In vitro antibacterial activities and mechanism of sugar fatty acid esters against five food-related bacteria

Lei Zhao *, Heyan Zhang, Tianyang Hao, Siran Li

Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University, Beijing 100048, China
Beijing Laboratory for Food Quality and Safety, Beijing Technology and Business University, Beijing 100048, China

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A B S T R A C T

The objective of this study was to evaluate the antibacterial activities of sugar fatty acid esters, with different fatty acid and saccharide moieties, against five food-related bacteria including Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhimurium. Sucrose monocaprate showed the strongest antibacterial activity against all tested bacteria, especially Gram-positive bacteria. The minimum inhibitory concentrations (MICs) for Gram-positive bacteria and Gram-negative bacteria were 2.5 and 10 mM, respectively. The minimum bactericidal concentrations (MBCs) for Gram-positive bacteria were 10 mM. Time-kill assay also showed that sucrose monocaprate significantly inhibit the growth of tested bacteria. The permeability of the cell membrane and intracellular proteins were both changed by sucrose monocaprate according to cell constituents' leakage, SDS–PAGE and scanning electron microscope assays. It is suggested that sucrose monocaprate, with both emulsifying and antibacterial activities, have a potential to serve as a safe multifunctional food additive in food industries.

1. Introduction

Sugar fatty acid esters are widely used as emulsifiers in food industries since they are tasteless, odorless, non-toxic, non-irritant and biodegradable (Gumel, Annuar, Heidelberg, & Chisti, 2011; Šabeder, Habulin, & Knez, 2005). They can be synthesized from renewable resources, such as fatty acids and carbohydrates, by an esterification reaction. The United States Food and Drug Administration (FDA) has allowed sucrose fatty acid esters to be added to certain processed foods (21CFR 172.859). In recent years, sugar fatty acid esters have attracted much attention because of their biological activities, including insecticidal (Puterka, Farone, Palmer, & Barrington, 2003), antitumor (Ferrer, Perez, Plou, Castell, & Ballesteros, 2005a) and antimicrobial properties (Ferrer et al., 2005b; Furukawa, Akiyoshi, O’Toole, Ogihara, & Morinaga, 2010; Habulin, Šabeder, & Knez, 2008; Xiao et al., 2011; Zhang, Wei, Cao, & Feng, 2014).

The antibacterial activity of sugar fatty acid esters has been studied extensively, yet variable results have been reported on different bacterial species. Some studies reported inhibition of Gram-negative bacteria (Ferrer et al., 2005b; Nobmann, Smith, Dunne, Henehan, & Bourke, 2009; Xiao et al., 2011), while others reported inhibition of only Gram-positive bacteria (Devulapalle et al., 2004; Piao, Kawahara-Aoyama, Inoue, & Adachi, 2006; Watanabe, Katayama, Matsubara, Honda, & Kuwahara, 2000). Sugar fatty acid esters can be both bacteriostatic (Shearer, Dunne, Sikes, & Hoover, 2000) and bactericidal (de Lamo-Castellvi, Ratphitagsanti, Balasubramaniam, & Yousef, 2010), depending on the dose. The antibacterial activity of sugar fatty acid esters is dependent on the nature of the carbohydrate core, number and type of esterified fatty acid and degree of esterification (Wagh, Shen, Shen, Miller, & Walsh, 2012), Watanabe et al. (2000) reported that among the carbohydrate esters synthesized, galactose and fructose laurates showed the highest growth-inhibitory effect against Streptococcus mutans, while the other analogs of hexose laurates showed no antibacterial activity. Smith, Nobmann, Henehan, Bourke, and Dunne (2008) found that lauric ester derivatives of methyl α-mannopyranoside and methyl β-glucopyranoside showed the best inhibitory effects against Staphylococcus aureus. Karlová, Polakova, Smidril, and Filip (2010) reported that the antimicrobial effects of fatty acid fructose esters decreased rapidly as the length of fatty acid chain increased and caprinoylfructose proved to be the most active. Recently, we also found that sugar fatty acid esters exhibited good antifungal activities against Penicillium oxalicum and Aspergillus tubingensis (Zhao et al., 2014).

The survival of microorganism in food can lead to spoilage or cause infection and illness. Of all the microorganisms, bacteria
are the most troublesome and important biological foodborne hazard for the food industry, even in developed countries. Commercial sucrose esters are applied mostly in Japan in canned beverages to provide sterilization-stable emulsions and inhibit the germination of spore-forming bacteria (Mitsubishi-Kagaku Foods Corporation, Japan). Meanwhile, the increasing demand of consumers for healthy, non-toxic and diversified processed foods (such as beverages, desserts and dairy products) has led to research on multifunctional food additives. Therefore, development of sugar fatty acid ester with antibacterial activity for the prevention of food contamination is becoming increasingly attractive. Although the antibacterial activities of sugar fatty acid esters have been studied, this information is still limited. The antibacterial activity of sugar fatty acid esters with different saccharide and fatty acid moieties against food related bacteria has not been well defined. Most importantly, to the best of our knowledge, the antibacterial mechanism of sugar fatty acid esters is far from clear.

The objective of this study was to determine the antibacterial activities of sugar fatty acid esters. By varying either the carbohydrate moiety (glucose, fructose, sucrose and maltose) or the fatty acids chain length (C10, C12, C14, C16 and C18), respectively, eight sugar fatty acid esters were synthesized. The antibacterial activities of sugar fatty acid esters against five common food-related bacteria were evaluated by measuring growth inhibition zone diameters, MIC and MBC values and kill-time curves. The potential antibacterial mechanisms against representative strains were determined by permeability and integrity of cell membrane, SDS–PAGE, scanning electron microscopy observation as well as DNA binding assay.

2. Materials and methods

2.1. Chemicals

Immobilized lipase Novozym 435 from Candida antarctica B (EC 3.1.1.3) was obtained from Novo Nordisk AS (Copenhagen, Denmark). Glucose, fructose, sucrose, maltose, capric, lauric, myristic, palmitic, stearic acid, bovine serum albumin and 3,5-dinitrosalicylic acid were purchased from Sigma–Aldrich (Shanghai, China). Coomassie brilliant blue G-250 was purchased from Sigma–Aldrich (Shanghai, China). Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MHB) were purchased from Beijing Aoboxing Bio-tech Co. Ltd. (Beijing, China). All other chemicals of analytical grade were purchased from Beijing Chemical Reagent Co., Ltd. (Beijing, China).

2.2. Bacterial strains and growing conditions

Five common food-related bacterial strains (three Gram positive and two Gram negative) were selected for the study. The Gram positive bacteria were Bacillus cereus AS1.1846, Bacillus subtilis AS1.1849 and S. aureus AS1.89 while the Gram negative bacteria were Escherichia coli AS1.90 and Salmonella typhimurium AS1.1174. All strains were obtained from the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China) and maintained on MHA slants at 4 °C. Cells were prepared by 16-h culture in MHB at 37 °C. A 16-h culture was diluted with MHB to an inoculum of 1 × 10^6 CFU/ml approximately. The number of cells in the suspensions was determined by duplicate plating from 10-fold serial dilution on MHA and counting the colonies after incubation at 37 °C for 24 h.

2.3. Sample preparation

Sugar esters with different fatty acids (capric, lauric, myristic, palmitic and stearic acid) and saccharides (glucose, fructose, sucrose and maltose) were synthesized as described by Zhao et al. (2014). Sugar fatty acid esters were prepared by the reverse hydrolysis in nonaqueous medium. The direct esterification of saccharide and fatty acid was performed with a molar ratio of 1:3 in the presence of acetone as organic solvent. Commercial immobilized lipase (Novozym 435) was used as the biocatalyst (15 g/l). Reactions were performed at 45 °C with orbital shaking (170–180 rpm) in the presence of 4 Å molecular sieves (70 g/l) for 72 h. The crude products were purified over a silica gel column using ethyl acetate/methanol as the eluent. Sugar fatty acid esters were dissolved in ethanol and diluted to the desired concentrations. The final concentration of ethanol in growth medium was below 2% (v/v), a level at which ethanol did not affect the bacterial growth based on preliminary experiments.

2.4. Paper disk diffusion assay

The antibacterial activity of sugar fatty acid esters was determined by the paper disc diffusion method described by Özer et al. (2007) with some modification. MHB cultures of bacteria were grown at 37 °C for 12 h. Suspensions (100 µl) of the bacteria, adjusted to final concentration of 10^6–10^7 CFU/ml, were added into Petri dishes with 9.9 ml of sterile MHA media (about 55 °C), and the mixture was mixed immediately. The disks (6 mm in diameter) impregnated with 10 µl of the ethanol solution of sugar fatty acid esters (255 mM) were placed on the inoculated agar. The plates were incubated at 4 °C for 2 h to allow diffusion of samples in the medium. Negative controls were prepared with the same solvents employed to dissolve the sugar fatty acid esters. Incubation of plates was performed at 37 °C for 24 h. The diameter of the clear zone in which no growth of bacteria was observed was measured using a vernier caliper to represent the antibacterial activity of sugar fatty acid esters. All experiments were performed in duplicate.

2.5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Sucrose monopropionate with the highest antibacterial activity in the previous experiment was selected for further study. MIC and MBC were determined by broth macrodilution assay (Wilson et al., 2005). Serial two fold dilutions of sucrose monopropionate were prepared in MHB at concentrations of 40, 20, 10, 5, 2.5 and 1.25 mM. One tube with the same volume of MHB was set as control. Inocula were added into all the tubes to achieve an initial inoculum of approximate 5 × 10^6 CFU/ml. All tubes were incubated at 37 °C for 24 h, a 1 ml portion was removed from each tube for colony counting by decimal dilution in 0.85% (w/v) sodium chloride solution, and plated out onto MHA. All experiments were conducted in duplicate. MIC is defined based on the logarithmic difference in population (logDP). The logDP is expressed by the following equation (Fang & Lin, 1995):

$$\log DP = \log(N/N_0) = \log(N) - \log(N_0)$$

N is the population after incubation for 24 h and N_0 is the initial population. MIC is defined as the lowest concentration resulting in maintenance or reduction of inoculums viability (logDP ≤ 0). The minimum bactericidal concentration (MBC) is defined as the concentration where 99.9% or more of the initial inoculums are killed (logDP ≥ −3).

2.6. Time-kill analysis

The time-kill test was performed based on D’Arrigo et al. (2010) to determine the killing kinetics of sucrose monopropionate against
five tested bacteria. Cultures of bacteria with a density of 5 × 10^5 CFU/ml were exposed to sucrose monocaprate broth dilutions with the final concentrations ranging from 1 × MIC to 4 × MIC. MHB broth was used as the control. After incubation, all the solutions were incubated at 37 °C under shaking conditions (160 rpm). After 0, 1, 2, 4, 8, 12, 16, 20, 24 and 48 h of incubation, aliquots of 100 μl were taken out, serially diluted, and inoculated on MHA plates for 24 h at 37 °C, and the number of survivors (CFU/ml) was determined by counting the colonies. Time-kill curves were constructed by plotting the log_{10} CFU/ml versus time. The experiments were conducted in triplicate.

2.7. Cell constituents’ release

The cell integrity is examined by determining the release of cell constituents into supernatant according to the method described by Lv, Liang, Yuan, and Li (2011), Diao, Hu, Zhang, and Xu (2014) with some modifications. Cells from the working culture of tested bacteria were collected by centrifugation for 10 min at 5000g, washed three times with 0.1 M phosphate buffer solution (PBS, pH 7.2), and resuspended in the same buffer. Twenty-five milliliters of cell suspension were incubated at 37 °C under agitation for 6 h in the presence of sucrose monocaprate at the MIC concentrations. Then, 2 ml aliquots of 100 ml were collected and centrifuged at 10,000 g for 5 min. Control groups containing bacterial supernatant without sucrose monocaprate treatments were tested similarly. The amounts of DNA and RNA released from the cytoplasm in supernatant were estimated by the detection of absorbance at 260 nm. The concentrations of proteins in supernatants were determined by Bradford assay (Bradford, 1976). A mixture of 1 ml of diluted supernatant and 5 ml of Coomassie brilliant blue G-250 reagent was made and its absorbance was measured at 595 nm. The protein concentration was determined with a calibration curve prepared with bovine serum albumin. Reducing sugars in supernatants were determined by 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959) with some modifications. A mixture of 1 ml of diluted supernatant and 1 ml of DNS reagent was heated in boiling water for 10 min. After cooling to room temperature under running tap water, 10 ml of distilled water was added. The absorbance of the reaction mixtures at 540 nm was recorded on a spectrophotometer (GBC, Australia) and compared with a glucose calibration curve to quantify the concentration of reducing sugars.

2.8. SDS–PAGE of whole-cell proteins

SDS–PAGE of the bacterial proteins was carried out according to the method of Li, Han, Feng, Tian, and Mo (2014) with some modifications. Cells of bacteria were prepared and treated as described in Section 2.7. Controls were run without sucrose monocaprate. The cells were collected by centrifugation at 5000g for 10 min, and resuspended in 0.1 M PBS (pH 7.2). After that, cell suspensions of the treated and control groups were adjusted to the same cell density (OD_{600}). Equal amounts of cell suspensions were centrifuged and the pellets were resuspended in sample dilation buffer. After heating in boiling-water for 10 min, the samples were subjected to SDS–PAGE using a vertical electrophoresis apparatus Bio-Rad (Hercules, CA, USA). The SDS–PAGE was performed with a 5% stacking gel and a 12% separating gel followed by Coomassie brilliant blue staining.

2.9. Scanning electron microscopy (SEM) analysis

To determine the efficacy of the sucrose monocaprate and the morphological changes of bacteria strains, SEM studies were carried out as previously reported with some modifications (Lv et al., 2011; Moosav et al., 2008). Logarithmic growth phase cells of 3 tested bacteria (OD_{600} ≈ 1.0) were treated with sucrose monocaprate at MIC value. The control (without sucrose monocaprate) and samples were incubated at 37 °C for 6 h. After incubation, cells were harvested by centrifugation (10,000g, 10 min) and washed twice with 0.1 M phosphate buffer solution (PBS, pH 7.2), and then fixed with 2.5% (v/v) glutaraldehyde in PBS overnight at 4 °C. After centrifugation, the cells were further dehydrated using a graded series of ethanol (30%, 50%, 70%, 80%, 90% and 100%) followed by drying with hexamethyldisilazane. Finally, the samples were fixed on SEM support, and then sputter-coated with gold under vacuum, followed by microscopic examinations using a scanning electron microscope (Tescan s.r.o., Brno, Czech Republic).

2.10. DNA binding assay

2.10.1. Ultraviolet spectroscopy

Interaction of sucrose monocaprate with DNA was studied by using a UV spectrophotometer method with little modification (Tang, Shi, Zhao, Hao, & Le, 2009). The pBR322 plasmid DNA and sucrose monocaprate were dissolved in 10 mM Tris–HCl buffer (pH 7.2) and then mixed to obtain various DNA/sucrose monocaprate samples with constant DNA concentration (400 μg/ml) and increasing sucrose monocaprate concentrations (0, 2.5, 5, 10 and 20 mM). After incubation at 37 °C for 10 min, absorbance of the mixed solutions was measured in the range of wavelengths 230–350 nm (Shimadzu UV-2450 spectrometer).

2.10.2. Agarose gel electrophoresis

Agarose gel electrophoresis assay for the detection of DNA-binding activity was performed based on the procedures described by He, Yang, Yang, and Yu (2010). The pBR322 plasmid DNA (0.2 μg) in Tris–HCl/EDTA buffer (pH 7.2) was treated with the

Table 1

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Inhibition zone diameter (mm)</th>
<th>MIC (mM)</th>
<th>MBC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucre monostearate</td>
<td>Sucre palmitate</td>
<td>Sucre myristate</td>
</tr>
<tr>
<td>Bacillus cereus (G+)</td>
<td>nd</td>
<td>nd</td>
<td>9.7 ± 0.3</td>
</tr>
<tr>
<td>Bacillus subtilis (G+)</td>
<td>nd</td>
<td>nd</td>
<td>10.8 ± 0.5</td>
</tr>
<tr>
<td>Staphylococcus aureus (G+)</td>
<td>nd</td>
<td>nd</td>
<td>10.0 ± 0.3</td>
</tr>
<tr>
<td>Escherichia coli (G-)</td>
<td>nd</td>
<td>nd</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>Salmonella typhimurium (G-)</td>
<td>nd</td>
<td>nd</td>
<td>6.5 ± 0.2</td>
</tr>
</tbody>
</table>

Data (means ± SD, n = 3) within a row with different superscripts are significantly different (p < 0.05). Each sugar fatty acid ester was tested at 2.55 μmol/disc.

1 Inhibition zone diameter (mm) including diameter of sterile disc (6 mm).
2 nd, not detected.
3 MIC, minimal inhibitory concentration of sucrose monocaprate.
4 MBC, minimum bactericidal concentration of sucrose monocaprate.
different concentrations of sucrose monocaprate, followed by dilution with the Tris–HCl buffer to a total volume of 20 µl. Then, the reaction mixtures were incubated at 37 °C for 1 h before being loaded onto a 1% agarose gel, and the electrophoresis was performed for 35 min under 110 V at room temperature.

2.11. Statistical analysis

Data were expressed as means ± SD. Statistical analysis was performed using the Duncan test at the 95% significance level to express the difference between two groups. p < 0.05 was considered statistically significant. Analysis was performed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Antibacterial activity

Initial screening of the antibacterial activity of the investigated sugar fatty acid esters against five tested food-related bacteria was conducted using the paper disk diffusion assay, which was determined by the presence or absence of inhibition zones. As shown

![Fig. 1. Time-kill curves of sucrose monocaprate against five food-related bacteria. (A) Bacillus cereus; (B) Bacillus subtilis; (C) Staphylococcus aureus; (D) Escherichia coli; (E) Salmonella typhimurium.](image-url)
in Table 1, sugar fatty acid esters with different fatty acid and saccharide moieties displayed a variable degree of antibacterial activity against different tested strains. The inhibition zone diameters of sugar fatty acid esters against Gram-positive bacteria were larger than that of Gram-negative bacteria, indicating stronger antibacterial effects against Gram-positive bacteria. The antibacterial activities of sucrose fatty acid esters with different fatty acid moieties decreased in the order: sucrose monocaprate > sucrose monolaurate > sucrose myristate. However, sucrose palmitate and sucrose monostearate exhibited no antibacterial activity against all the tested bacteria. Similar results were reported by Karlová et al. (2010), who indicated that the antibacterial activity of sugar fatty acid esters decreased rapidly as the chain length of fatty acid increased. From another point of view, the antibacterial activities of monocaprates with different saccharide moieties were in the order: sucrose monocaprate > maltose monocaprate > fructose monocaprate > glucose monocaprate. The results suggested that disaccharide monoesters exhibited better antibacterial activity than monosaccharide monoesters, which was in accordance with the results of Ferrer et al. (2005b). Sucrose monocaprate showed the strongest antibacterial activity against all the tested bacteria, especially against B. cereus, B. subtilis and S. aureus, with the inhibition zone diameters of 19.7 ± 0.2, 22.7 ± 0.7 and 22.3 ± 0.3 mm, respectively. In our previous study, sucrose monocaprate also exhibited strong antifungal activities against P. oxalicum and A. tubingensis, as was shown by the reduction in the size, thickness and pigmentation of mold colonies (Zhao et al., 2014). Therefore, sucrose monocaprate was selected for further investigated in this study.

3.2. MICs and MBCs of sucrose monocaprate

The MICs and MBCs of sucrose monocaprate against the tested bacteria are shown in Table 1. The MICs and MBCs for Gram-positive bacteria were 2.5 and 10 mM, respectively. The MICs for E. coli and S. were both 10 mM, which were 3-folds higher than that of Gram-positive bacteria. Unfortunately, the MBCs of sucrose monocaprate for Gram-negative bacteria were not gained within the selected concentration range (1.25–40 mM). The results suggested that Gram-negative bacteria were more resistant to sucrose monocaprate than Gram-positive bacteria due to their outer membrane, which restricted diffusion of sucrose monocaprate through their lipopolysaccharide covering. A similar conclusion has also been drawn by Al-Zoreky (2009), Jouki, Mortazavi, Yazdi, and Koocheki (2014).

3.3. Time-kill analysis

To further investigate the antibacterial activities of sucrose monocaprate, time-kill assays were carried out to study the concentration and time-dependent killing effect. The results obtained for the time-kill curves are summarized in Fig. 1. For all five selected bacteria, higher concentration of sucrose monocaprate led to a more rapid decrease in bacterial number. The growth of bacteria was suppressed by sucrose monocaprate at 1 × MIC, indicating a bacteriostatic effect. In the treatments at 2 × MIC concentration, the numbers of viable cells of five bacteria were significantly lower than the initial value after 24 h. When sucrose monocaprate concentration was set at 4 × MIC, there were 3.92, 4.55 and 4.23 log CFU/ml drops in colony counts after 24 h for B. cereus, B. subtilis and S. aureus, respectively (Fig. 1A–C). Sucrose monocaprate displayed bactericidal effect against Gram-positive bacteria and over 99.9% of bacterial reduction was observed. The time to kill 3-log of B. cereus and B. subtilis was 16 h (Fig. 1A and B), while that of S. aureus was 12 h (Fig. 1C). However, a 4 × MIC concentration of sucrose monocaprate was insufficient to achieve a bactericidal effect (>99.9% [3-log] reduction in bacterial inoculum) for E. coli and S. typhimurium within

![Fig. 2. SDS–PAGE of tested bacteria cells treated with sucrose monocaprate. (A) Bacillus cereus; (B) Staphylococcus aureus; (C) Escherichia coli. Lane 1: untreated bacteria; lane 2: sucrose monocaprate-treated bacteria at MIC value for 6 h.](image-url)
When the incubation time was extended to 48 h, sucrose monocaprate showed a bactericidal effect against Gram-negative bacteria as there was more than 3-log CFU/ml decrease in colony counts.

3.4. Integrity of cell membrane

Based on the sensitivity, representativeness and characteristics of the tested food-related bacteria, two Gram-positive (B. cereus and S. aureus) and one Gram-negative bacteria (E. coli) were selected for further study to explore the antibacterial mechanism of sucrose monocaprate. The integrity of the cell membrane was determined by measuring the release of cell constituents including protein, reducing sugar and the absorbance at 260 nm of the supernatant of tested bacteria. Table 2 shows the release of cell constituents when B. cereus, S. aureus and E. coli were treated with sucrose monocaprate for 6 h. After sucrose monocaprate was added to tested bacteria, the release of cell constituents increased significantly. Compared to the control, the leakage of proteins from B. cereus, S. aureus and E. coli treated with sucrose monocaprate increased by 34.4, 27.3 and 17.2 times, respectively. Similarly, the leakage of reducing sugar from B. cereus, S. aureus and E. coli treated with sucrose monocaprate were 3.2, 1.6 and 1.5 times as high as that of the control; in addition, the OD$_{260}$ of supernatant from B. cereus, S. aureus and E. coli treated with sucrose monocaprate were 3.0, 2.1 and 1.5 times as high as that of the control. The leakage of cell constituents of three tested bacteria were in the order: B. cereus > S. aureus > E. coli. The tendency was in accordance with the results of antibacterial activity. The above results indicated that damage to the cytoplasmic membranes might occur, which led to the losses of cell constituents and inhibition of cell growth.

Fig. 3. Effect of sucrose monocaprate on the morphology of Bacillus cereus (A, B), Staphylococcus aureus (C, D) and Escherichia coli (E, F). (A, C, E) untreated; (B, D, F) treated with sucrose monocaprate at MIC value for 6 h.
3.5. SDS–PAGE patterns of proteins from sucrose monocaprate treated bacteria

SDS–PAGE profiles of bacterial soluble proteins from B. cereus, S. aureus and E. coli treated with sucrose monocaprate are shown in Fig. 2. The protein profiles of bacteria treated with sucrose monocaprate differed from those of the control. The protein bands of untreated bacteria showed strong intensities. After treatment with sucrose monocaprate at 1 × MIC for 6 h, the protein bands faded or even disappeared. Similar results were also found in bacteria cells treated by certain antibacterial compounds, such as chitosan (Tao, Qian, & Xie, 2011), glycine (Sitohy, Mahgoub, & Osman, 2012) and lactic acid (Wang, Chang, Yang, & Cui, 2015). Considering the increasing protein contents in the cell-free supernatant (Table 2), it was suggested that sucrose monocaprate could decrease the content of cellular soluble proteins by permeating and disrupting cell membranes.

In the case of B. cereus (Fig. 2A), there were 18 major bands in lane 1 for untreated bacteria. By contrast, nine bands (R1, R2, R4–R8, R12 and R15) disappeared in lane 2 for bacteria treated with sucrose monocaprate. One new band (R10, approximately 49.3 kDa) appeared in lane 2. As for S. aureus (Fig. 2B), there were two thick bands (R3 and R5, about 61.6 and 50.1 kDa) among 18 major bands in lane 1 for untreated bacteria. After treated with sucrose monocaprate, the amount of R3 significantly reduced, while six bands (R2, R5, R7, R9, R12 and R14) disappeared in lane 2. Five new bands (R4, R8, R10, R11, R13), especially R4 (approximately 53.3 kDa) appeared in lane 2. In addition, a similar phenomenon was also observed in sucrose monocaprate treated E. coli. As shown in Fig. 2C, there were 30 major bands in lane 1 for untreated bacteria. However, ten bands (R4, R8, R12, R18, R20, R22, R24, R26, R28 and R30) disappeared in lane 2 for sucrose monocaprate treated bacteria. Twelve new bands (R5–R7, R9, R11, R13, R19, R21, R23, R25, R27 and R29) appeared in lane 2. According to Cloete, Thantsha, Maluleke, and Kirkpatrick (2009), Li et al. (2014), the explanation for disappearance of protein bands might be that sucrose monocaprate interfered with cell proteins synthesis of bacterial, or resulted in the leakage of proteins from bacterial cells. In addition, the appearance of new protein bands was probably attributed to degradation or aggregation of bacterial proteins by sucrose monocaprate.

3.6. Scanning electron microscopy (SEM) analysis

The morphological changes of B. cereus, S. aureus and E. coli were evaluated by SEM analysis. The electron micrographs of both untreated and sucrose monocaprate treated bacterial cells are shown in Fig. 3. Untreated cells showed regular and typical morphology, with a plump and smooth surface, and were uniform in size and distribution (Fig. 3A, C, and E). In contrast, B. cereus cells treated with sucrose monocaprate at MIC concentration (5 mM) revealed a severe damaging effect on the cell morphology, showing an irregularly wrinkled and coarse outer surface (Fig. 3B). This indicated that sucrose monocaprate treatment may result in damage to the B. cereus cell wall and cytoplasmic membrane. However, sucrose monocaprate had little or no effect on the external morphology of S. aureus and E. coli cells when viewed by SEM at 20 k× magnification (Fig. 3D and F). In order to fully understand the antibacterial mechanism of sucrose monocaprate, morphological changes of bacterial cells should be further studied by transmission electron microscopy at higher magnification.

3.7. DNA binding study

The interaction between sucrose monocaprate and DNA was studied, since it had a potential to reach the inner structure of cells through a damaged membrane. Absorption spectroscopy is one of the most useful techniques to study the binding of complexes to DNA. Fig. 4A illustrates that with increased concentration of sucrose monocaprate, the maximum absorption intensity decreased significantly. The spectral change process reflected the corresponding changes in conformation and structure of DNA after binding to sucrose monocaprate. Hypochromism results from the contraction of DNA in the helix axis as well as from the conformational change of DNA (Shahabadi, Kashanian, & Fatahi, 2011). It was suggested that sucrose monocaprate may bind onto the phosphate group of DNA by hydrogen bonding, which could reduce the charge density of main chain and increase the contraction of DNA structure.

Since sucrose monocaprate showed binding effect with pBR322 DNA, agarose gel electrophoresis of free DNA and DNA with increasing amounts of sucrose monocaprate was used to evaluate whether it had DNA cleavage ability. When circular plasmid DNA is subjected to electrophoresis study, the fastest migration will be observed for the super coiled form (Form I). If one strand is cleaved, the super coils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form will be generated which migrates in between (Kannan & Arumugham, 2012). As shown in Fig. 4B, the bands of plasmid pBR322 DNA treated with different concentrations of sucrose monocaprate were similar to that of control (lane 1). The result indicated that sucrose monocaprate had no ability to cause DNA cleavage.

![Fig. 4](image-url) Interaction of pBR322 plasmid DNA with increasing amounts of sucrose monocaprate. (A) Ultraviolet spectroscopic measurements. (B) Agarose gel electrophoresis of pBR322 plasmid DNA treated with different concentrations of sucrose monocaprate. Lane 0: control; and lanes 1–7: 80, 40, 20, 10, 5, 2.5 and 1.25 mM of sucrose monocaprate.
4. Conclusion

The antibacterial activities of eight sugar fatty acid esters (succrose monopurate, sucrose monolaurate, sucrose myristate, sucrose palmitate, sucrose monostearate, maltose monopurate, fructose monopurate and glucose monopurate) against five food-related bacteria were evaluated in this study. Sucrose monopurate showed the strongest antibacterial activity against the tested bacteria, especially against Gram-positive bacteria. The release of cell constituents and SEM observation suggested that sucrose monopurate exerted its antibacterial effect through affecting the permeability of the cell membrane, leading to leakage of some cellular components such as proteins, reducing sugars and 260 nm absorbing materials. The change in protein patterns and contents analyzed by SDS–PAGE suggested that sucrose monopurate might interfere with the synthesis of bacterial cell proteins or induce degradation or aggregation of bacterial proteins. Moreover, sucrose monopurate had no direct effect on cellular DNA migration profiles. In conclusion, sucrose monopurate has a potential to be used in the control of food-related bacteria and serve as a safe multifunctional food additive with both emulsifying and antibacterial properties in the food industry. Further studies are required to fully understand the antibacterial efficacy of sucrose monopurate, such as its effect against other food-related bacteria and interactions with food ingredients, in order to justify the real application in food systems.

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