Olive oil polyphenol extract inhibits vegetative cells of *Bacillus cereus* isolated from raw milk

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ABSTRACT

This study was conducted to analyze the antibacterial effect of olive oil polyphenol extract (OOPE) against vegetative cells of *Bacillus cereus* isolated from raw milk and reveal the possible antibacterial mechanism. The diameter of inhibition zone, minimum inhibitory concentration, minimum bactericidal concentration, and survival counts of bacterial cells in sterile normal saline and pasteurized milk were used to evaluate the antibacterial activity of OOPE against *B. cereus* vegetative cells. The changes in intracellular ATP concentration, cell membrane potential, content of bacterial protein, and cell morphology were analyzed to reveal possible mechanisms of action. Our results showed the diameter of inhibition zone, minimum inhibitory concentration, and minimum bactericidal concentration of OOPE against *B. cereus* vegetative cells were 18.44 ± 0.55 mm, 0.625 mg/mL, and 1.25 mg/mL, respectively. *Bacillus cereus* GF-1 vegetative cells were decreased to undetectable levels from about 8 log cfu/mL after treatments with 0.625 mg/mL of OOPE in normal saline at 30°C for 3 h and in pasteurized milk at 30°C for 10 h. The antibacterial mechanisms of OOPE against *B. cereus* GF-1 vegetative cells may be due to the reduction of intracellular ATP concentrations, cell membrane depolarization, decrease of bacterial protein content, and leakage from cytoplasm. These findings illustrated that OOPE could be used to prevent the growth of contaminating *B. cereus* cells in dairy products.

Key words: *Bacillus cereus* vegetative cells, olive oil polyphenol extract, raw milk, antibacterial effect

INTRODUCTION

Bacillus cereus, a gram-positive endospore-forming bacteria, is considered one of the important spoil-

age microorganisms in the dairy industry and can contaminate dairy products through the production environment (Kumari and Sarkar, 2016). This organism secretes lipase and protease, which cause loss of nutrients from dairy products and shorten shelf life (Nemeckova et al., 2009). Moreover, as a result of the production of enterotoxins and emetic toxin, *B. cereus* can cause emesis and diarrhea severe enough to result in hospitalization or even death (Schoeni and Wong, 2005). Several measures have been taken in the dairy industry to reduce product contamination by *B. cereus*, but cases of *B. cereus* contamination are still reported frequently (Gundogan and Avci, 2014; Alnakip, 2016; Spanu et al., 2016; Fossi et al., 2017).

Currently, thermal sterilization technology and synthetic preservatives are the most common methods to inhibit the growth of *B. cereus* strains in dairy products (Rendueles et al., 2011; Kumari and Sarkar, 2016). However, heat treatment can have many negative effects on the quality of milk and dairy products, including undesirable protein denaturation, nonenzymatic browning, loss of vitamins and volatile flavor compounds, freezing point depression, and flavor changes (Coutinho et al., 2018). Moreover, considering serious health risks, such as cancer, allergies, migraines, liver and kidney damage, birth defects, and brain damage, consumers are understandably wary about synthetic preservatives added to their food (Parke and Lewis, 1992; Demirci et al., 2008). Therefore, recent studies have focused on natural extracts as potential preservatives to inhibit microbial growth (Lacombe et al., 2010; Li et al., 2016; Cetin-Karaca and Newman, 2018). Lacombe et al. (2010) found that the sugars, organic acids, phenolics, and anthocyanins extracted from American cranberry have strong antimicrobial activity against *Escherichia coli* O157:H7. Li et al. (2016) reported that *Cronobacter sakazakii* in powdered infant formula can be reduced by tea polyphenols acidified with 4 mol/mL of hydrochloric acid (pH = 3.55), from 7 log cfu/mL to an undetectable level within 7 h. Cetin-Karaca and Newman (2018) showed that phytochemicals, including *trans*-cinnamaldehyde, (–)-epigallocatechin gallate,

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and [10]-gingerol, can effectively inhibit the growth of *B. cereus* in reconstituted infant rice.

Olive oil polyphenol extract (OOPE), composed of polyphenols, hydroxytyrosol, tyrosol, and phenolic acids, has attracted much attention on account of its antioxidant properties, which are considered to be the main reason that natural extracts can inhibit the growth of pathogenic microorganisms (Visioli et al., 1998; Gordon et al., 2001; O'Dowd et al., 2004; Gupta et al., 2012). As an example, tea polyphenols or tea extracts with antioxidant capacity have been confirmed to have significant antimicrobial activity against *Staphylococcus aureus*, *Salmonella* Enteritidis, *Serratia marcescens*, *B. cereus*, and *C. sakazakii* (Friedman et al., 2006; Lacombe et al., 2010; Yi et al., 2014; Zhang et al., 2014). Therefore, enough evidence exists to show OOPE as a potential preservative to inactivate the growth of *B. cereus* (Fei et al., 2018; Yakhlef et al., 2018). However, few reports have been published on the antimicrobial activity and mechanism of action of OOPE against *B. cereus* strains.

The aim of our study was to analyze the antimicrobial activity of OOPE against vegetative cells of *B. cereus* strains isolated from raw milk and clarify the possible mechanism of action. The diameter of inhibition zone (DIZ), MIC, and minimum bactericidal concentration (MBC) were determined to evaluate the antibacterial effect of OOPE against *B. cereus* cells. The reduction of viable *B. cereus* cell counts after treatment with OOPE in sterile normal saline (NS) and pasteurized milk (PM) were measured to assess the antibacterial ability in the medium with and without milk. The changes in intracellular ATP concentration, membrane integrity, cell morphology, and cell membrane potential were determined to explore a possible mechanism of action.

MATERIALS AND METHODS

OOPE Material

The OOPE used in our study was provided by Shanghai Kai Da Biotechnology Co. Ltd. (Shanghai, China). The polyphenols were extracted from olive oil using a supercritical fluid extraction, and dried into powder with a vacuum freeze-drying method. The final moisture of the OOPE was less than 7%. Detailed chemical composition of the compound is shown in Table 1.

Bacteria and Culture Conditions

Ten *B. cereus* strains were used in our study. One of these strains, *B. cereus* ATCC 14579, was obtained from American Type Culture Collection (ATCC, Manassas,

VA). The other 9 were isolated from different raw milk samples, identified using 16S rDNA sequencing by our team, and named GF-1, GF-2, GF-3, GF-4, GF-5, GF-6, GF-7, GF-8, and GF-9, respectively. One hundred microliters of cultures were transferred to 10 mL of brain heart infusion (BHI) broth, and incubated at 30°C for 24 h. The cultures were streaked onto mannitol egg yolk polymyxin (MEYP) agar plates, adding mycosin B and yolk emulsion, and incubated at 30°C for 20 h. A typical colony was selected and incubated in BHI broth at 30°C for 24 h to obtain the pure culture of *B. cereus* strains.

Disk Diffusion Assay

The antimicrobial effect of OOPE against *B. cereus* cells was evaluated using the disk diffusion method with minor modification (Barry and Barry, 1976). One hundred microliters of bacterial suspension (10^6 cfu/mL) was spread evenly on the BHI agar. Fifteen microliters of OOPE (10 mg/mL) was added to filter paper disks (6 mm diameter), placed on the BHI agar inoculated with *B. cereus* strains, and incubated at 30°C for 24 h. Each treatment was performed with 3 replications. An equal part of the sterilized distilled water was used as a negative control. The DIZ was measured to evaluate the antibacterial effect of OOPE.

Determinations of MIC and MBC

The MIC values of OOPE for 10 *B. cereus* strains were determined using the agar dilution method as described by the European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Diseases (2000). The OOPE was mixed with warm (about 50°C) MEYP agar in the sterile 24-well plates. The final concentrations of OOPE were adjusted to 0, 0.3125, 0.625, 1.25, 2.5, and 5 mg/mL, respectively, the mixture in wells was mixed by gentle blowing, and 0.1 mg/mL of ampicillin was used as positive control. Two microliters of *B. cereus* culture (10^6 cfu/mL) was inoculated on the solidified MEYP agar. After the cultures were dried,

Table 1. Chemical composition of olive oil polyphenol extract (OOPE) used in this study

Composition	Content (% wt/wt)
Total polyphenols	≥30
Hydroxytyrosol	≥6
Tyrosol	≥0.8
Phenolic acids	≥1.5
Residual solvent content	
Ethanol	<0.1

the 24-well plates were incubated at 30°C for 24 h. The MIC of OOPE against *B. cereus* is defined as the lowest concentration at which test organisms cannot grow on MEYP agar. After the MIC of OOPE against each strain was determined, 100 μ L of culture was treated with ≥ 1 MIC of OOPE and spread on the MEYP plates, followed by incubation at 30°C for 24 h. The lowest concentration of OOPE that causes the absence of colonies was identified as the MBC of OOPE against *B. cereus* vegetative cells.

Reduction of *B. cereus* GF-1 by OOPE in NS and PM

Two milliliters of *B. cereus* GF-1 culture was centrifuged at $8,000 \times g$ for 10 min at 4°C to obtain the working cell pellets and stored at 4°C for further study. The pellets were transferred in 1 mL of sterile NS and colonies were enumerated on MEYP plates after gradient dilution. The density of the working culture was then adjusted to 10^8 cfu/mL with sterile NS and 10^6 cfu/mL with PM. The OOPE was added to the working cultures to obtain the final concentrations of 0, 0.5, 1, 1.5, and 2 MIC. Samples treated with OOPE in NS were incubated at 30°C for 0.5, 1, 3, and 5 h. Meanwhile, samples treated with OOPE in PM were incubated at 30°C for 2, 4, 6, 8, and 10 h. One hundred microliters of each sample after different treatments was spread on MEYP plates in triplicate to calculate survival populations of *B. cereus* GF-1 cells.

Measurement of Intracellular ATP Concentrations

The intracellular ATP concentrations of *B. cereus* GF-1 cells with different treatments were measured according to the method described by Sánchez et al. (2010) and Shi et al. (2016c). The OOPE was added to 2 mL of bacterial suspension (10^7 cfu/mL) to achieve final concentrations of 0, 1, and 2 MIC. The mixtures were incubated at 30°C for 30 min followed by disintegration using ultrasonic treatment, then kept at 100°C for 2 to 3 min to inactivate the ATPase. To obtain the supernatants, the samples were centrifuged at $5,000 \times g$ for 5 min at 0°C using a high-speed freezing centrifuge (Shanghai Centrifuge Institute Co. Ltd., Shanghai, China). Finally, the supernatants were added to the white 96-well microtiter plates to determine the intracellular ATP concentrations of samples using an ATP assay kit (Beyotime Bioengineering Institute, Shanghai, China).

Determination of Membrane Potential

The effect of OOPE on membrane potential of *B. cereus* GF-1 cells was analyzed as described by previous reports (Sánchez et al., 2010; Shi et al., 2016c).

One hundred microliters of bacterial suspension (10^7 cfu/mL) was added to black 96-well microtiter plates and incubated at 30°C for 30 min. The OOPE was mixed in the bacterial suspension to obtain the final concentrations of 0, 1, and 2 MIC, followed by incubation at 30°C for 30 min. Bis-(1, 3-dibutylbarbituric acid) trimethine oxonol [DiBAC4 (3), Beijing Solarbio Sciences and Technology Co. Ltd., Beijing, China] was used as membrane potential-sensitive fluorescent probe. Fluorescence was measured at excitation and emission wavelengths of 492 and 515 nm, respectively, according to the manufacturer's instructions (Fei et al., 2018).

SDS-PAGE

Bacterial proteins of *B. cereus* GF-1 cells were analyzed using SDS-PAGE according to Bradford (1976). Approximately 10^7 cfu/mL of *B. cereus* GF-1 cells were treated with 1 MIC of OOPE at 30°C for 3, 6, 9, and 12 h. The supernatants were prepared by centrifugation at $8,000 \times g$ at 4°C for 10 min and mixed with SDS-PAGE loading buffer. The samples were heated at 95°C for 10 min and analyzed by SDS-PAGE using a 5% stacking gel and a 15% separating gel. The protein bands were stained with Coomassie brilliant blue R-250 (Beijing Solarbio Sciences and Technology Co. Ltd.) in the gel, and scanned with an HP scanner (HP 1000, Hewlett-Packard Co. Ltd., Beijing, China). As a negative control, untreated *B. cereus* cells were analyzed with the same processing.

Transmission Electron Microscopy Analysis

According to previous studies, transmission electron microscope was used to observe the cell morphology of *B. cereus* GF-1 cells after treatments with OOPE at 0, 1, and 2 MIC for 4 h (Karlsson et al., 2007; Li et al., 2016). The cultures were centrifuged at $3,000 \times g$ for 5 min at 4°C to prepare the working cells. The cells were fixed using 0.1 M sodium phosphate buffer (2.5% glutaraldehyde) at 4°C for 12 h, and washed 3 times with NS. They were further fixed in 1% osmium tetroxide at 4°C for 2 h followed by washing 3 times with NS. The cells were dehydrated with a graded series of ethanol (50, 70, 90, and 100%) for 10 min, and embedded in Epon Lx-112 (Ladd Research, Williston, VT), followed by double staining with uranyl acetate and lead citrate. Finally, all samples were observed under the transmission electron microscope (Hitachi, Tokyo, Japan).

Statistical Analysis

Five experiments, including disk diffusion assay, determinations of MIC and MBC, reduction of *B. ce-*

reus GF-1 by OOPE in NS and PM, measurement of intracellular ATP concentrations, and determinations of membrane potential, were carried out in triplicate. Statistical analyses were performed by ANOVA using the SPSS 20.0 software (IBM Corp., Armonk, NY). The data were expressed as mean values $\pm$ standard deviations. Significant differences ($P < 0.05$) between treatments were determined using Tukey's multiple range test.

RESULTS

Antimicrobial Effects of OOPE on *B. cereus* Strains

The OOPE showed satisfactory antimicrobial effects against vegetative cells of 10 strains, as shown in Table 2. The mean DIZ of OOPE against the 10 strains was 18.44 ± 0.55 mm. No significant differences ($P > 0.05$) in DIZ values of OOPE against *B. cereus* strains were found. The MIC and MBC of OOPE against the 10 strains were 0.625 and 1.25 mg/mL, respectively. We found no significant difference in the antibacterial effect of OOPE against 10 *B. cereus* isolates. Considering that it is of more practical significance to determine the antibacterial mechanism of OOPE using *B. cereus* strains isolated from raw milk, *B. cereus* GF-1 was randomly chosen for future studies.

Reduction of *B. cereus* GF-1 by OOPE

The antimicrobial effects of OOPE against *B. cereus* GF-1 cells in NS and PM were evaluated and are shown in Figure 1. Compared with the control group, the growth of *B. cereus* GF-1 cells in NS and PM was significantly inhibited by treatments with 1 and 2 MIC of OOPE ($P < 0.05$). Furthermore, the concentration of

B. cereus cells was reduced to 0 from 8 and 6 log cfu/mL, respectively, with 2 MIC (1.25 mg/mL) of OOPE in NS after 0.5 h or in PM after 6 h.

Changes in Intracellular ATP Concentrations

Figure 2 shows that, compared with those of control group, the intracellular ATP concentrations of *B. cereus* GF-1 cells treated with OOPE at 1 and 2 MIC for 0.5 h were significantly reduced ($P < 0.05$). In addition, with the increase of OOPE concentrations, the concentrations of intracellular ATP of tested cells were significantly decreased ($P < 0.05$).

Changes in Membrane Potential

As shown in Figure 3, the fluorescence of *B. cereus* GF-1 cells treated with OOPE was compared with that of untreated cells. The result indicated that, compared with control group, a significant increase in fluorescence occurred in the cells treated with 1 and 2 MIC of OOPE ($P < 0.05$), which suggested that OOPE led to the cell membrane depolarization of *B. cereus* GF-1 cells.

SDS-PAGE Analysis

The bacterial protein of *B. cereus* GF-1 cells treated with 1 MIC of OOPE was compared with that of untreated cells using SDS-PAGE analysis (Figure 4). The result showed that protein bands of *B. cereus* GF-1 cells treated with 1 MIC of OOPE were fainter compared with control protein bands. Furthermore, after a treatment with 1 MIC of OOPE for more than 6 h, almost all protein bands disappeared.

Table 2. Antimicrobial activity of olive oil polyphenol extract (OOPE) against all 10 *Bacillus cereus* strains

Strain	Source	Diameter of inhibition zone (mm)	MIC (mg/mL)	Minimum bactericidal concentration (mg/mL)
ATCC 14579	American Type Culture Collection (Manassas, VA)	18.34 ± 0.55^a	0.625	1.25
GF-1	Raw milk	18.26 ± 0.48^a	0.625	1.25
GF-2	Raw milk	18.54 ± 0.57^a	0.625	1.25
GF-3	Raw milk	18.41 ± 0.45^a	0.625	1.25
GF-4	Raw milk	18.28 ± 0.47^a	0.625	1.25
GF-5	Raw milk	18.32 ± 0.40^a	0.625	1.25
GF-6	Raw milk	18.29 ± 0.65^a	0.625	1.25
GF-7	Raw milk	18.56 ± 0.53^a	0.625	1.25
GF-8	Raw milk	18.43 ± 0.42^a	0.625	1.25
GF-9	Raw milk	18.50 ± 0.47^a	0.625	1.25

^aColumn values with the same letter are not significantly different ($P < 0.05$).

Transmission Electron Microscope Observation

As compared with the regular morphology of *B. cereus* GF-1 cells (Figure 5A), the cells treated with

OOPE displayed serious deformation and cytoplasmic leakage (Figure 5B and C). Furthermore, the degree of cell deformation and cytoplasmic leakage increased as the concentration of OOPE increased.

Figure 1. Reduction of *Bacillus cereus* GF-1 vegetative cells after treatment with different concentrations of olive oil polyphenols extract (OOPE) in normal saline (A) and pasteurized milk (B). Error bars denote SD. Different letters (a-e) denote significant differences between treatments within the same incubation time points ($P < 0.05$).

Figure 2. Change in intracellular ATP concentrations of *Bacillus cereus* GF-1 vegetative cells after treatments with 0, 1, and 2 MIC of olive oil polyphenol extract (OOPE). Values represent the means of independent triplicate measurements. Bars represent the SD ($n = 3$). Different letters (a–c) denote significant differences ($P < 0.05$).

DISCUSSION

Many studies have focused on the antimicrobial effects of natural extracts on food-borne pathogens, and some natural ingredients have been reported to inhibit the growth of *B. cereus* strains (Ultee et al., 2002; Valero and Salmeron, 2003; Ponce et al., 2008; Cetin-Karaca and Newman, 2018). Valero and Salmeron (2003) showed that the growth of *B. cereus* in refrigerated processed food products was inhibited for

Figure 3. Change in membrane potentials (measured as relative fluorescence units) of *Bacillus cereus* GF-1 vegetative cells following treatments with 0, 1, and 2 MIC of olive oil polyphenol extract (OOPE). Values represent the means of independent triplicate measurements. Bars represent the SD ($n = 3$). Different letters (a,b) denote significant differences ($P < 0.05$).

at least 60 d by adding 5 $\mu\text{L}/100\text{ mL}$ of cinnamon essential oil at $\leq 8^\circ\text{C}$. Cetin-Karaca and Newman (2018) reported that 0.83 log cfu/g *B. cereus* cells and 2.0 log cfu/g of *B. cereus* spores in reconstituted infant rice cereal decreased after a treatment with 0.05% of *trans*-cinnamaldehyde at 37°C for 24 h. In addition, tea extracts and phenolic hydroxyl groups of carvacrol were also used to inhibit growth of *B. cereus* (Ultee et al., 2002; Friedman et al., 2006).

Bacillus cereus has been reported to be one of the most common pathogenic microorganisms contaminating the dairy processing environment (Brandl et al., 2014). Once dairy products are contaminated with *B. cereus*, it can cause serious food-borne disease outbreaks and accelerate the spoilage of dairy products (Bedi et al., 2005; Chitov et al., 2008; Kumari and Sarkar, 2016). In addition, this pathogen secretes protease and lipase, which can cause nutritional loss and a decrease in sensory properties of dairy products (Kumari and Sarkar, 2016). Therefore, it is necessary to analyze the antibacterial effect of OOPE against *B. cereus* in dairy products. Our findings indicated that approximately 6 log cfu/mL of *B. cereus* cells in PM were reduced to 0 log cfu/mL after treatment with OOPE at 30°C for 6 h. This indicates that OOPE is a viable natural antibacterial substance for treating dairy products. A similar study (Li et al., 2016) evaluated the antimicrobial activity of acidified tea polyphenols against *C. sakazakii* in rehydrated powdered infant formula. In comparison with tea polyphenols, OOPE consists of more active substances, including phenolic acids, hydroxytyrosol, and tyrosol, which have been confirmed as the major components of OOPE with antibacterial effects (Gordon et al., 2001; O'Dowd et al., 2004; Cruz-Romero et al., 2013; Zhu et al., 2013).

In previous studies, the intracellular ATP concentration was analyzed as a primary consideration, because ATP provides energy for normal physiological activities in microorganisms (Shi et al., 2016a,b,c). In the current study, we found that intracellular ATP concentration of *B. cereus* GF-1 decreased significantly after treatment with OOPE as compared with control cells. The decrease of intracellular ATP concentration has been associated with leakage of intracellular ATP, depletion of the intracellular ATP pool, and dissipation of proton motive force components (Gill and Holley, 2004). Therefore, in conjunction with transmission electron microscopy results (Figure 5), our results suggest the reduction of *B. cereus* GF-1 cells might be related to the loss of ATP during destruction of cell membrane integrity, especially considering that similar results were found in the action of inhibiting *C. sakazakii* by lipoic acid, citral, and syringic acid, respectively (Shi et al., 2016a,b,c).

Figure 4. Sodium dodecyl sulfate-PAGE analysis of proteins of *Bacillus cereus* GF-1 vegetative cells treated with 1 MIC of olive oil polyphenol extract (OOPE). Lane M = marker; lanes 1, 3, 5, 7 = control for 3, 6, 9, and 12 h respectively; lanes 2, 4, 6, 8 = treated samples for 3, 6, 9, and 12 h, respectively.

Change of membrane potential is considered a factor in the natural extract antibacterial process due to its important role in cell antibiotic uptake and bactericidal action (Bot and Prodan, 2009). Our finding showed that OOPE caused membrane depolarization of *B. cereus* GF-1 cells, which is an important type of membrane damage associated with a less negative charge inside the cell (Gries et al., 2013). Similarly, *Vibrio cholerae* cells, after exposure to nopal cactus methanolic extracts, and *C. sakazakii* cells treated with liponic acid also resulted

in cell membrane depolarization (Sánchez et al., 2010; Shi et al., 2016b).

To further reveal the mechanism of action of OOPE against *B. cereus* cell, SDS-PAGE was used to analyze the change in protein levels in supernatants of tested strains after treatment with OOPE. Bacterial protein, as the basic OM of cells, is closely related to bacterial life activities and important physiological functions (Wang et al., 2015). Our findings showed that a significant reduction of protein was found in super-

Figure 5. Transmission electron microscopy images of *Bacillus cereus* GF-1 vegetative cells (40,000 $\times$) untreated for 4 h (A), treated with 1 MIC of olive oil polyphenol extract (OOPE) for 4 h (B), or treated with 2 MIC of OOPE for 4 h (C).

nant of *B. cereus* GF-1 cells, and all protein bands disappeared after treatment with OOPE for 6 h. These results suggest that the proteins leaked out of the cells due to loss of membrane integrity, as shown by the micrographs (Figure 5). This is similar to the results of sugarcane bagasse extract against *Staph. aureus*, sugar beet molasses polyphenols against *Staph. aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella Typhimurium* (Zhao et al., 2015; Chen et al., 2017).

Cellular morphology of bacteria under electron microscopy can reflect the state of bacteria under the stimulation of natural products (Joshi et al., 2014; Xu et al., 2017). Joshi et al. (2014) found that, after treatments with blueberry proanthocyanidin and commercial blueberry juice, pores and bubbles were observed on the cell surface of *C. sakazakii* strains under a scanning electron microscope. The study reported by Xu et al. (2017) showed that compared with untreated cells, the *Staph. aureus* cells treated with punicalagin became larger and had a rough surface. The antibacterial process of tea polyphenols caused serious cell deformation and cytoplasmic leakage in *C. sakazakii*, and American cranberry extract had a similar effect against *E. coli* O157:H7 (Lacombe et al., 2010; Li et al., 2016). Similarly, the cell morphology of *B. cereus* was obviously damaged after treatment with OOPE due to the leakage of cytoplasm caused by increased permeability of the cell membrane.

CONCLUSIONS

In general, OOPE had a significant antibacterial effect against vegetative cells of *B. cereus* strains isolated from raw milk. The antimicrobial mechanism of OOPE may be due to the reduction of intracellular ATP levels, cell membrane depolarization, a decrease in cellular protein, and cytoplasm leakage. Our findings indicated that OOPE can be considered as a natural food additive to prevent *B. cereus* contamination in the dairy industry. However, before adding OOPE to dairy products as a bactericidal substance, further studies, including safety experiments, dosage optimization, and analysis of the sensory characteristics of foods, should be considered.

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