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Inactivation of *Escherichia coli* phages by PEF treatment and analysis of inactivation mechanism

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ABSTRACT

Inactivation of phage by pulsed electric field (PEF) treatment using *Escherichia coli* phages (M13mp18 and λ phage) as the phage particle model was studied. *E. coli* phages were successfully inactivated, and the absence of degradation of biological molecules was confirmed by electrophoretic analysis of these molecules from the inactivated phages. The comparison of sensitivity to PEF treatment between the *E. coli* phage and the *E. coli* cells was also carried out. The *E. coli* phages were more sensitive to the PEF treatment than the *E. coli* cells, and this difference allowed the preferential inactivation of *E. coli* phages.

Introduction

Fermentation using bacteria is one of the most important unit operations in the industry. In addition to the production of traditional foods, recently, that of enzymes, pharmaceuticals and chemicals by fermentation using bacteria including genetically modified ones has been applied to practical use. In such industrial applications, infection of the fermentation bacteria by phages would result in the decrease in product amount and quality. Inactivation of phages has become increasingly important to prevent the contamination of fermentation cultures by phages. Bacterial inactivation methods, particularly heat treatment, are also applied for phage inactivation. The inactivation of heat treatment via the biomolecular denaturation of bacteria and phages has been established. However, heat treatment also denatures other heat-sensitive molecules such as vitamins, sugar, protein, flavor compounds and color components of fermentation feedstock. To avoid these problems, application of nonthermal methods for the inactivation of phages has also been investigated [1–4].

As one of nonthermal inactivation methods for bacteria, pulsed electric treatment (PEF) has been attracting attention. Nanosecond rise time and microsecond of half bandwidth of applied voltage characterize PEF and those do not generate a large amount of ohmic heat; with these characteristics, PEF was applied to liquid sterilization. The mechanism of inactivation of microorganisms in liquid by PEF is the destruction of the outer membrane structure of microorganisms due to electrical compression and fenestration [5,6]. PEF does not lead to the generation of active species that react with the components of fermentation feedstock.

In this study, the inactivation of phages by PEF treatment was investigated as a nonthermal method of phage inactivation. *E. coli* and its phages were selected as the host and phage models. The profile of the *E. coli* phage inactivation by PEF treatment was compared with that by heat treatment. Damage of phage DNA and protein was analyzed by electrophoresis. The sensitivities of *E. coli* cell and phages to PEF were also compared using a mixture solution of these cells and phages.

Materials and methods

Strains and media

The *E. coli* phages M13mp18 phage (Takara Bio Inc., Ohtsu, Japan) [7] and λ phage (NBRC 20016) were used as the inactivation targets of the PEF treatment. *E. coli* MV1184 (Takara Bio) was used as the host strain for *E. coli* phage infection. It was cultivated in Luria–Bertani (LB) medium [1% (w/v) Bacto peptone, 0.5% yeast extract and 0.5% sodium chloride] until OD_{660} = 0.5–0.8 at 35 °C.

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Then the phages were inoculated into *E. coli* broth, and cultivated for 4 h. The supernatants were centrifuged for 10 min at 18,000× *g* and 4 °C and used as the phage stock solutions.

Phage titer measurement

Phage titer (plaque formation unit: PFU) was measured by the plaque counting method. In brief, the *E. coli* cells cultivated for 12 h were collected by centrifugation for 5 min at 1000× *g* and suspended in sterilized distilled water. An appropriately diluted phage stock solution, an *E. coli* solution, and LB medium containing 0.6% agar, which was autoclaved and cooled down to 45 °C, were mixed well and overlaid onto LB medium plates containing 1.5% agar. Phage titer was calculated form the number of plaques formed on the plate after 12 h of cultivation at 37 °C.

PEF generation and PEF treatment apparatus

A high-voltage pulse generator with a rotary spark gap described in our previous report was used [8]. The capacitor capacity, pulse frequency, and peak applied voltages were 8 nF, 50 Hz, and 5 and 7 kV, respectively. The waveforms of 5 and 7 kV pulses are shown in Fig. 1. The rise time and pulse width of both applied voltages were approximately 50 ns and 25 μs, respectively. A schematic diagram of the treatment chamber used in this study is shown in Fig. 2. The treatment chamber consisted of two parallel acrylic plates that have plate stainless steel electrodes facing each other with a gap of 31 mm and an acrylic cylinder. The phage solution diluted to 10^7 PFU/ml was poured into the treatment chamber, and pulsed voltage was applied for 0–12 min. The phage titer of the treated phage solution was measured as described above. An *E. coli* cell suspension was also treated using PEF, and viable cell number (colony forming unit: CFU) was determined by colony counting. A mixture of *E. coli* cells (10^7 CFU/mL) and phages (10^7 PFU/mL) was treated with PEF at 5 kV, and then the treated mixture was inoculated into LB medium. The mixture was incubated at 35 °C and culture turbidity (optical density at 660 nm: OD_{660}) was measured at various time points.

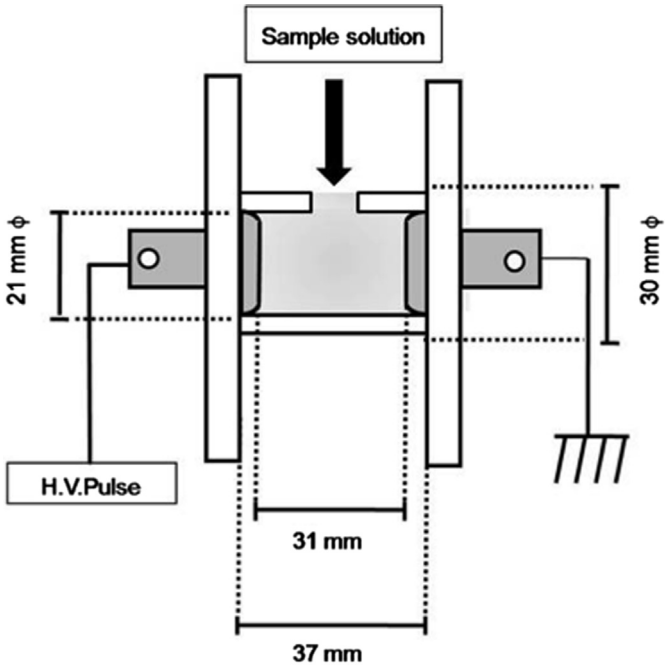

Fig. 2. Schematic illustration of PEF reactor used in this study.

The capsid proteins of phages were concentrated by ultrafiltration (cut off, 30 kDa) and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% gel. The proteins separated on the gel were stained with a silver stain II kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

The phage DNA was extracted from the proteins using phenol-chloroform, purified and concentrated by ethanol precipitation.

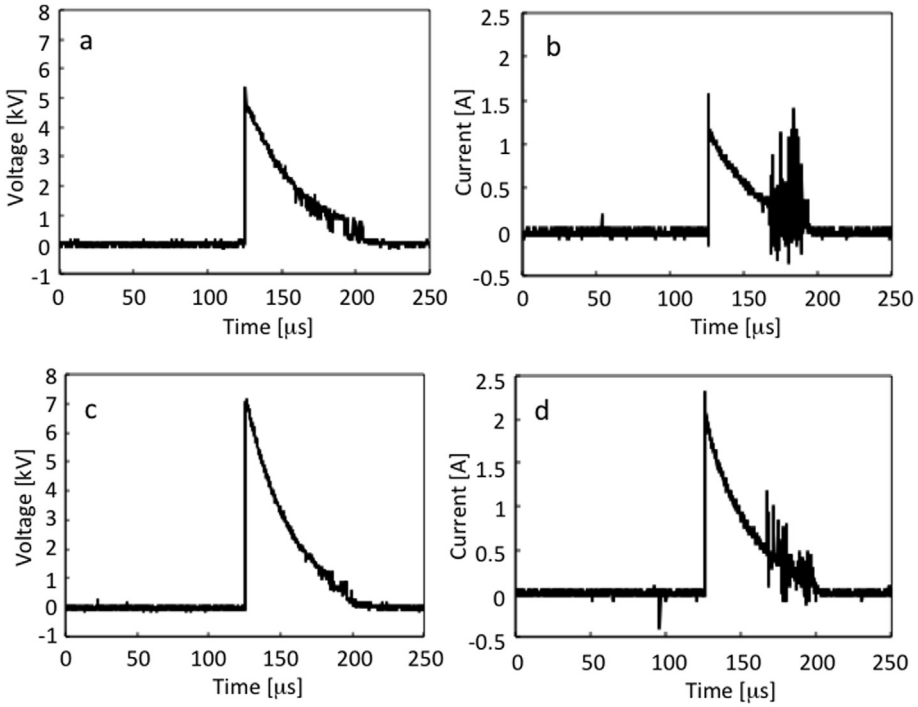

Fig. 1. The applied pulse voltage waveforms at 5 (a) and 7 kV(c), and the corresponding discharge current waveforms at 5 (b) and 7 kV (d).