Original paper

Antibacterial Effects of Monoglycerol Fatty Acid Esters and Sucrose Fatty Acid Esters on *Bacillus* spp.

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Fatty acid esters are food additives with strong antibacterial activity against spore-forming bacteria. The antibacterial activity of monoglycerol fatty acid esters (MGs) and sucrose fatty acid esters (SEs) with various fatty acid chain lengths was systematically investigated on four typical *Bacillus* species: *B. cereus*, *B. subtilis*, *B. megaterium*, and *B. coagulans*. Monoglycerol monolaurate (MG12) and monoglycerol monomyristate (MG14) showed relatively strong bactericidal effects on vegetative cells of these species among the MGs tested at both pH 6.0 and pH 8.0. Different SEs showed bactericidal effects on different species at pH 6.0, while the SEs had antibacterial effects only on *B. coagulans* at pH 8.0. SEs showed antibacterial effects on vegetative cells of these species, as was the case with MGs. In addition, it was found that MGs and SEs showed strong antibacterial effects on both *B. cereus* and *B. subtilis* in the logarithmic phase of growth, while the antibacterial activities of MGs persisted longer than those of SEs.

Keywords: monoglycerol fatty acid ester, sucrose fatty acid ester, antibacterial effect, bacterial spore, vegetative cell

Introduction

Some bacteria, including those of the *Bacillus* and *Clostridium* genera, form spores with high tolerance to various stresses, such as heat, antibacterial agents, and drying, that are lethal to vegetative cells. Because of the high heat tolerance, bacterial spores in foods may cause spoilage of food or food poisoning since these bacterial spores survive heat sterilization, and germinate and grow during distribution or storage of the contaminated food (Brown, 2000; Carlin, 2011; Granum, 1995). The control of spore-forming bacteria is crucially important in the food industry. Some fatty acid esters are used to control sporulating bacteria in various foods. For example, sucrose fatty acid ester is added to canned cafe au lait to control the growth of *B. coagulans* and *Geobacillus stearothermophilus*, which cause flat-sour spoilage of the drink (Conley and Kabar, 1973; Nakayama et al., 1977; Nakayama and Samo, 1980; Nakayama and Shinya, 1981; Nakayama et al., 1982; Piao et al., 2006; Shearer et al., 2000). Monoglycerol, diglycerol, and polyglycerol fatty acid esters also are reported to show antibacterial activity (Kato and Shibasaki, 1975; Miyamoto and Matsushita, 1988). These fatty acid esters show different antibacterial spectra depending on the backbone of their hydrophilic groups and the lengths of their fatty acids, but the reason for these differences in spectra remains unclear (Blaszyk and Holley, 1998; Kabara et al., 1972; Kato and Shibasaki, 1975; Koga and Watanabe 1968; Miyamoto and Matsushita, 1988; Nakayama et al., 2003), since there has been no single report directly comparing the antibacterial action of fatty acid esters with different structures on different bacterial species under the same...
In the present study, we systematically investigated the antibacterial activity of monoglycerol fatty acid esters (MGs) and sucrose fatty acid esters (SEs) with various fatty acid chain lengths when tested at pH 6.0 and pH 8.0 against four Bacillus species. Furthermore, to elucidate the mechanisms of the antibacterial action of these fatty acid esters, effects of fatty acid esters on spore germination and growth after germination were investigated in B. cereus and B. subtilis.

Materials and Methods

Strains and spore preparation  Bacillus cereus JCM2152 and B. subtilis JCM1465 were purchased from RIKEN BioResource Center, Tsukuba, Ibaraki, Japan. B. megaterium DSM 319 was purchased from DSMZ, Braunschweig, Germany. B. coagulans No. 1180 was purchased from the Japan Canners Association, Tokyo, Japan. (No. 1180 is the number for the culture collection of the Research Laboratory of the Japan Canners Association.)

The bacterial strains were cultured with shaking (130 rpm) in 100 mL BBL™ Trypticase™ Soy Broth (TSB) medium (Becton, Dickinson & Company, Franklin Lakes, NJ, USA) for 18 h at 30°C for B. cereus JCM2152, B. subtilis JCM1465, and B. megaterium DSM 319, and at 50°C for B. coagulans No. 1180. Aliquots of the cultures then were plated on Standard Method Agar medium (Nisui, Japan) and incubated at the respective temperature for 1 to 2 weeks to produce spores. When the ratio of free spores to whole cells was >80% (as determined by phase contrast microscopy), the colonies were harvested, suspended in cold sterilized water, and centrifuged at 4°C and 6000 × g for 15 min. The pellets were washed three times with cold sterilized water, resuspended in sterilized water, and heated at 80°C for 10 min to kill all of the vegetative cells. Spore suspensions with spore concentrations of 1.0×10⁷ cells/mL (counted by hemocytometer) were stored at 4°C and used for experiments within 2 weeks.

Preparation of fatty acid ester solution  Monoglycerol fatty acid esters with C8, C10, C12, C14, C16, and C18 saturated fatty acid chains were provided by Taiyo Kagaku Co., Ltd., Japan. These compounds are referred to as MG8, MG10, MG12, MG14, MG16, and MG18, respectively. Sucrose fatty acid esters with C8, C10, C12, C14, and C16 saturated fatty acid chains were provided by Mitsubishi-Kagaku Foods Co., Japan. Sucrose stearic acid (saturated C18) ester was provided by Daiichi Kogyo, Co., Ltd., Japan. These compounds are referred to as SE8, SE10, SE12, SE14, SE16, and SE18, respectively. Each fatty acid ester was dissolved in distilled water at a concentration of 1.0 mg/mL, autoclaved at 121°C for 15 min, and stored pending use in the following experiments.

Measurement of viable cell counts  To determine viable counts, samples (100 μL each) including bacterial cells were plated on BBL™ Trypticase™ Soy Agar (TSA) medium (Becton, Dickinson & Company) after dilution with phosphate-buffered saline (PBS) if needed. After the plates were incubated at their optimal growth temperature (described above) for 48 h, viable cell counts were calculated from the number of colonies formed. For measurement of viable cell counts of samples including MG or SE, samples were 10-fold serially diluted with diluent with lecithin & polysorbate 80 “DAIGO” (LP solution, Wako Pure Chemical Industries, Ltd., Japan) to avoid the effects of fatty acid esters on the damaged bacterial cells. One hundred μL of the dilution was plated. For measurement of spore counts, samples were heated at 80°C (in a hot water bath) for 10 min before plating. Spores were considered to have germinated when spore counts decreased over time without a corresponding decrease in the total viable counts.

Antibacterial activity test  The fatty acid ester solutions were diluted with 50% Luria Broth (LB) medium (Nihon Pharmaceutical Co., Ltd, Japan) to obtain 4 mL of two-fold dilution series with concentrations ranging from 2 ng/mL to 500 ng/mL. Spore suspensions (20 μL each) were inoculated to 50% LB broth with or without fatty acid esters, and then cultivated statically at their optimal growth temperature (described above; and also at 37°C for B. coagulans No. 1180) for 48 h. Viable cell counts were measured by plating at 0 and 48 h of cultivation. In the present paper, we defined a decrease in the viable cell count to <10% of the initial count as a “bactericidal effect”, and a change in the viable cell count to ≥10% to <1000% of the initial count as a “bacteriostatic effect”. The minimum concentrations at which a bactericidal effect and a bacteriostatic effect were observed were defined as the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC), respectively.

Effects of the timing of addition of fatty acid esters on growth after start of incubation of spores  The spore suspension of B. cereus JCM2152 or B. subtilis JCM1465 (20 μL) was inoculated into 2 mL of 50% LB broth in test tubes and mixed thoroughly. Viable cell and spore counts were measured after cultivation for 0, 1, 2, 3, 4, 5, 6, and 48 h. MG12 and SE16 were added to the culture of B. cereus JCM2152 to a final concentration of 16 ng/mL. MG12 or SE12 were added to the culture of B. subtilis JCM1465 to final concentrations of 16 ng/mL or 250 ng/mL, respectively. The fatty acid esters that showed the highest antibacterial activity against the test bacteria were used, and were added at MBC.

Electron microscopy  B. cereus JCM2152 spore suspension (100 μL) was dropped on an ISOPORÉ membrane filter (pore size 0.2 μm; Merck Millipore, Billerica, MA, USA), and spores were collected onto the filter under vacuum using a filtration bottle (NALGENE 115-mL Filter Unit, Thermo Fisher Scientific Inc., Waltham, MA, USA). Then, 100 μL of 50% LB broth was placed on the filter and incubated at 30°C for from 6 to 12 h to induce spore germination. Subsequently, the cells were washed continuously with 10 mM HEPES buffer (pH 7.0) at 4°C, preventing the cells from drying. The filter was then soaked in 10 mM HEPES buffer (pH 7.0) containing 2.5% glutaraldehyde and incubated at 4°C for 24 h. After sample fixation, cells on the
collection filter were dehydrated by continuous drop-wise addition of a graded ethanol series (50%, 70%, 80%, 90%, 95%, 100%, and 100%). Substitution was performed with the drop-wise addition of t-butyl alcohol to the filter at 30°C. Following substitution, the filters were removed from the filtration system and frozen at −20°C for 15 min. The frozen samples on filters were dried in a freeze dryer (ES-2030; Hitachi Ltd. Tokyo, Japan) at −20°C, and each filter was cut to a suitable size and affixed to the aluminum specimen holder of a scanning electron microscope (SEM; S-4300SE/N; Hitachi Ltd. Tokyo, Japan) using double-sided carbon tape for SEM observations.

The procedures for preparing samples for transmission electron microscopy (TEM) were the same up to the dehydration step. Aliquots of 20 μL dehydrated cell suspension were distributed into BEEM embedding capsules (Nissin EM Co., Japan) and the ethanol was substituted with propylene oxide (Nissin EM Co., Japan). Following propylene oxide substitution, 0.05 mL resin (Q-615; Nissin EM Co., Japan) was added and the samples were degassed in a sonicator (Branson 5200; Yamato Scientific Co., Japan) for 30 s. The samples then were infiltrated with resin in a desiccator under negative pressure at room temperature for 48 h. After resin substitution was complete, the BEEM capsules were heated in a block heater (Cool Thermo Unit CTU-N Neo; Tai-tec Co., Saitama, Japan) at 40°C for 24 h, and then at 70°C for 48 h to harden the resin. Ultra-thin sections (70–90 nm) were cut from the capsules with a diamond knife (Sumi Knife; Okenshoji Co., Tokyo, Japan) using a microtome (Ultracut-S; Leica Microsystems, Tokyo, Japan) and collected on copper mesh (Sheet Mesh 150-A; Nissin EM Co., Tokyo, Japan) for TEM examination. After drying, the mesh containing the collected sample was examined by TEM (H-7650; Hitachi Ltd. Tokyo, Japan).

Results

Effects of fatty acid esters on viability of Bacillus spp. The antibacterial effects of the MGs and SEs on the four Bacillus species are shown in Tables 1-5.

Against B. cereus JCM2152, MG12 (MBC: 16 ng/mL) showed the strongest effect among the MGs tested at both pH 6 and 8 (Table 1). At both pH 6 and 8, the esters with longer fatty acid chain length (MG 14, 16, and 18), showed no effects on viability of the bacterium. In the case of SE, the antibacterial activity differed between pH 6 and 8. SE16 showed the strongest bactericidal effects (16 ng/mL), but at pH 8.0, none of the SEs showed bactericidal effects.

B. subtilis JCM1465 also was most susceptible to MG12 (MBC: 16 ng/mL) among MGs tested at both pH 6 and 8 (Table 2). MG16 and 18 showed no effects on B. subtilis. Among SEs tested, only SE12 at pH 6 showed bactericidal effect on the bacterium at 250 ng/mL.

B. megaterium DSM 319 was resistant to the bactericidal action of MGs and SEs; MG14 and SE14 showed bactericidal effects at 500 and ≤32 ng/mL, respectively, at pH 6 (Table 3(a)). Since most of the fatty acid esters tested did not show bactericidality (except for MG14 and SE14 at pH 6.0), MICs were determined (Table 3(b)). MG12 and 14 showed the strongest bacteriostatic effect on B. megaterium at ≤32 ng/mL at pH 6 and MG12 at pH 8. Among SEs tested, SE14 showed the strongest bactericidal action at ≤32 ng/mL at pH 8. On the other hand, none of the SEs showed bacteriostatic effects at pH 8.0.

The MBCs of the fatty acids were determined at both 37 and 50°C against B. coagulans No. 1180. At 37°C, MG14 showed the strongest bactericidal effect at 4 ng/mL at both pH 6 and 8 (Table 4). In the case of SEs, SE16 showed the strongest effect at pH 6 among SEs tested; MBC was determined to be 16 ng/mL. At pH 8, SE12, 14, and 16 showed strong bactericidal action at 63 ng/mL. As shown in Table 5, at 50°C, MG12 and 14 showed the strongest bactericidal effect at 63 ng/mL at pH 6. In contrast, only MG14 at pH 8 showed bactericidal effect at 250 ng/mL. Among SEs tested, SE16 showed the strongest effect at pH 6; MBC was determined to be 16 ng/mL. Among SEs tested at pH 8, SE18 showed the

Table 1. The MBC of the MGs and SEs on B. cereus JCM2152 spores

<table>
<thead>
<tr>
<th>Ester</th>
<th>pH</th>
<th>C8</th>
<th>C10</th>
<th>C12</th>
<th>C14</th>
<th>C16</th>
<th>C18</th>
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<td>16</td>
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<tr>
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<td>-</td>
<td>500</td>
<td>32</td>
<td>16</td>
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</table>

, MBC is over 500 ng/mL; MG, Monogrycerol ester; SE, Sucrose ester

Table 2. The MBC of the MGs and SEs on B. subtilis JCM1465 spores

<table>
<thead>
<tr>
<th>Ester</th>
<th>pH</th>
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<th>C10</th>
<th>C12</th>
<th>C14</th>
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</tbody>
</table>

, MBC is over 500 ng/mL; MG, Monogrycerol ester; SE, Sucrose ester

Table 3. The MBC (a) and MIC (b) of the MGs and SEs on B. megaterium DSM 319 spores

(a)

<table>
<thead>
<tr>
<th>Ester</th>
<th>pH</th>
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<th>C10</th>
<th>C12</th>
<th>C14</th>
<th>C16</th>
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</thead>
<tbody>
<tr>
<td>MG</td>
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<td>500</td>
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<tr>
<td>SE</td>
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<td>≤32</td>
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</table>

, MBC is over 500 ng/mL; MG, Monogrycerol ester; SE, Sucrose ester

(b)

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<th>C10</th>
<th>C12</th>
<th>C14</th>
<th>C16</th>
<th>C18</th>
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<tr>
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<td>250</td>
<td>63</td>
<td>≤32</td>
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<td>8</td>
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<td>63</td>
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, MIC is over 500 ng/mL; MG, Monogrycerol ester; SE, Sucrose ester
strongest bactericidal effect at 63 ng/mL. None of the SEs had bactericidal effects at pH 6.0.

Throughout these experiments, MG12 and MG14 exhibited stronger antibacterial effects on the four *Bacillus* strains than did the other MGs examined. On the other hand, the bactericidal effects on each strain differed between SEs with different fatty acid chains. SEs exhibited greater bactericidal effects on *B. coagulans* No. 1180 than on the other strains, especially at 37°C.

Washing with LP solution did not change the viable cell count in almost all the experiments (data not shown).

**Fig. 1. Effects of MG12 on growth after start of incubation of spores of *B. cereus* and *B. subtilis***

Spores were incubated in 50% LB broth (●) and MG12 was added at 0(■), 1(●), 2(▲), 3(▲), 4(▲), 5(■), and 6(■) h after the start of incubation. Total viable (closed symbols) and spore (open symbols) counts were determined before and immediately after the addition, and after incubation for 48 h. Total viable count is the sum of counts of vegetative cells and spores. MG12 was added to the cultures of *B. cereus* JCM2152 (A) and *B. subtilis* JCM1465 (B) at a final concentration of 16 ng/mL.

**Fig. 2. Effects of SE on growth after start of incubation of spores of *B. cereus* and *B. subtilis***

Spores were incubated in 50% LB broth (●) and SE was added at 0(■), 1(●), 2(▲), 3(▲), 4(▲), 5(■), and 6(■) h after the start of incubation. Total viable (closed symbols) and spore (open symbols) counts were determined before and immediately after the addition, and after incubation for 48 h. Total viable count is the sum of counts of vegetative cells and spores. SEs used for *B. cereus* JCM2152 (A) and *B. subtilis* JCM1465 (B) were 16 ng/mL SE16 and 250 ng/mL SE12, respectively.
not decrease (Fig. 1 and Fig. 2). The spore counts further decreased until 3 to 5 h of incubation without increase in the total viable counts, indicating germination of *B. cereus* JCM2152 and *B. subtilis* JCM1465 spores continued until 3 to 5 h of incubation in the broth. Thereafter, the total viable counts increased gradually for both bacteria, indicating growth of vegetative cells.

In the case of *B. cereus* cultured in 50% LB broth supplemented with MG12 at 16 ng/mL (Fig. 1(A)), total viable counts decreased at 48 h when MG12 was added at 0, 1, 2, or 3 h. However, the total viable counts did not decrease to lower than one-tenth of the initial counts at 48 h, when added at 4, 5, or 6 h of incubation. On the other hand, the spore counts showed a large decrease immediately after start of incubation, and the counts did not increase in the presence of MG12. Both total viable and spore counts did not change immediately after the addition of MG12, regardless of the timing of addition of MG12.

Both total viable and spore counts of *B. subtilis* showed large decreases (to less than 100 CFU/mL at 48 h), regardless of the timing of addition of 16 ng/mL MG12 (Fig. 1 (B)). Although total viable counts decreased immediately after the addition of MG12 at 2, 3, 4, 5, or 6 h of incubation, the spore count did not change.

As shown in Fig. 2 (A), total viable counts of *B. cereus* at 48 h decreased when SE16 was added at 16 ng/mL and 0 h of incubation. On the other hand, the total viable counts were essentially the same as those of a control at 48 h when SE16 was added at 1, 2, 3, 4, 5, or 6 h of incubation. The spore counts showed large decreases at 48 h when added at 0, 1, 2, or 3 h of incubation, but not when added at 4, 5, or 6 h. Although total viable counts decreased immediately after the addition of SE16 at 4, 5, or 6 h of incubation, the spore count did not change.

Spore counts of *B. subtilis* at 48 h showed large decreases (to about 100 CFU/mL) regardless of the timing of addition of SE12 at 250 ng/mL (Fig. 2(B)). In contrast, total viable counts did not decrease immediately after addition of SE12 at 0, 1, or 2 h of incubation and increased to the same level as that of the control at 48 h. Although total viable counts decreased immediately after addition of SE12 at 3 or 4 h of incubation, the viable counts increased at 48 h. When added at 5 or 6 h, total viable counts decreased immediately after the addition; the counts in these cultures either did not increase (5 h) or decreased (6 h) after incubation for 48 h.

**Electron micrographs of *B. cereus* spores after incubation for 6 or 12 h** Electron micrographs of *B. cereus* JCM2152 spores after incubation of for 6 or 12 h are shown in Fig. 3(A)(B) and Fig. 3(C)(D), respectively. The thickness of the cell wall was estimated to be 25 – 30 nm for the spores at 6 h of incubation. The thickness was half of that of vegetative cells (50 – 70 nm) after incubation for 12 h. The surface of the cells at 6 h showed a smooth structure, whereas that of the cells at 12 h showed a scale-like and rough structure compared to that observed at 6 h.

**Discussion**

Here we investigated the antibacterial effects of various MGs and SEs on bacterial spores just after the start of incubation. The spore counts of *B. cereus* JCM2152 and *B. subtilis* JCM1465 in 50% LB broth just after addition of MG and SE at the concentration with an antibacterial activity decreased with increase in the incubation time, as did those of the control (Fig. 1 and Fig. 2), indicating that these fatty acid esters did not inhibit spore germination. These results are consistent with previous reports (Buňková et al., 2011; Kuwana et al., 2011).

TEM revealed that the cell wall of cells at 6 h after the start of incubation was thinner than that at 12 h. In the case of *B. subtilis*, when MG or SE was added at 3 to 6 h after the start of incubation, the viable cell count decreased immediately after the addition (Fig. 1(B) and Fig. 2(B)). This phenomenon indicated that the fatty acid esters act strongly on cells with thin cell wall just after germination.

On the other hand, the same phenomenon was observed on *B. cereus* with SE (Fig. 2(A)) but not with MG (Fig. 1(A)). Although the antibacterial action of MG12 was not exhibited on *B. cereus* immediately after the addition, the total viable counts decreased after incubation for 48 h. This result suggests a difference in the affinity of the fatty acid esters for vegetative cells. Compared with SE, MG appeared to take a longer time to bind stably to vegetative cells of *B. cereus*.

Thus, the antibacterial action of MG was not exhibited immediately on germinating *B. cereus*. In contrast, MG caused large decreases in the *B. subtilis* total viable counts at 48 h when MG was added at 0 to 3 h of incubation. On the other hand,
although SE decreased viability of both *B. cereus* and *B. subtilis* immediately after the addition at 3 to 6 h from the start of incubation of the spores, the total viable counts largely decreased at 48 h only when SE was added at the start of incubation. SE seemed to be inactivated by some enzyme(s) produced by the bacteria; we postulated that this activity is an esterase, given that *Bacillus* spp. exhibit high esterase activity (Sugimoto, et al., 1998). In contrast, it seems that MGs were stable and not inactivated by an activity produced by the bacteria. Although further detailed investigation is required, it seems likely that SE reduced total viable count immediately after induction of germination only when SE was present prior to the induction of spore germination; antibacterial activity was not seen when SE was added after the initiation of germination. Assessment of the esterase activities of various strains on MGs and SEs would help to clarify the lack of persistence of SE’s antibacterial effect.

Most microorganisms have some enzymes for using fatty acids as an energy source, and obtain energy by degrading fatty acids into water and carbon gas (Sugimoto, et al., 1998). When excess amounts of fatty acids are present around microorganism cells, the cells cannot metabolize all of the fatty acids, and intermediate metabolites accumulate inside the cells. This accumulation inhibits the activities of enzymes and growth of the microorganism. This process is reported to be one of the mechanisms underlying the antibacterial effects of fatty acids and their esters (Kato and Shibasaki, 1975). The uptake speed of fatty acids partly relies on their affinity with the lipid bilayer membrane of the microorganism. Among monoglycerol short-chain fatty acid esters, the longer the fatty acid chain, the higher the hydrophobicity of the fatty acid ester. These hydrophobic fatty acid esters that have high affinity for the lipid bilayer membranes of microorganisms are well incorporated by microorganisms, and show strong antibacterial effects (Buňková et al. 2011). Fatty acid esters also may adsorb to the bacterial surface, where the esters induce damage and the leakage of intracellular substances (Kuwana et al., 2011). These facts indicate that interactions with the bacterial surface are crucial for the antibacterial effects of the fatty acid esters. Among food additives, non-ionic fatty acid esters, including MGs and SEs, have antibacterial effects at lower concentrations than cationic or anionic fatty acid esters. In this study, MG12 and MG14 showed the strongest effect (among the examined MGs) on the four *Bacillus* strains at both pH 6.0 and pH 8.0 (Tables 1 to 5). In addition, whereas MGs had bactericidal effects on *B. cereus JCM2152*, *B. subtilis JCM1465*, and *B. coagulans* No. 1180, MGs exhibited bacteriostatic effects on *B. megaterium DSM 319*. This distinction may reflect the high affinity of MG12 and MG14 for the surfaces of the four strains, which therefore show high adsorption of MG12 and MG14. On the other hand, different SEs showed bactericidal effects on different species. We presume that this pattern is due to the chemical characteristics of non-ionic fatty acid esters (e.g., lower critical micelle concentration than cationic or anionic fatty acid esters), thereby facilitating their absorption to bacterial surfaces.

Interestingly, SEs exhibited weak antibacterial activity against mesophiles (*B. cereus JCM2152*, *B. subtilis JCM1465*, and *B. megaterium DSM 319*), but strong antibacterial activity against *B. coagulans* No. 1180 at 37°C. Antibacterial effects of SEs on *B. coagulans* No. 1180 at 50°C were weaker than those at 37°C. Further studies to investigate the antibacterial mechanisms of fatty acid esters by their interaction with the bacterial membranes are planned. Proposed studies would include measuring the composition of bacterial lipid bilayers, and determining the critical micelle concentrations and hydrophilic lipophilic balance values of fatty acid esters. A deeper understanding of the antibacterial mechanism of fatty acid esters, and of the interaction between the esters and food ingredients (which interfere with the antibacterial action of the esters) will facilitate effective application of MGs and SEs as safe food additives in foods and beverages.

Acknowledgments We thank members of Research and Development Center of MITSUBISHI-KAGAKU FOODS CO. for preparing and providing sucrose fatty acid esters with C8, C10, C12, C14, and C16 saturated fatty acid chains.

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Antibacterial Effects of MG and SE on Bacillus spp.


