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Action mechanism of pulsed magnetic field against *E. coli* O157:H7 and its application in vegetable juice

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A B S T R A C T

Compared with traditional thermal sterilization process, pulsed magnetic field (PMF) is a non-thermal sterilization technology with superior advantages of maintaining the nutrition and flavor of food. But little is known about its antimicrobial mechanism. To explore its antimicrobial mechanism, the variation of cell membrane, intracellular biological macromolecules and respiratory metabolism of *E. coli* O157: H7 after PMF treatment, were investigated. The results showed that PMF could cause cell membrane damage and increase cell membrane permeability, reducing the content of three intracellular macromolecules (protein, ATP and DNA), as well as exhibiting inhibition for ATPase activity. The inhibition rate and superposition rate test confirmed the pathway that affecting the respiratory metabolism of *E. coli* O157:H7 treated with PMF was hexose monophophate pathway (HMP), via reducing the activity of key enzyme glucose-6-phosphate dehydrogenase in HMP. Besides, PMF also exhibited superior antibacterial effect on *E. coli* O157:H7 when it was applied in vegetable juices.

1. Introduction

Vegetable juice is swiftly welcomed in the global due to its good flavor and low calories. However, vegetables are often contaminated by *E. coli* O157:H7 (Cui, Ma, & Lin, 2016), causing the growth of *E. coli* O157: H7 in the unsterilized juice prepared by contaminated vegetables (Oyarzábal, Nogueira, & Gombas, 2003). In recent years, most outbreaks of *Escherichia coli* O157: H7 infections are associated with consumption of contaminated juice productions (Bang et al., 2014). *E. coli* O157: H7 is an enteric pathogen that can cause severe gastrointestinal diseases in humans, such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (Silagyi et al., 2009). *E. coli* O157:H7 caused 63,153 cases of foodborne diseases and led to 20 deaths each year in the United States, leading to annual economic loss of 255 million U.S. dollars (Batz, Hoffmann, & Morris, 2012). Hence, it is urgent to seek effective ways to control the contamination of *E. coli* O157:H7.

Traditional thermal reaction is a commonly applied method to restrict the proliferation of bacteria, which destroys the biological functions of bacteria through heating. In comparison with the thermal treatment, the so-called non-thermal treatment does not increase the apparent temperature of organism, while it's physiological and biochemical changes occur. Non-thermal sterilization technology elicited widespread interest for its advantage of maintaining the appearance and quality of food due to the absence of heating process (Yang et al., 2016).

As a kind of the latest non-thermal sterilization technology, pulsed magnetic field (PMF) attracts increased attentions because of its high sterilization rate and less impact on food nutrition and flavor. The inhibitory effect of PMF on food-borne pathogenic bacteria originates from the dielectric blocking principle of strong electric pulse. The strong magnetic field accelerates the charged particles to hit other molecules, resulting in the decomposition and electrolysis of molecules to become anions and cations. These ions pass through the cell membrane and act on intracellular substances to inhibit the growth of microorganisms (Liu & Ma, 2006). In addition, using PMF to treat bacteria for a long time will alter its physiological parameters, including increment, transcription, synthesis and secretion of growth factors, etc. (Chang et al., 2003, 2005; Schwartz, Fisher, Lohmann, Simon, & Boyan, 2009). Most of previous studies focused on the application of pulsed magnetic field sterilization in foods, such as water, milk and mushroom meal (Narsetti at al., 2006; Gao, Ma, & Guo, 2005). To the best of my knowledge and thorough literature survey, few researches have involved in the deeply antibacterial mechanism of PMF against bacteria. Herein, the purpose of this study was to explore the inactivation mechanism of PMF treatment on *E. coli* O157:H7, followed by detecting the morphology and metabolism of bacterial cells. Finally, the antibacterial application of PMF in vegetable juices against *E. coli* O157:H7 was also assessed.

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2. Methods and materials

2.1. Materials and apparatus

Escherichia coli EHEC O157:H7 CICC 21530 was obtained from China Center of Industrial Culture Collection, and stored in liquid paraffin wax at 4 °C. Strain was cultured at 37 °C for 24 h in nutrient agar (NA) or nutrient broth (NB) medium. Pulsed magnetic field (PMF) generator (Magnetic field intensity: 0–10 T, Voltage: 0–15kv, Pulse interval: 2min) was manufactured in Jiangsu University (Zhenjing, China).

2.2. PMF treatment

PMF is supplied by a well stably DC power supply and generated by automatically alternating the charge and discharge to the chamber coil using series of capacitances. The cooling device monitors the temperature and maintains the temperature at 25 °C. The intensity of PMF in the space was recorded by a Tesla meter (Lianzhong, Hunan, China). The pulse number was manually counted. Firstly, the *E. coli* O157: H7 was inoculated and cultured in 10 mL NB for 24 h, then diluted the bacteria to 10^3 CFU/mL with phosphate buffer. The tubes containing *E. coli* O157:H7 (10^3 CFU/mL) were treated with the magnetic field intensity of 0, 2, 4, 6, and 8T with 20 pulses. Each treatment was repeated three times to determine the optimal magnetic field intensity. After determining the optimal magnetic field intensity, the *E. coli* O157:H7 was treated with 10, 20, 30, 40, 50, 60 pulse numbers in that intensity.

2.3. Determination of cell membrane permeability

Firstly, the *E. coli* O157: H7 was inoculated and cultured in 10 mL NB for 24 h. Then the bacteria suspension was suffered from PMF treatment (experiment group). The sample without PMF treatment was regarded as the control. After that, 4 mL of each bacteria suspension was centrifuged at 4000 rpm for 15 min to collect the supernatant. Finally, the supernatant was diluted 20 times to determine its conductivity by a conductivity meter on the basis of the following equation (Cui, Zhao, & Lin, 2015).

$$R(\%) = \frac{R_a - R_c}{R_c} \times 100\%$$

R referred to conductivity change rate, Ra and Rc were the conductivity of experiment group and control group.

Protein contents of the treated bacteria strains were measured by the BCA Protein Assay Kit (Jiancheng Bioengineering Institute, Jiangsu, China).

2.4. Transmission electron microscopy (TEM) analysis

The morphological changes of *E. coli* O157: H7 treated with PMF were observed via a TEM (Model-JEM-2100, JEOL, Tokyo, Japan) (Lin, Zhang, Zhao, & Cui, 2016). The tubes containing bacteria strains were treated with the PMF at 8T, 60 pulses once, twice, three times. The sample without PMF treatment was regarded as the control. Subsequently, treated bacteria strains were collected by centrifugation at 8000*g* for 10 min and washed three times with 0.03 M Phosphate buffer saline (PBS). Finally, the *E. coli* O157: H7 samples were separately dyed with 3% (v/v) phosphotungstic acid and dried for TEM observation.

2.5. Sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed to detect the soluble protein content and protein leakage from *E. coli* O157:H7 treated by PMF at 8.0 T, 60 pulses (Wang, Chang, Yang, & Cui, 2015). The sample without PMF treatment was regarded as the control. All samples were centrifuged at 5000 rpm, 4 °C for 10 min, the precipitated *E. coli* O157:H7 were rinsed and resuspended with sterile water. They were treated through ultrasonic at 150 W for 5 min to break the cells, then boiled for 8 min. Finally, they were centrifuged (5000 rpm) at 4 °C for 10 min to collect the soluble protein of *E. coli* O157:H7. 100 μL of the collected sample was mixed with 25 μL of the loading buffer (250 mmoL/L Tris-HCl (pH 6.8), SDS (10%, w/v), bromophenol blue (0.5%, w/v), glycerine (50%, v/v) and β-mercaptoethanol (5%, v/v)). Next, the mixture was boiled for 8 min, cooled immediately and centrifuged (8000 rpm) at 4 °C for 10 min. The generated supernatant was collected and analyzed by the SDS-PAGE.

2.6. Determination of ATP concentration and adenosine triphosphatase (ATPase) activity

E. coli O157:H7 were incubated and cultured in 15 mL of NB for 24 h to obtain the log-phase bacteria. Then the bacteria suspension was centrifuged at 4000 g for 5 min, the precipitate was collected and washed thrice with 0.03 M phosphate buffer saline (PBS), followed by resuspending in 4 mL PBS (Lin, Mao, Sun, & Cui, 2018). Subsequently, the suspension was treated by PMF (8T, 60 pulses). The sample without PMF treatment was regarded as the control. After treating, the bacterial suspension was centrifuged at 4000 g for 10 min to remove supernatant. The precipitate was added with 500 μL lysozyme and 500 μL TE buffer solutions. Finally, bacteria cells were broken by an ultrasonic cell crusher (Nanjing Immanuel Instrument Equipment Co., Ltd, Nanjing, China) to determine ATP concentration.

The ATPase activity was measured by the ATPase Assay Kit (Jiancheng Bioengineering Institute, Jiangsu, China). Absorbance values at 660 nm detected by an ultraviolet spectrophotometer were obtained to compare the activity of ATPase.

2.7. Determination of oxidative respiratory metabolism

The oxidative respiration rate is the amount of oxygen consumed per unit mass of microorganism. Briefly, 3.6 mL of PBS (0.03 M, pH 7.3), 0.4 mL of glucose and 1 mL of 10^6 bacterial suspensions with or without PMF treatment were prepared and add into tubes as the initial solution. After solution was exposed to air for 5 min, the dissolved oxygen content of the solution was determined by the dissolved oxygen meter (Electric Scientific Instrument Co., Ltd. Shanghai, China) to calculate the initial respiratory rate according to dissolved oxygen content. Then three kinds of typical inhibitor were added into the initial solution, respectively. The tubes were gently shaken and the dissolved oxygen content was measured to calculate the respiratory rate. Inhibiting rate (I_R) was calculated according to the following equation:

$$I_R = \frac{R_0 - R_1}{R_0} \times 100\%$$

Where R_0 represented the initial respiratory rate, R_1 represented the respiratory rate of the bacteria treated by PMF or three kinds of typical inhibitors.

Subsequently, three kinds of typical inhibitors were added into the tubes containing bacteria suspension treated by PMF. The superpose rate (D_R) was calculated according to the following equation:

$$D_R = \frac{R_1 - R_2}{R_1} \times 100\%$$

Where R_1 represented the respiratory rate of bacteria in the presence of CCO, R_2 represented the respiratory rate of bacteria in the presence of CCO and inhibitor.

2.8. Determination of intracellular DNA content

Firstly, the log-phase bacteria after different treatment were centrifuged at 4000 g for 10 min. Then, the collected precipitate was washed three times with equal volume of phosphate buffer (PBS) and re-

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