasma coagulation test. Those verified *S. aureus* were stored at -80°C in TSB supplemented with 20% (v/v)glycerol for further study. Working cultures were maintained in TSA stored at 4°C. Briefly, each strain was grown at 37°C for 48 h in TSB. Cultures were diluted, to 0.5 McFarland standard (approximately 108CFU/ml) prior to following tests.

Biofilm Formation Assay: Biofilm assay of all *S. aureus* were quantified followed previous study with some modifications [12]. Briefly, after cultures were diluted (10^6 CFU/mL), the bacterial suspension (1 µl) was added to each well of the 96-well polystyrene microtiter plates. Each well was also filled with 100 µl of fresh TSB containing different concentrations of sucrose esters range from 0.05 mg/ml to 7mg/ml (referenced and designed by the hygiene standards for use of food additives, then all samples were incubated at 37°C for 48 h under aerobic conditions. Wells with no additives were used as negative controls. The absorbance of wells was measured at 630nm in a spectrophotometer (Bio-Rad, Hercules, CA). After unattached bacteria were removed by washing three times through submerging the plates in sterile deionized water, the plates were air-dried for 30 min and stained with 0.1% (wt/wt) crystal violet for 20 min, and then rinsed six times with deionized water to remove unbound crystal violet. After air-drying for 30 min, ethanol (95%, 100 µl) was added to each well, and the plate was placed on a shaker for 30 min. The absorbance was determined at 492 nm. The ratio (B) of absorbance at 492 and 630 nm was used to assess biofilm formation. The isolates were classified according to scale values of B. No biofilm producer=B<0.1; weak biofilm producer=0.1 ≤ B<0.5; moderate biofilm producer =0.5 ≤ B<1; strong biofilm producer=B ≥ 1. Biofilm-forming rate represents the percentage of the No. of strains producing biofilm (B ≥ 0.1) to that of total strains.

Bacterial Hydrophobicity Assay: The modified bacterial adherence to hydrocarbon–xylene (BATH) test was performed as originally proposed by Rosenberg et al [13]. The surface hydrophobicity of bacterial cells treated with two concentrations (3 mg/ml and 4mg/ml) of three types of sucrose esters as well as untreated cells was assessed. Briefly, 5 ml of the cell suspension (106 CFU/ml) were mixed with TSB (1:100 dilution, v/v) containing sucrose esters, placed in 10ml tubes and incubated for 48 h at 37°C in a rotary shaker at 120 rpm. Bacterial sediments obtained from treated as well as from control cultures were rinsed twice in 0.1 M phosphate-buffered saline (pH 7.2, 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4, two-fold dilution with deionized water prior to experiments). After suspending by deionized water, and absorbance value of *S. aureus* were tested at 630 nm (A630t), 200 µl Xylene was added to 4ml *S. aureus* suspension. Then the samples were vortexed for 60 s, followed by incubation for 15 min at 25°C. After samples were separated into two layers, the aqueous layer was removed carefully, and measured at absorbance 630 nm (A630d). The percentage of decrease was shown as the absorbance of the lower aqueous phase to the absorbance of the initial cell suspension. The result of CSH was analyzed according to the following formula: % CSH=[(A630t-A630 d) × 100]/A630t [14]. The strain was considered hydrophilic when it expressed a percentage of adhesion to xylene ≤ 35% [15].

Statistical Analysis: All statistical tests were conducted using SPSS 17.0 (SPSS Inc, Chicago, IL, USA). Data presented in all studies were repeated three times using duplicate samples. The statistical significance of associations between variables in different categories of isolates (strong, moderate and weak biofilm-producing × ratio CSH) was calculated using the two related sample-test analysis of variance test. The statistically significant differences for Independent-samples were generated using T test (P<0.05).

RESULTS AND DISCUSSION

Identification of Bacteria: A total of 12 isolates were screened and identified as *S. aureus* from 30 samples. According to the GB 4789.10-2010, samples were spread on Baird – Parker Agar (BPA) plate. Positive samples formed white, grey or black colonies, which are surrounded by an opaque halo of precipitation. Haemolytic activity was performed by streaking strains on blood agar plates. Identification of *S. aureus* was confirmed by Gram staining and coagulase reaction. Twelve (40%) samples yielded *S. aureus*, which were isolated all from beef. The results showed that high present of *S. aureus* positively were among beef samples. The latest reports showed that *S. aureus* contamination was common among in raw beef meat (15%) [16]. In this study, the high percentage contamination of *S. aureus* presented might be caused due to the use of expired meat samples instead of raw meat.

Biofilm-forming ability and CSH analysis: All concentrations of sucrose esters range from 0.05 mg/ml to 7mg/ml tested had no significant effect growth of all *S. aureus* according to the absorbance of wells at 630nm (data not shown).

The ability of biofilm-formation of *S. aureus* was assessed based on its adhesion rate (B).Results of biofilm-forming rate (BFR) with sucrose ester treatment are showed in **Figure 1**. Various levels of biofilm formation inhibitory by sucrose esters were observed against all the isolates.

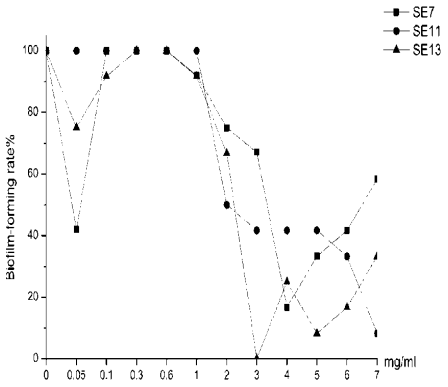

Figure 1. Inhibitory effects of sucrose ester emulsifiers on biofilm formation of *Staphylococcus aureus*. Each value is a mean of three tests run in duplicate ± SD.

At the low concentration of 0.05 mg/ml, SE7 and SE11 exhibited moderate biofilm inhibiting capability, whereas SE11 at the concentration of 0.1 ~ 1 mg/ml did not decrease the bacterial adhesion. When SE7 reached 4 mg/ml, the biofilm formation rate fell to the minimum value. It showed the lowest biofilm-forming rate at the high concentration of 7 mg/ml for SE11. However, with the addition of 3 mg/ml SE13, BFR was significantly reduced and showed 100% inhibition. Although the value increased up to 3 mg/ml for SE13, a significant BFR reduction was also observed from **Figure 1**. At concentrations of 0.3 ~ 4 mg/mL, biofilm formation assay results **(Figure 1)** indicated that SE7 and SE13 showed reduction of BFR, while the BFR of SE11 was the overall downward trend. In the previous study, few commercial sanitizers available are permissible to use for the decontamination of vegetables and fruits or use to eliminate the pathogens adhered to the foods; therefore, food additives could be a new viewpoint as a safe alternative for the decontamination of foods. Adhesion inhibitory effects of several food additives, such as SE on several pathogenic bacteria onto microtiter plate have been shown [17,18]. The registered food additives such as sucrose fatty acid esters can be used as emulsifiers in processed foods are promising agents for inhibiting biofilm formation by food poisoning bacteria [19], which supported the same effect of our results. In our study, the concentrations of 3 mg/ml and 4 mg/ml of three types of sucrose esters was abided by food safety standards, based on the research conducted so far, we believe that commercially available SE could be used in the future as a preventative measure, to prevent the establishment of a biofilm, or as a method used to eradicate an already existing biofilm infection.

Next, we examined the changes in the cell surface hydrophobicity of *S. aureus* after the treatments with three types of sucrose esters were also tested at concentration of 3 mg/ml and 4 mg/ml (no effect on growth). Levels of *S. aureus* hydrophobicity differed depending on various species of pathogen and sucrose ester types [20]. Hydrophobicity of *S. aureus* was shown significantly reduced in comparison to non-treated bacterial except for 3 strains (St.2, St. 3, St.4) in some concentrations **(Figure 2)**. The effect of 3 mg/ml SE7 was decreased from 10.67 to 90.32%, four (33.3%) strains displayed less effective influence with percentages lower than 50%, while six (50%) strains were reaching 51.61% or higher than this. All *S. aureus* strains exhibit a dramatic reduction at concentration of 4 mg/ml SE7, ranged from 49.01 to 98.26%.

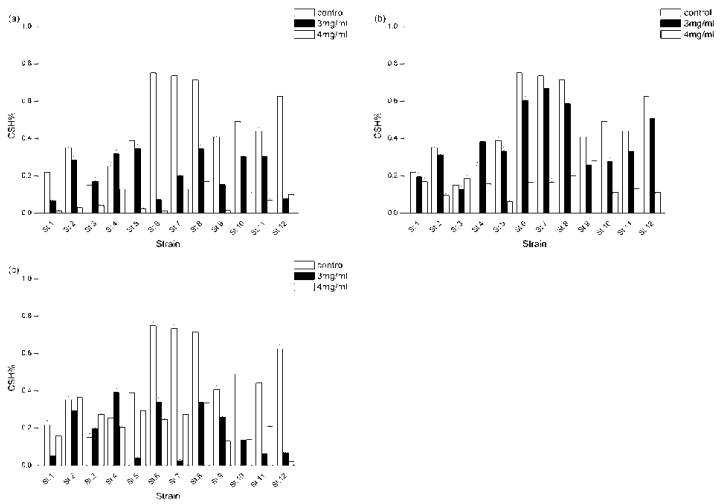

Figure 2. The effects of different concentrations of SE13 on cell surface hydrophobicity of *S. aureus* (mean ± SD) (a) SE 7, (b) SE11, (c) SE13. Each value is a mean of three tests run in duplicate ± SD.

Correlation between Biofilm-Forming Ability and CSH: It is clear that there is a remarkable correlation between biofilm-forming ability and the ratio CSH (P=0.002). The results of Independent-samples output T-test of variance is showed in **Table 1**. Sucrose esters of 3 mg/ml and 4 mg/ml had inhibitory effect on biofilm formation of food-borne *S. aureus* (P1<0.05).

Table 1. Statistics analysis of B and CSH values in 12 *S. aureus*.

	Control	SE7		SE11		SE13	
	0 mg/ml	3 mg/ml	4 mg/ml	3 mg/ml	4 mg/ml	3 mg/ml	4 mg/ml
B range	0.103~1.147	0.068~0.285	0.007~0.833	0.056~0.277	0.029~0.352	0.008~0.125	0.007~0.542
CSH range	0.151~0.736	0.069~0.348	0.013~0.169	0.196~0.668	0.063~0.282	0.027~0.342	0.021~0.364
P1		0.014	0.005	0.02	0.011	0.001	0.001
P2		0.006	0	0.01	0	0.002	0.004

P-values are the One-way analysis of variance between different groups: P1: comparison control versus other concentration of B values; P2: comparison control versus other concentrations of CSH.

As showed in **Figure 2**, a majority of strains (10/12) had measurable decrease in their CSH status compared to controls inoculated in the presence of the SE. Meanwhile, a significant (P2 ≤ 0.010, **Table 1**) effect of SE in the cell surface hydrophobicity of *S. aureus*. In further experiments, the changes in the cell surface hydrophobicity of *S. aureus* after the treatment with two concentrations of sucrose esters were also tested.

Hydrophobicity of the bacterial surface is important for the adhesion of bacteria to water-insoluble substrates. It is hypothesized that bacterial adhesion enhances with an increasing bacterial hydrophobicity and decreases with reduced hydrophobicity [21,22]. Bacterial attachment is influenced by cell surface charge [23], hydrophobicity [24] and structures that include extracellular polysaccharides and fibrils or fimbriae [25], but the fundamental mechanisms for bacterial adhesion are still not well understood. Previous observations have suggested that the term of hydrophobicity in the interpretation of bacterial adhesion is often used, because it has been observed that adhesion increases with increased hydrophobicity of bacteria [21-28]. In latest report, the author demonstrated that a positive correlation was verified between hydrophobicity and biofilm biomass for *C. parapsilosis* [29]. In our experiments, SE also obviously decreased the cell surface hydrophobicity of different *S. aureus* strains obviously. Furthermore, the results of B values and CSH values assay **(Figures 1 and 2)** and statistical analysis **(Table 1)** indicated that SE inhibited biofilm formation of *S. aureus* by interfering with the cell surface hydrophobicity of strains. They can decrease the CSH of *S. aureus* and destroy the cell membrane permeability. Our results are agreeable with the previous reports, in which sugar fatty acid esters inhibited the initial attachment of the *S. aureus* cells to the abiotic surface [19]. Another literature reported that sugar fatty acid esters at some concentrations inhibit the adhesion of *Salmonella enteritidis* [30]. However, the mechanism underlying how sucrose esters inhibit the biofilm-forming is still unclear. At this point, our results speculated that SE inhibited the film formation of *S. aureus* by interfering with the cell surface hydrophobicity of strains, and bacterial hydrophobicity is likely an important one of many parameters, which determine the ability of a cell to adhere, invade, and cause damage.

CONCLUSION

In conclusion, the present study showed that SE have anti biofilm ability, and can be further used as a preventative measurement to prevent attachment of food borne pathogens or as a method to eradicate an already existing biofilm infection. Although further mechanism of inhibiting biofilm formation by food additives is not fully understood, our study has shown a desirable application of safe food additives for the prevention of food contamination.

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REFERENCES

1. Raad II and Hanna HA. Intravascular catheter-related infections: new horizons and recent advances. Arch Intern Med. 2002; 162: 871-878.
2. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998; 339: 520-551.
3. Donlan RM. Biofilms and device-associated infections. Emerg Infect Dis. 2001; 7: 277-281.
4. Gotz F. Staphylococcus and biofilms. Mol Microbiol. 2002; 43: 1367-1378.
5. Fitzpatrick F, et al. The genetics of biofilm formation-will a greater understanding of pathogenesis lead to better management of device-related infection? Clin Microbiol Infect. 2005; 11: 967-973.
6. Zegans ME, et al. Bacterial biofilms and ocular infections. Ocul Surf. 2005; 3: 73-80.
7. Fux CA, et al. Survival strategies of infectious biofilms. Trends Microbiol. 2005; 13: 34-40.
8. Hall-Stoodley L, et al. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004; 2: 95-108.

9. Revis AN, et al. Inactivation of Listeria monocytogenes biofilms using chemical sanitizers and heat: Biofilms in the Food Environment. Blackwell Publishing, Iowa, USA. 2007; 2: 73–104.

10. Branen JK and Davidson P. Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediamineteteaacetic acid and lactoferrin. Int J Food Microbio. 2004; 90: 63–74.

11. Mbandi E, et al. Antilisterial effects of free fatty acids and monolaurin in beef emulsions and hot dogs. Food Microbiol. 2004; 21: 815–818.

12. Rode TM, et al. Different patterns of biofilm formation in Staphylococcus aureus under food-related stress conditions. Int J Food Microbiol. 2007; 116: 372–383.

13. Rosenberg M, et al. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol Lett. 1980; 9: 29–33.

14. Pan WH, et al. The correlation between surface hydrophobicity and adherence of Bifidobacterium strains from centenarians'faeces. Anaerobe. 2006; 12: 148-152.

15. Savoia D, et al. Aspecific adherence and cytotoxicity of Escherichia coli strains. Microbios. 1996; 87: 21-30.

16. Osman KM, et al. Prevalence and antimicrobial resistance profile of Staphylococcus species in chicken and beef raw meat in egypt. Foodborne Pathogens and Disease. 2015; 12: 406-413.

17. Islam MT, et.al. Combined effects of selected food additives on adhesion of various foodborne pathogens onto microtiter plate and cabbage leaves. Food Control. 2014; 46: 233-241.

18. Miyamoto T, et al. Inhibition of Adhesion of Several Bacteria onto Microtiter Plate by Selected Food Additives. Japan Journal of Food Microbiology. 2011; 28: 157-166.

19. Furukawa S, et al. Sugar fatty acid esters inhibit biofilm formation by food-borne pathogenic bacteria. Int J Food Microbiol. 2010; 138: 176-180.

20. Choi N-Y, et al. Biofilm formation, attachment, and cell hydrophobicity of foodborne pathogens under varied environmental conditions. Journal of the Korean Society for Applied Biological Chemistry. 2013; 56: 207-220.

21. Hall-Stoodley L, et al. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews Microbiology. 2014; 2: 95-108.

22. Van Loosdrecht MCM, et al. The role of bacterial cell wall hydrophobicity in adhesion. Appl Environ Microbiol. 1987; 53: 1893-1897.

23. Dickson JS and Koohmaraie M. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl Environ Microbiol. 1989; 55: 832-836.

24. Dahlback B, et al. The hydrophobicity of bacteria an important factor in their initial adhesion at the air-water interface. Arch Microbiol. 1981; 128: 267-270.

25. Fletcher M and Floodgate GD. An electron-microscopic demonstration of an acid polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J Gen Microbiol. 1973; 74: 325-334.

26. Bussher HJ, et al. Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. Appl Environ Microbiol. 1984; 48: 980-983.

27. Van Loosdrecht MCM and Zehnder AJB. Energetics of bacterial adhesion. Experientia. 1990; 46: 817-822.

28. Fletcher M and Pringle JH. The effect of surface free energy and medium surface tension of bacterial attachment to solid surfaces. J Coil Interfac Sci. 1985; 104: 5-13.

29. Silva-Dias A, et al. Adhesion, biofilm formation, cell surface hydrophobicity, and antifungal planktonic susceptibility: relationship among Candida spp. Front Microbiol. 2015; 6: 205-212.

30. Miyamoto T, et al. Inhibitors of adhesion ability of Salmonella Enteritidis. J Jpn Soc Food Sci. 2009; 56: 200–208.