Study of lightning-mediated irreversible electroporation on *E. coli* L10

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Duration of the experiments: day 1: 90 min; day 2: 30 min
Max. number of participants: 4
Location: Microbilogical Laboratory 1
Level: Basic

PREREQUISITES

Participants should be familiar with the Safety rules for handling with chemicals and Rules for sterile/aseptic work in microbiological labs. The basic knowledge of handling microorganisms/bacteria is required.

THEORETICAL BACKGROUND

When living matter is exposed to strong electric field (hundreds of V/cm or more), a considerable increase of their membrane permeability (electroporation) and/or their merger (electrofusion) can be observed. If the field is neither too strong nor too long-lasting, electroporation is reversible, while otherwise it becomes irreversible, resulting in cell death.
Electric fields with amplitudes and durations adequate for electroporation and electrofusion can also occur in natural environments when these are hit by a lightning stroke. But unlike with the standard laboratory studies and applications of electroporation and electrofusion, a stroke proceeds through a highly conductive channel (electric arc) created by electrical breakdown of the air separating the cloud and the ground, and the time course of the electric current and the electric field induced by it as it flows through the ground are neither rectangular nor purely exponentially decaying. Furthermore, in the ground the current does not flow towards a well-defined electrode, but dissipates downward and outward from its point of entry, and consequently the amplitude of the electric field it induces decreases rapidly with increasing distance from this point. To study lightning-mediated electroporation, we have developed an exposure system (Figure 1) that allows to emulate lightning-mediated electroporation of living matter in a downscaled but otherwise realistic manner.

The aim of this laboratory practice is to emulate lightning-mediated irreversible electroporation on bacterial *E. coli* culture using our exposure system.

EXPERIMENT

We will demonstrate irreversible lightning-mediated electroporation on bacterial *E. coli* culture using our exposure system (Figure 1). We will load agar plate covered with confluent *E.coli* cells to the exposure system and apply several electrostatic discharges as shown on left panel in Figure 2. We will be able to observe the area of irreversible electroporation as seen on right panel in Figure 2.

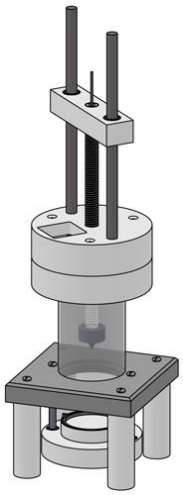

Figure 1. The exposure system for investigation of lightning-mediated electroporation on living matter.

Protocol 1/2 (Exposure of *E. coli* to electrostatic discharges): Escherichia coli K12 ER1821 strain (New England BioLabs, Frankfurt, Germany) will be used as a model of bacterial cells. The overnight culture of *E. coli* will be mixed with Luria broth (Sigma-Aldrich, Munich, Germany) in an Erlenmeyer flask and placed into an incubator with continuous shaking (I-50, Kambič Laboratory Equipment, Slovenia) for 24 hours at 37°C. After incubation, the flask will be removed from the incubator and centrifuged for 15 min at 4°C at 4200 RPM. Supernatant will then carefully be removed, and pure water (Aqua B. Braun, Braun Melsungen, Germany) will be added and stirred gently to suspend the cell pellet, and diluted further with additional pure water to a concentration of 5.5 x 10^8 CFU/ml to yield the final suspension of *E. coli*.
On agar plates cooled to 21°C, the final suspension of *E. coli* will be poured uniformly over the surface of the plates at 1 ml per plate. After 1 min the suspension that will not get absorbed into agar will be removed with a pipette. The agar plates will then be left uncovered for additional 10 min in the laminar, allowing the remaining suspension to evaporate. At this stage there will be no fluid present on the agar that would allow bacterial cells to float and migrate.
For electric discharge application, an agar plate with bacterial cells prepared as described above will be loaded into the exposure system, and 10 electric discharges will be applied. The control agar plate will be loaded into the exposure system in the same manner as the exposed plates, but no discharge will be applied. All plates will then be incubated for 24 h at 37°C.

Protocol 2/2 (Assessing the area of irreversible electroporation): The region of irreversible electroporation of *E. coli* will clearly be detectable by normal eye sight 24 h after exposure (Figure 2, right panel).

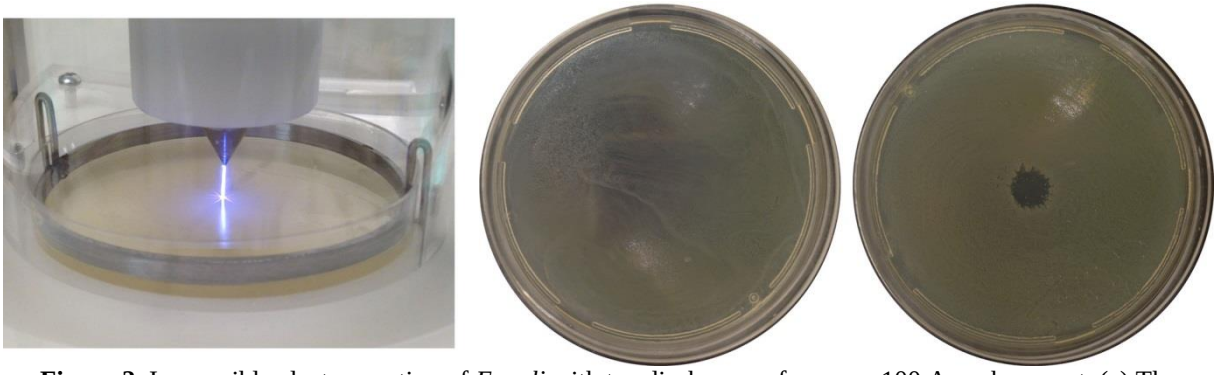

Figure 2. Irreversible electroporation of *E. coli* with ten discharges of approx. 100 A peak current. (a) The exposure. (b) The control plate (loaded into the system, but no discharge applied). (c) The exposed plate. The transparent areas correspond to regions with absence of *E. coli* colonies.

FURTHER READING:

Kotnik, T. Lightning-triggered electroporation and electrofusion as possible contributors to natural horizontal gene transfer. *Phys. Life Rev.* 2013.

Marjanovič, I., Kotnik, T., An experimental system for controlled exposure of biological samples to electrostatic discharges. Bioelectrochemistry. *vol. 94. pp. 79–86.*

Dower, W.J., J.F. Miller, and C.W. Ragsdale. High efficiency transformation of E.coli by high voltage electroporation. *Nucleic Acids Res. 16*:6127 –6145. 1988

NOTES & RESULTS