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Electropermeabilization responses in Gram-positive and Gram-negative bacteria

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ABSTRACT

Effects of 3-kJ kg^{-1} nanosecond pulsed electric fields (PEFs) on cellular permeabilization of *Salmonella enterica* and *Staphylococcus aureus* were observed. It was seen that bacterial responses depend on both the electrical pulse attribute and the cell plasma membrane structure. For traditional permeabilization, the responses involved the thickness of the peptidoglycan layer where a maximum of 2.5 log reduction in *S. enterica* population was achieved. Meanwhile, in the area of selective permeabilization, it showed insignificant reduction in both pathogens. Such inactivation mechanisms were described through the behavior of potential across plasma membrane and intracellular organelles by PSPICE simulations incorporating PEF-cell interaction model.

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1. Introduction

Recent trends in food technology have shown more attention to pulsed electric field (PEF) as an alternative method on an inhibition of microorganism growth. The efforts in the past few decades involved the uses of high-intensity electrical pulses to focus on a reduction of microbial fractions in foods [1–6]. Its mechanism basically involves an application of electrical pulses in a momentary time on objects placed between two electrically conductive electrodes. As a result, the bacterial reduction remains effective while major heating effects are minimized. As a matter of fact, the transmembrane potential induced by the applied field causes the cell membrane to damage. The cell membrane being subjected to high voltage pulses with the duration longer than its membrane charging time constant can lose its impermeability. The potential across the outer membrane, once beyond a critical value, eventually leads to the formation of pores on the membrane surface, resulting in cell breakdown [7–9]. The increased membrane potential ΔV_m upon exposing with pulsed electric fields E of duration τ can be expressed as

$$\Delta V_m(t) = fE\frac{D}{2}\cos\theta\left(1 - e^{-t/\tau_m}\right) \quad (1)$$

where D is the cell diameter, θ is the angle with respect to the direction of the electric field, and f is the shape factor, which is 1.5 for spherical cells. For rod-shaped cells, this factor equals $l_c/(l_c - 0.33D)$, where l_c is the length of the cell. According to Cole [10], the time required to charge the outer cell membrane τ_m is dependent on the electrical parameters of both cell and medium. For a cell with homogeneous cytoplasm, the charging time constant is simplified to

$$\tau_m \approx \frac{3}{4}\rho C_m D \quad (2)$$

where the resistivities ρ of the medium and cell cytoplasm are assumed equal to each other, and C_m is the capacitance of the membrane per unit area. Owing to the size of bacteria, which is on the order of one micron or less, much higher PEF strengths are required for membrane breakdown compared to those of larger mammalian cells. The cause of cell death due to the disintegration of the cell membrane portion was verified by the correlation between cell viability and membrane permeabilization of *L. plantarum* and *E. coli* under stresses [11,12].

In contrast to traditional PEF technique, considerably short pulses compared with the cytoplasm membrane charging time have been recently considered in many applications, especially in the areas of medicine and biology [13–15]. Such ultra fast pulses allow the voltage to develop across subcellular structures. As

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described by the cross-sectional view of a bacterial cell with nonmembrane-bound nucleoid in Fig. 1, if the duration of applied pulses is long compared to the charging time associated with the membrane capacitance, only the outer membrane is charged. On the other hand, if the penetrating field is sufficiently fast with the duration of shorter than the membrane charging time, it can have pronounced effects on intracellular organelles, potentially leading to the malfunctionality of nucleoids. In this study the PEF areas of interest are the traditional permeabilization ($\tau > \tau_m$) and the selective permeabilization ($\tau < \tau_m$) where we compared the inactivation responses of *Salmonella enterica* and *Staphylococcus aureus*. *S. enterica* is a rod-shaped Gram-negative bacterium and is commonly found on raw egg shells and in red meat [16]. *S. aureus*, on the other hand, is a spherical Gram-positive bacterium that is capable of producing multiple arrays of enterotoxins [17]. As ones of the most serious bacterial threats in food industries, these species can cause diseases ranging from nausea, vomiting, diarrhea, abdominal pain, intoxication to typhoid fever. Following the introduction, we report the configurations of the high-intensity PEF systems and the preparation of materials. The results detailed in Section 3 are discussed and concluded in Section 4.

2. Methodology

2.1. Generation of high-intensity nanosecond pulses

Temporal profiles of ~500- and ~30-ns pulses are shown in Fig. 2a and b, respectively. For a generation of 500-ns pulses, the pulse forming network (PFN) was created from series of two 16-kV, 0.022 μF metalized polyester capacitors (ASC Capacitors, USA) in conjunction with the 15-AWG coil (Bangkok Cable, Thailand) wound on an air core. On the other hand, 30-ns pulses were generated by the pulse forming line (PFL) composed of a set of three 1-m long coaxial cables (RG8/U, 10.29-mm ø, 96.79 pF/m, 52 Ω) connected in parallel with the maximum rating of 10 kV. Each cable has a solid polyethylene as an insulator material. For both systems, an SL300 high voltage DC source (Spellman, Inc., Hauppauge, New York, USA) supplies the energy to pulse forming circuits through the decoupling resistor of 100 MΩ. Both pulse profiles at the repetition rate of ~5 Hz were monitored by a P6015A high-voltage probe (Tektronix, Inc., Beaverton, OR, USA), reading through a DSO5054A digital oscilloscope (Agilent Technologies, Inc., Santa Clara, CA, USA). The self-breakdown switch is a spark gap with a few nanoseconds delay in rise time. The treatment chamber is a 1-mm commercial electroporation cuvette (VWR Scientific Products, West Chester, PA, USA) that can offer homogeneous electric fields across the entire active region. The pulse amplitudes were adjusted so that both systems can serve the load with nearly identical specific energy of 3 kJ kg^{-1} per pulse. That is, the shorter

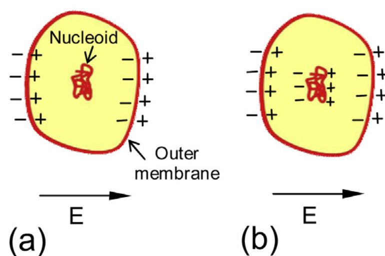

Fig. 1. Cross-sectional views of a bacterial cell where only nonmembrane-bound nucleoid represents subcellular components. The application of pulsed electric field results in charge distributions (a) only on the outer membrane for traditional permeabilization ($\tau > \tau_m$) and (b) on both the outer membrane and subcellular structures for selective permeabilization ($\tau < \tau_m$).

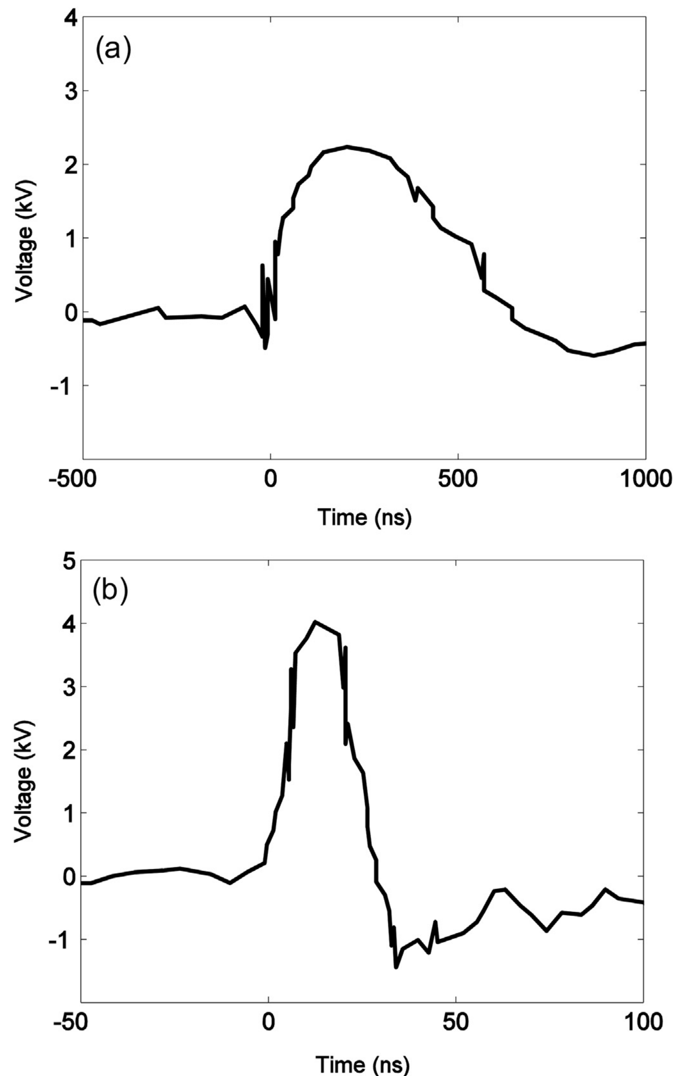

Fig. 2. Temporal plots of (a) ~500-ns and (b) ~30-ns high-voltage pulses from measurements across the treatment chamber.

the pulse duration, the higher the main pulse amplitude is required. The corresponding pulse strengths are ~25 and ~100 kV cm^{-1} for the pulse durations of 500 and 30 ns, respectively. The mismatch between the impedances of the pulse forming circuits and the load causes small fluctuations after the main pulses.

2.2. Material preparation and data analysis

Bacterial strains used in this study were *S. enterica* serotype Typhimurium LT2 and *S. aureus* ATCC25923. Stock cultures of both bacteria were stored at −80 °C in Tryptic Soy broth (TSB) with 15% glycerol (v/v) and streaked onto TS agar plates prior to each experiment. Plates were incubated overnight at 37 °C. Single isolate colonies were inoculated into 5 ml of TSB and incubated overnight (15–18 h) at 37 °C while kept shaking at 200 rpm. Prior to performing experiments, 1:10 serial dilutions were performed on overnight cultures in order to determine the colony forming units CFU/ml. For our study, cultures were diluted to approximately 10^3–10^4 CFU/ml, mimicking medium load of bacterial contamination in foods. An aliquot of 160 μl of diluted cultures were used in each treatment condition. Each PEF treatment was performed in duplicate. After each treatment, treated culture of 50 μl was used in serial

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