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Effect of Electric Pulse on Bacterial growth and Adaptation as an Environmental Factor

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The lightening is a natural phenomenon and makes an electric pulse in water and soil environments, which can be one of various environmental factors affecting microbial cells living in ecosystem. We generated an electric pulse in bioreactor by using electrochemistry. Titanium anode and cathode were?set up in?a bioreactor, to which DC 3, 6 or 9 volt of electricity was charged. To make electric pulse between two electrodes, anode and cathode were periodically exchanged at intervals of 15 min. A kanamycin-resistant *Pseudomonas* sp. and kanamycin-susceptible *E. coli* were?used as test strains. *E. coli* and *Pseudomonas* sp. was mixedly cultivated in electric field using LB medium containing 100 ß¹/ml of kanamycin for 48 hr. *E. coli* mixedly pre-cultivated with *Pseudomonas* sp. in 6 or 9 volt of electric field was grown on McConkey agar plate containing kanamycin, which is showing that *E. coli* was physiologically converted to kanamycin-resistant, and electric pulse may activate physiological transformation of *E. coli*.

Key words: kanamycin, *Pseudomonas*, *E. coli*, electric pulse, physiological transformation

1. Introduction

Various environmental factors such as temperature, ionic strength, osmotic pressure, acidity, oxygen concentration, pressure, water activity and light have been known to influence on all organisms living in natural ecosystem. However, lightening being frequently generated in biosphere has not been recognized as an environmental factor. The lightening can make electric pulse between striking zone and around that, by which microorganisms living in soil and water can be directly affected. An adaptation capability of microorganism against disadvantageous environmental factors is dependent upon their genetic functions. Various microorganisms have been known to exchange their genetic elements intra-species or inter-species. Natural genetic exchange (transformation) is the active uptake of free DNA by bacterial cells and the heritable incorporation of its genetic information. The cellular processes involved in transformation have been studied extensively by *in vitro* experimentation with a few transformable species.[1)] It is known that plasmid DNA and linear duplex DNA molecules are adsorbed to chemically purified mineral grains of sand and to particles of several clay fractions.[2,3)] The DNA absorbed to mineral particles can be easily transformed by bacterial cells. Laboratory experiments have shown that natural transformation occurs in many bacterial genera such as *Azotobacter, Bacillus, Haemophilus, Pseudomonas,* and *Acintobacter.*[1,4)] Studies in soil extracts, fresh and marine waters and aquifer material have provided evidence that transformation of bacteria also can occur in natural environments.[5,6,7,8)] For most natural transformation, an organism becomes competent in vitro through starvation and/or other manipulations of culture conditions.[9)] The natural competence for transformation in *Legionella pneumophila* is dependent on growth conditions such as temperature, ionic strength, acidity, pressure and oxygen concentration and is induced under conditions similar to those for expression of specific genes.[10,11,12)] Wouters *et al.*[13,14)] developed the electric transformation method and examined efficiency of method for gene expression in transformant. Generally, higher than 1000 volt or 1

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mega hertz of electric pulse is required for electric transformation. In natural ecosystem, it is possible that lightening discharged to earth or water surface may also function as electric pulse and electric field may be generated around the lightening-striking zone. Effect of electric pulse on bacterial growth,[15] cell surface properties,[16] membrane permeability,[17] bacterial lethality[13] and DNA hybridization[19] was examined *in vitro* but on genetic transformation was not. In this study, we examined effect of low voltage of electric pulse on genetic transformation. For easy achievement of this experimental purpose, a kanamycin-resistant gene of *Pseudomonas* sp. was used as a target for transformation of *E. coli*.

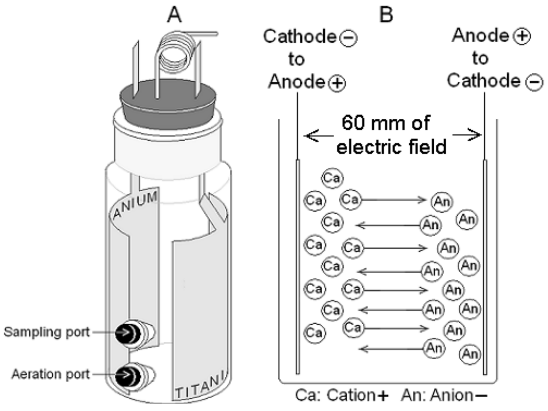

Fig. 1. Real structure of electrochemical bioreactor (A) with two electrodes, to which DC 3, 6 or 9 volts of electricity was charged. The distance (electric field) between was adjusted to 60 mm. Anode and cathode are exchanged at intervals of 15 min. At the second that electric poles are being exchanged, large quantity of anion and cation has to move to opposite electrode (B), by which electric pulse can be produced between anode and cathode. The electric pulse generated outside bacterial cells may affect genetic transformation.

2. Materials and Methods

2.1. Electrochemical bioreactor

An electrochemical bioreactor was composed of glass bottle (500 ml volume) and titanium electrodes (50 mm × 150 mm, 1mm thickness) as shown in Fig. 1A. Distance between two electrodes was adjusted to 60 mm, which is electric field. Anode and cathode were periodically exchanged at intervals of 15 min, to which DC 3, 6 or 9 volts of electricity was charged. At the second that electric poles are being exchanged, large quantity of anions and cations has to move to opposite electrode (Fig. 1B), by which electric pulse can be generated in the electrical bioreactor as shown in Fig. 2.

2.2. Condition for transformation test

For exclusion of other factors except electric pulse influenced on bacterial environment such as growth temperature (37°C), dissolved oxygen (8-10 mg/L),

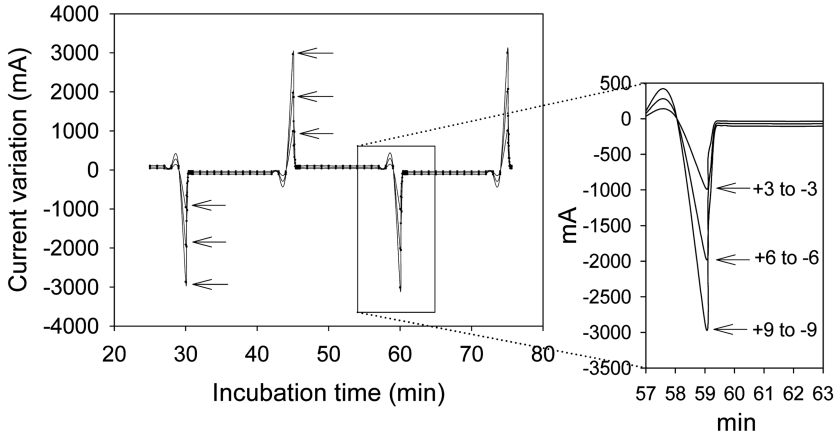

Fig. 2. The electric pulse generated by conversion of anode to cathode (lower peaks) or cathode to anode (upper peaks) at the intervals of 15 min. At the second that electric poles are being exchanged, large quantity of anions and cations were move to opposite electrode, which is expressed as the sudden current variation. The range of current (mA) variation is proportional to the potential (volt) charged to electrodes as shown in figure of right side.

ionic strength (170 mM Na^+), acidity (pH 7.0) and medium ingredients (10 g/L peptone, 5 g/L yeast extract and 10 g/L NaCl) were adjusted based on the conventional method.

2.3. Organsims and cultivation

E. coli was used as a DNA receptor and a kanamycin-resistant *Pseudomonas* sp. was used as a DNA donor for transformation test. Two bacterial species were mixedly cultivated with Luria-Bertani (LB, Difco, U.S.A.) medium in electric field, and MacConkey sorbitol agar containing kanamycin (Difco, U.S.A.) was used for selective detection of *E. coli* transformed in electric field. To induce effective transformation of *E. coli* in electric field, fresh cells (0.75 g as dry weight) harvested from 16 hr old culture of *E. coli* by centrifugation (5,000 g for 30 min) were periodically added to culture of *Pseudomonas* sp. in electrochemical bioreactor at the intervals of 3 hr for 24 hr and then mixed culture of *Pseudomonas* sp. and *E. coli* was incubated for more 24 hr without addition of *E. coli* cells. Periodic addition of *E. coli* to culture of *Pseudomonas* sp. is required to increase transformation probability because kanamycin-susceptible *E. coli* is difficult to grow or survive in culture containing kanamycin before genetic transformation is generated.

3. Results

3.1. Determination of inhibition zone on agar plate

The minimum inhibitory concentration (MIC) of kanamycin against *Pseudomonas* sp. is 100 μg/ml and kanamycin concentration contained in paper disc was adjusted to 2 mg, by which theoretical mean concentration of kanamycin contained in agar medium (20 ml) can be calculated as 100 μg/ml, however, which is not real concentration because gradient concentration of kanamycin from center to margin was formed. Accordingly, inhibition zone of kanamycin against *Pseudomonas* has to be determined to compare with that against *E. coli* on agar plate. As shown in Fig. 3, inhibition zone of kanamycin against *Pseudomonas* sp. was 45 mm, which was used as a criterion to detect kanamycin-resistant *E. coli* genetically transformed in the electric field.

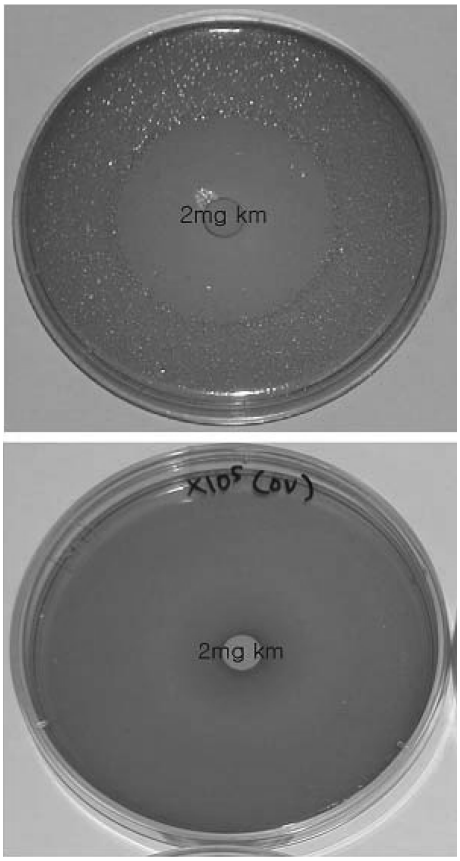

Fig. 3. Growth pattern of a kanamycin-resistant *Pseudomonas* sp. (upper) on LB agar plate and kanamycin-susceptible *E. coli* (lower) on MacConkey sorbitol agar plate containing kanamycin (km). Kanamycin concentration contained in paper disc was adjusted to 2 mg. Minimum inhibitory concentration (MIC) of kanamycin for *Pseudomonas* sp. is 100 mg/ml. The kanamycin concentration in clear zone around km disc has to be more than MIC, which is an indicator to estimate the resistance of *E. coli* against kanamycin. Kanamycin-susceptible bacterium can not grow at any location of agar plate (lower).

3.2. Influence of electric pulse on bacterial growth

When *E. coli* and *Pseudomonas* sp. were cultivated with LB medium in 9 volt of electric field, bacterial growth was not negatively influenced by electric field as shown in Fig. 4. However, bacterial growth was greatly depressed in the than 11 volt of electric field (data were

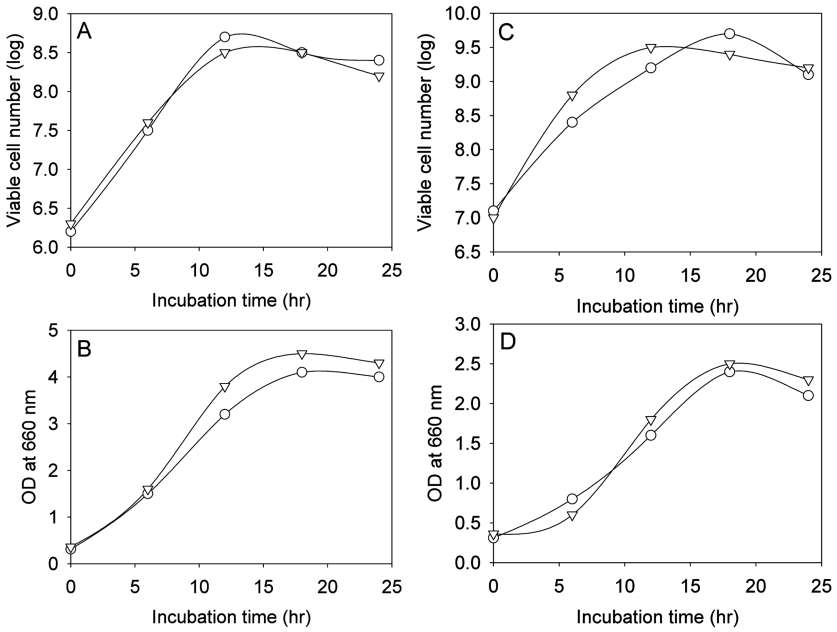

Fig. 4. Growth of *Pesudomonas* sp. (A and B) and *Escherichia coli* (C and D) on LB broth in the conventional growth condition (○) or in the electric field (▽), to which 9 volts of electric pulse was charged at intervals of 15 min. The bacterial growth was not inhibited by the electric field.

not shown). This result means that less than 9 volt of electric field is not possible to be another interfering factor except a function for transformation of *E. coli* by *Pseudomonas* sp.

3.3. Influence of electric pulse on transformation

The kanamycin-resistant gene of *Pseudomonas* sp. was used for easy achievement of transformation and easy detection of *E. coli* genetically transformed. The MacConkey agar containing kanamycin is useful for selective detection of kanamycin-resistant *E. coli* because colonies of *E. coli* growing on MacConkey agar is changed to red and kanamycin-susceptible *E. coli* can not grow on the medium. As shown in Fig. 5 (lower), red colored colonies were observed on agar plate when

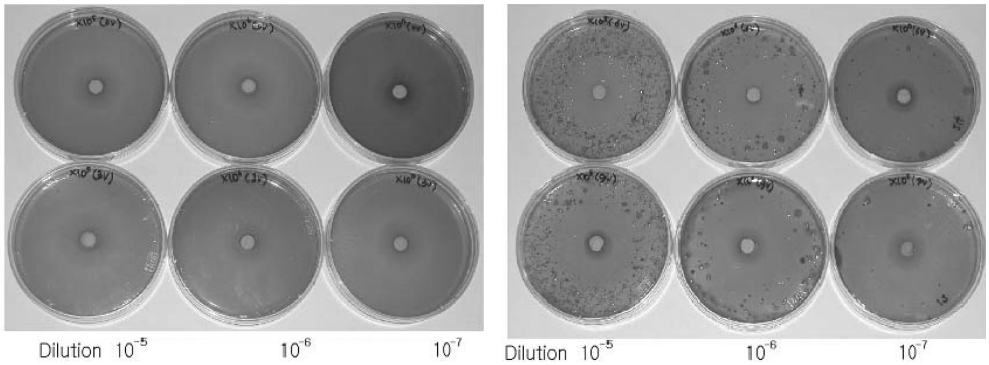

Fig. 5. Growth pattern of *Escherichia coli* on MacConkey sorbitol agar medium containing kanamycin. *Escherichia coli* was mixedly pre-cultivated with kanamycin-resistant *Pseudomonas* sp. in Luria-Bertani broth under conventional growth condition (upper line) or under the electric field (lower line), to which 3, 6 and 9 volts of electric pulse was charged at intervals of 15 min for 48 hr. Red colored colonies of *E. coli* were observed in the lower plates but not in the upper plates. This means that the genetic transformation of *E. coli* by *Pseudomonas* sp. may be activated under the electric field. Each paper disc contained 2mg kanamycin.

Table 1. Viable cell numbers of *Escherichia coli* grown on MacConkey sorbitol agar plate containing kanamycin. *Escherichia coli* was mixedly pre-cultivated with kanamycin-resistant *Pseudomonas* under conventional growth condition and in the 9 volt of electric field for 48 hr.

Pre-cultivation condition	Viable cell numbers (dilution rates)		
	(10^{-5})	(10^{-6})	(10^{-7})
In the conventional condition	13	2	0
In the electric field	1000<	142	11

E. coli was cultivated on MacConkey agar medium containing kanamycin after mixedly pre-cultivated with *Pseudomonas* sp. in 6 and 9 volt of the electric field for 48 hr. However, several colonies of *E. coli* in conventional condition and in 3 volt of the electric field was observed as shown in Fig. 5 (upper). Table 1 shows number of *E. coli* grown on MacConkey agar plate containing kanamycin after mixedly pre-cultivated with *Pseudomonas* sp. in convenient condition and 9 volt of electric field, respectively.

4. Discussion

This study describes effect of electric field on transformation of *E. coli*. The results revealed that *E. coli* was transformed to kanamycin-resistant by incoming an kanamycin resistant gene from *Pseudomonas* sp, and transformation of *E. coli* was activated in the electric field. The genetic transformation was occurred in 6 and 9 volt of electric field but was not in 3 volt of electric field or in conventional culture. Generally, any bacterial cells may be naturally competent cells for genetic transformation[5,11,20] but efficiency of transformation was different or uncertain according to species.[4] Our data also demonstrate that probability of natural transformation is very rare (Table 1). The natural competence for transformation is dependent on growth conditions such as temperature, ionic strength, acidity, pressure and oxygen concentration.[10,11,12] The ionic strength or acidity variation activates or inhibits membrane-bound transferase and influences on membrane structure.[21,22] It is uncertain whether variation of membrane structure by ionic strength and acidity is related or unrelated to efficiency of genetic transformation, however, it is certain that electric pulse can activate movement of anion and cation in opposite direction, by which ionic strength or proton concentration of micro-environment around bacterial cells may be changed. In 9 volt of electric field, about 10^8 cells of *E. coli* was transformed to kanamycin resistant, however, growth of Pseudomonas and *E. coli* was not inhibited.

The electric field was reported to inhibit bacterial growth,[15] change the membrane permeability,[13] cell surface structure[16] and electroporation efficiency,[23] however, no result concerning genetic transformation of bacterial cell in less than 9 volt of electric field was reported. In case of electro-transformation method, at least higher than 1,000 volt of electric field or the higher than mega hertz of electric pulse has to be required, which is a technique for incoming of short DNA fragment into competent cells *in vitro* but different from genetic transformation of bacterial cell. On the basis of this study, we propose a hypothesis that the electric field formed by the lightening frequently generated may be an environmental factor to activate genetic transformation in natural ecosystem.[18] Fig. 6 shows an imaginary picture synthesized from pictures of the lightening and the ocean. As shown in Fig. 6, the

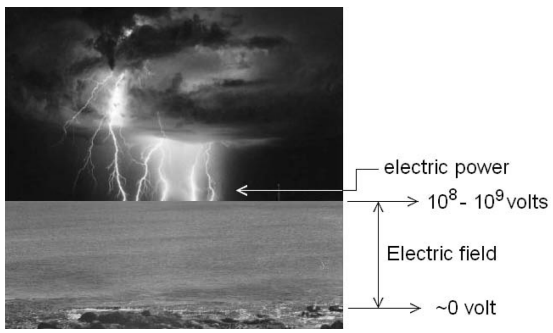

Fig. 6. Lightening functions like electric power for production of electric pulse between water surface and shore, and the sea or lake water functions as electrolyte. The electric power is about 108-109 volts at the lightening-striking point but getting lesser in proportional to the distance from the lightening-striking zone to the shore. It is possible that some microorganisms may be genetically affected by the electric field generated around zone struck by lightening in natural ecosystem.

electric field can be generated around the zone struck by lightening when lightening with gigantic electric energy is discharged to surface of the earth or water.

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