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Comparative proteomic analysis of *Escherichia coli* O157:H7 following ohmic and water bath heating by capillary-HPLC-MS/MS

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DNA-binding transcriptional regulator (GI: 15832303)
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co-chaperonin GroES (GI: 15834377)
DNA replication protein DnaC (GI: 15834575)
periplasmic chaperone (GI: 15829434)
arginine ABC transporter substrate-binding protein (GI: 15830200)
detox protein (GI: 162139768)
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ABSTRACT

Escherichia coli O157:H7 is an important food-borne pathogenic microorganism that has been used as a model organism for studying microbial inactivation effects and inactivation mechanism in various sterilization technologies. The objective of this study was to investigate the effects of high voltage short time ohmic- (HVST), low voltage long time ohmic- (LVLT), and water bath- (WB) heating on inactivation and proteome changes of *E. coli* O157:H7 cells at the same endpoint temperature of 72 °C, and to analyze whether a non-thermal death effect existed in ohmic heating. The inactivation effect of *E. coli* cells after HVST was comparable to WB, and the largest inactivation was observed after LVLT. There was lower intracellular protein content detected in LVLT and HVST samples than those of WB ($P < 0.05$). Quantitative proteomic profiles using capillary-HPLC-MS/MS technology identified 2626 proteins, among them, a total of 142 (62 up-regulated and 80 down-regulated), 129 (37 up-regulated and 92 down-regulated), and 61 (20 up-regulated and 41 down-regulated) differential proteins were obtained by comparisons of HVST vs. CT (control), LVLT vs. CT, and WB vs. CT samples, respectively, and revealing a strongest cell response to HVST followed by LVLT and WB. Compared with WB samples, more protein changes in HVST and LVLT samples were mainly attributed to the leakage of intracellular proteins due to the damage of cell membrane by current of ohmic heating. Bioinformatics analysis indicated that the differential proteins were mainly involved in transcription, translation, cell wall and membrane biogenesis, amino acid, carbohydrate, and lipid metabolism. KEGG enrichment analysis indicated that the ribosome, terpenoid backbone biosynthesis, glycerophospholipid metabolism, ABC transporters, and folate biosynthesis were significantly enriched. Overall, the application of both HVST and LVLT treatments had the potential to inactivate *E. coli* cells, especially HVST with a shorter heating time, and the results in this study presented an important step toward understanding the response of *E. coli* cells to ohmic heating on proteome level.

1. Introduction

Ohmic heating is defined as a process where heat is generated when electric current passes directly through food-stuff (Icier and Ilicali, 2005), which has received much attention from researchers and the food industry due to its shorter heating time, uniform temperature distribution, and energy efficiency (Pootao and Kanjanapongkul, 2016; Shirsat et al., 2004). Ohmic heating could be widely applied in liquid, semi-solid, and solid food sterilization (Nistor et al., 2015), but most published studies focus primarily on the inactivation effects and inactivation kinetics of ohmic heating on microorganisms and, with few reports on the molecular responses of the microorganism (Kim et al., 2017; Pereira et al., 2007; Ryang et al., 2016; Sagong et al., 2011; Tola and Ramaswamy, 2014). High temperature stress is well known to cause denaturation and the function loss of essential proteins, and in order to cope with these stresses, a cellular response occurs (Hongsthong et al., 2009; Kwon et al., 2008). The effects of ohmic heating on microorganisms were primarily ascribed to the thermal death effect, but some studies reported that additional effects due to the electric current also contributed to the death of microorganisms; as a consequence, microorganisms could be destroyed at a temperature below their thermal death temperature (Cho et al., 2016; Shynkaryk, 2006; Uemura and Isobe, 2002). Therefore, proteins of microorganisms subjected to ohmic heating might suffer different changes compared with conventional heat treatment due to the effect of electric current. However, so far, there was no research focused on the response of

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microbial proteins to ohmic heating.

Some cell behaviors are attributable to large fractions of the proteome and not just a single protein or gene or pathway (Ghosh et al., 2016). In recent years, it has become clear that, in addition to the regulation of specific gene expression, the proteomic studies enable one to determine not only the level of the protein but also posttranslational modifications, which appear to constitute an integral part of the stress response (Rosen and Ron, 2002). Therefore, monitoring protein expression at the scale of the proteome can better describe the microbial metabolisms (Bièche et al., 2012). Several studies on response of protein have shown drastic changes when microorganisms were exposed to limited nutrient, salts, high pressure, heat, cold, and osmotic stress etc., using proteomic technology (Bihan et al., 2013; Hong et al., 2016; Jia et al., 2015; Kirkpatrick et al., 2001; Pittman et al., 2014; Shui et al., 2015). *Escherichia coli* is well characterized gram-negative bacterium and is the main cause of food-borne diseases, which is spread mainly through water and various food, such as beef, chicken, and dairy products. *E. coli* has been used as a model organism for studying bacterial genetics and physiology, because it is composed of the fewest protein types and its proteome and osmotic adjustment mechanisms are well understood. Therefore, *E. coli* was selected as a model in this study to elucidate the mechanism of ohmic heating on microbial response on molecular level.

In our previous study, we investigated the bacterial ecology of long term ohmic- (LTOH, 10 V/cm, 22 min, 72 °C) and water bath (WB, 42 min, 72 °C) cooked pork during refrigerated storage by culture-dependent and amplicon sequencing of 16S rRNA gene, and the results indicated that LTOH-cooked meat has a similar shelf-life to WB treated ones (Tian et al., 2016). So, in this study, 10 V/cm and 5 V/cm were selected to match the heating time with water bath heating and to compare the effects of different voltage gradients on *E. coli* cells. The objective of this study was to investigate the differential changes in protein expression profiles of *E. coli* O157:H7 cells in the mid-logarithmic growth phase after high voltage short time ohmic- (HVST), low voltage long time ohmic- (LVLT) and water bath- (WB) heating using HPLC-MS/MS, in order to have a better understanding of the response of microorganisms subjected to ohmic heating.

2. Materials and methods

2.1. Bacterial strains and sample preparation

E. coli O157:H7 strain (NCTC 12900) was obtained from Shanghai Huiyun Biological Co., Ltd., China and was stored at −80 °C in 50% glycerol. Cells were subcultured onto nutrient agar (NA) at 37 °C for 24 h, and then a single colony was inoculated into 20 mL of nutrient broth (NB) and incubated in a shaker with 180 rpm at 37 °C for 12 h. Then 100 μL of overnight culture was transferred to 20 mL NB for the same incubation conditions until the mid-logarithmic growth phase was acquired (4 h). *E. coli* cells were centrifuged with 5000 ×*g* at 4 °C for 12 min, and the pelleted cells were washed by PBS (pH, 7.2 ± 0.1, 0.01 M NaH_2PO_4, 0.03 M Na_2HPO_4, and 0.09 M NaCl, electrical conductivity 1.23 ± 0.09 S/m) twice, and then re-suspended in PBS with final concentration about 2×10^8 colony-forming units per milliliter (CFU/mL) before treatment.

2.2. Experimental design

2.2.1. Ohmic heating

Ohmic heating was performed using a batch ohmic heater (3.5 kW power supply, 15 A, 0–250 V, 50 Hz) as described by Dai et al. (2013). The heating cell consisted of a Teflon cylinder (inner diameter, 4.2 cm; length, 16 cm) and two parallel food stuff-grade stainless steel electrodes (electrode gap, 10 cm). About 160 ± 5 mL cell suspension was assigned to the heating unit, and the temperature of samples was recorded at 0.5 min interval using a homemade thermoelectric probe encased with isolative ceramic during heating. The samples were ohmically heated to a target temperature of 72 °C at the geometrical center by 10 V/cm and 5 V/cm voltage gradients, as shown in Fig. 1, which needed 3 min and 13 min, respectively, therefore, these two processes were named as HVST (10 V/cm, 3 min) and LVLT (5 V/cm, 13 min) in this study. After heating, the samples were cooled to 4 °C in an ice-water bath immediately and were thoroughly blended with vortex generator for plate count and proteomic analysis. Samples without any heat treatment were served as control (CT). Each treatment was performed in three replicates.

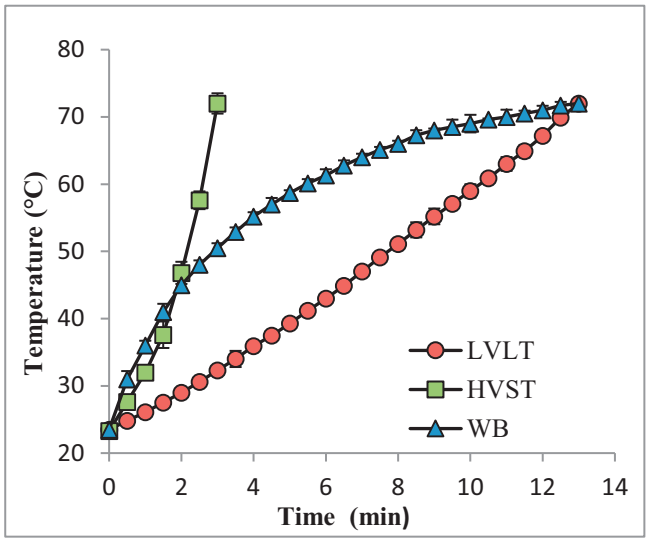

Fig. 1. Time-temperature profiles of HVST, LVLT and WB-treated *E. coli* O157:H7 cells in PBS.

2.2.2. Water bath heating

WB was carried out by a thermostatic water bath (Ke Xi Instrument Co., Ltd., China). In brief, approximately 160 ± 5 mL cell suspension was poured into a plastic casing (diameter, 4.2 cm; length, 10 cm) and heated at an 80 °C water bath. During heating, the plastic casing was constantly shaken, to avoid local overheating at the outer layers. The temperature was recorded at 0.5 min intervals by a temperature probe, and it took 13 min for samples to reach the core temperature of 72 °C. After heating, samples were cooled and blended for analysis using the same method as the HVST and LVLT samples.

2.3. Determination of bacterial inactivation

The inactivation of *E. coli* cells was determined by Improved-MacConkey sorbitol agar (IMSA) according to a modification of the method of Park and Kang (2013). 25 mL of sample was transferred into a sterile stomacher bag containing 225 mL of sterile 0.85% physiological saline (PS) and homogenized for 1 min with a clapping homogenizer (IUL, Spanish). After homogenization, 1 mL aliquots of sample were 10-fold serially diluted with 9 mL sterile 0.85% PS, and an appropriate sample dilution of 100 μL was spread on the plate. Each dilution was carried out in duplicate, and the plate was incubated at 37 °C for 24–48 h before enumeration.

2.4. Protein extraction, quantification, and digestion

Cell suspensions were centrifuged at 4 °C and 5000 ×*g* for 12 min, and the intracellular protein extraction was performed according to Liao et al. (2011), then protein was quantified using the Bradford kit (Dingguo Biological, China). After protein quantification, following the addition of 5 μL 1 M DTT, 60 μg of protein fractions in the centrifuge