Irreversible electroporation of bacterial cells L5

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Duration of the experiments: day 1: 90 min; day 2: 60 min
Max. number of participants: 4
Location: Microbiological laboratory
Level: Basic

PREREQUISITES

Participants should be familiar with Safety rules and Rules for aseptic work in microbiological laboratory. No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

Irreversible electroporation is recently being tested as potential alternative treatment of different water samples. Short, high voltage electric pulses cause cell membrane disruption and lead to loss of viability and reduced number of bacteria in the treated sample. Different water samples were treated by irreversible electroporation and recently the method was tested also for hospital wastewater. Regardless of the application, optimization of electric pulse parameters is needed for effective treatment.

Irreversible electroporation is a phenomenon based on the fact that when the cell is exposed to the external electric field of sufficient amplitude and duration, its membrane is permeabilized. Increasing amplitude of electric field increases the level of cell membrane permeabilization. When electric field parameters used are below the critical value, after some time cell membrane reseals and treated cells survive. If electric pulses exceed the critical value, the damage cannot be repaired and as a result cell viability is affected. The process is known as irreversible electroporation and is also used in food processing technologies.

The efficiency of irreversible electroporation can be monitored by plating the treated sample on nutrient agar and counting the number of colony-forming units (CFU). Each colony may arise from a group of cells rather than from one individual cell and they represent the cells that survived electric pulse treatment.

The aim of this laboratory practice is to demonstrate irreversible electroporation of *Escherichia coli* in distilled water, using different electric pulse parameters.

EXPERIMENT

We will detect irreversible electroporation by CFU count. The effect of electroporation on reduced cell viability will be determined for chosen sets of electric pulse parameters.
Our experimental organism will be *E.coli* K12 (ER1821, New England BioLabs) cultured 12-16 hours in Luria Broth (L3522, Sigma Aldrich) at 37 °C with vigorous shaking.

Protocol 1/2 (Electroporation of bacteria): On the first day of experiment 20 ml of bacterial cell culture will be centrifuged at 4200 rpm and the pellet will be resuspended in 20 ml of distilled water. Control sample will be the untreated suspension of bacteria. To determine the number of bacterial cells

you will need to prepare serial dilutions of resuspended bacteria ranging from 10^{-1} to 10^{-7}. Dilute 100 µl of bacterial suspension in tubes containing 900 µl of sterile distilled water. First plate the control. Pipette 100 µl of dilutions 10^{-6} and 10^{-7} on LB agar in Petri dishes; spread them evenly with sterile glass rod.

For electroporation of the samples we use 10^{-1} dilution. Electric pulses will be applied with electric pulse generator HVP-VG (Igea, Italy). Samples for treatment are placed in cuvettes with integrated aluminium electrodes, electrode distance 1 mm. The volume of the treated sample is 90 µl. After electroporation take 50 µl of the sample from cuvette for serial dilutions. Spread 100 µl of dilutions 10^{-4}, 10^{-5}, 10^{-6} on LB agar in Petri dishes and spread it evenly with sterile glass rod. All agar plates will be incubated overnight at 37 °C.

Protocol 2/2 (Counting bacterial colonies): Count colony-forming units 24 hours after the treatment. From the data obtained determine the reduction of bacterial survival. When calculating the CFU/ml, you should take into consideration all dilutions that were made, so you can estimate the number of bacteria in original water sample.

Number of viable cells per ml = number of colonies × dilution factor of plating and pipetting

Control represents total cell count and you will calculate the reduction in cell count for samples exposed to different electric pulse parameters.

FURTHER READING:

Teissie J. et al. Recent biotechnological developments of electropulsation. *Biolectrochemistry* 55: 107-112, 2002.
Gusbeth C. et al. Pulsed electric field treatment for bacteria reduction and its impact on hospital wastewater. *Chemosphere* 75:228-33, 2009.
Saulis G. Electroporation of cell membranes: The fundamental effects of pulsed electric fields in food processing. *Food Engineering Reviews* 2:52-73, 2010.
Žgalin M.K., Hodžić D., Reberšek M., Kandušer M. Combination of microsecond and nanosecond pulsed electric field treatments for inactivation of *Escherichia coli* in water samples. *The Journal of Membrane Biology* 245:643-650, 2012.

NOTES & RESULTS

electric pulse parameters *n* x *t* (µs) *E* [kV/cm], *f* [Hz]	8 x 100 µs 7,5 kV/cm 1 Hz	8 x 100 µs 15 kV/cm 1 Hz	8 x 100 µs 30 kV/cm 1 Hz
number of viable *cells per ml*			
reduction of E.coli [order of magnitude]			

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