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Electroduction on *Azospirillum brasilense* Sp7 and *Chromobacterium violaceum*

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ABSTRACT

Electroduction is a fast, simple and very efficient transformation method with a wider range of bacteria strains which involved physical process by exposing to high electricity. A successful transfer of a 21.6 kb cosmid vector pLAFR1 into *Azospirillum brasilense* Sp7 and *Chromobacterium violaceum* was achieved with the maximum efficiency of about 35 X 10^2 and 24 X 10^2 transformants, respectively, at the electrical field strength of 5kV/cm with longer pulse length range between 8.3 and 8.5 milliseconds. Doubling the pulse length and a decrease or increase in the electrical field strength of 5kV/cm resulted in a reduction in the total number of transformants. The electroduction parameters were very important to make this transformation method works efficiently.

INTRODUCTION

Electroduction is a physical mechanism of genetic transformation that has an advantages and ability to solve many problems that were facing in traditional and old transformation methods [1,2]. Electroduction is able to direct transfer the vector or genetic materials from a bacteria donor cells into another bacteria recipient cells. Recipient cells are possible from the same species or a completely different species or genus from the donor cells. The ability to direct transfer a vector or genetic materials between two different types of bacteria have many advantages such as being able to bypass the need of vector or plasmid purification, saving time for analytic procedures, harvest the vector or other genetic materials from the bacteria that resist to regular cell lysis procedure, increase the yield of vector, reduce the bacteria contamination rate [3,4] and easier multiple host for functional screening of heterologous genes. These advantages make the electroduction method a very useful and powerful tool in molecular biology or genetic engineering.

To achieve high efficiency from the electroduction, selection of electric pulse parameters is very important. Each type of bacteria required a different range of parameters and experience to achieve a high efficiency of electroduction [2,4,5]. A large size of cosmid vector pLAFR1 is a very useful vector which is much larger than a common plasmid vector and able to insert a large DNA fragment up to 20 kb size [6,7]. To study the electroduction of cosmid vector pLAFR1 into other bacteria is the key to enhancing the limitation of the bacteria and to become more useful in genetic engineering.

The objective of this study was aimed to direct transfer a cosmid vector pLAFR1 into the recipients *Azospirillum brasilense* Sp7 and *Chromobacterium violaceum* by electroduction from the donor *Escherichia coli* K12 MM294 with maximum transformation efficiency.

MATERIALS AND METHODS

Bacteria strains, Cosmid DNA and Electroporation apparatus

Azospirillum brasilense Sp7 and *Chromobacterium violaceum* were chosen as a recipient because of their different colonial morphology compare to donor for easy identification. *Escherichia coli* K12 MM294 which contained pLAFR1 [6,7] a cosmid broad host range vector of 21.6 kb was chosen as a donor. pLAFR1 contained a cos site and a relaxation complex site with Tc^r as a marker. Gene Pulser Unit equipped with Gene Pulse Controller and electroporation cuvettes with interelectrode distance of 1 mm (BioRad) was used in all experiments. The capacitor and pulse controller resistance were set to 25 µF and 400 Ω to get a long pulse length at 10 milliseconds and additional setting at 25 µF and 800 Ω to get a

longer pulse length at 20 milliseconds for field strength 3kV/cm and 4kV/cm for comparison.

Preparation of Donor and Recipient for Electroduction

A donor cells *Escherichia coli* K12 MM294 containing cosmid pLAFR1 were grown on LB-agar plates containing 20 µg/mL tetracycline antibiotic in an incubator for 2 days at 30 °C. One loopful of *E. coli* colony from the LB-agar plates was inoculated into the LB broth and was grown for 16 h at 30°C with shaking until it reached a mid-logarithmic phase. Then, 1 mL of *E. coli* cells from the LB broth was transferred to a 1.5 mL tube and placed on ice for between 15 and 30 min and then centrifuged at 3622 ×g for 2 min. The pelleted cells were washed three times with cold sterile distilled water and lastly washed with 10% glycerol. Then the cells were resuspended in 0.2 mL 10% glycerol and kept on ice for 30 min. Recipient cells of *A. brasilense* Sp7 and *C. violaceum* were prepared in the same manner as the donor cells in LB media but without antibiotic. Both donor and recipient cells were mixed together in a 1.5 mL tube and vortexed at high speed for 3 s and was kept on ice again for 30 min.

Electroduction

The electroduction procedure was according to Summers and Withers [8] and Eynard and Teissie [9]. Exactly 60 µL aliquots of the prepared mixtures electroduction were transfer to the ice cold 0.1 cm electroporation cuvettes. The mixture was pulsed under Gene Pulser and a control where the pulse was omitted. 1 mL LB broth was added into the cuvette then transferred to 1.5 ml tube and incubated for only 3 h to prevent any genetic exchange through conjugation during the incubation period. One hundred-fold dilutions from the incubated tube were spread onto LB-agar plates containing 20 µg/mL tetracycline antibiotic with an alcohol-flamed glass rod. The Petri dish plates were incubated at 30 °C for 3 d. Only tetracycline-resistant recipient bacterial cells which had acquired the pure cosmid directly from donor bacteria cells by electroduction would grow on these plates.

RESULTS AND DISCUSSION

By using lower electric field strength and longer pulse length, the cosmid from donor *Escherichia coli* K12 MM294 was successful directly transferred into both recipients *Azospirillum brasilense* Sp7 (**Fig. 1**) and *Chromobacterium violaceum* (**Fig. 2**) by electroduction. In control experiment where the pulse was omitted, none of the electroductant of *A. brasilense* Sp7 and *C. violaceum* were obtained. The result of electroduction for *A. brasilense* Sp7 was shown on (**Table 1**) and *Chromobacterium violaceum* on (**Table 2**). Maximum transformation efficiency was obtained for *A. brasilense* Sp7 and *C. violaceum* at electric field strength of 5kV/cm with long pulse length range at 8.3 to 8.5 milliseconds with total number of electroductants 35 X 10^2 and 24 X 10^2 (**Fig. 3**). A decrease or increase in the electric field strength of 5kV/cm resulted a reduction in the total number of transformants.

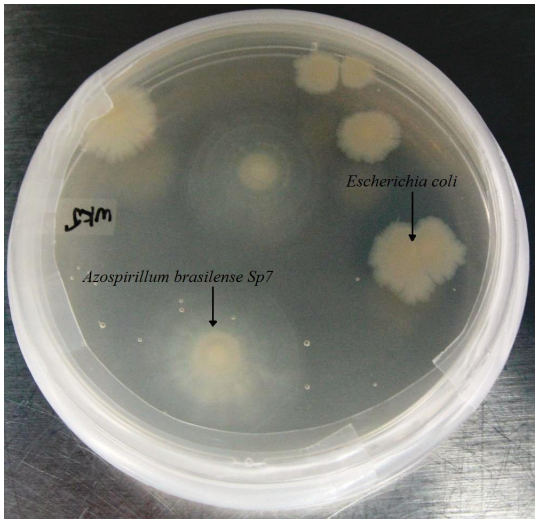

Fig 1. Electroductants of *Azospirillum brasilense* Sp7 and donor *Escherichia coli K12 MM294 coli* on LB agar plate contained tetracycline antibiotic.

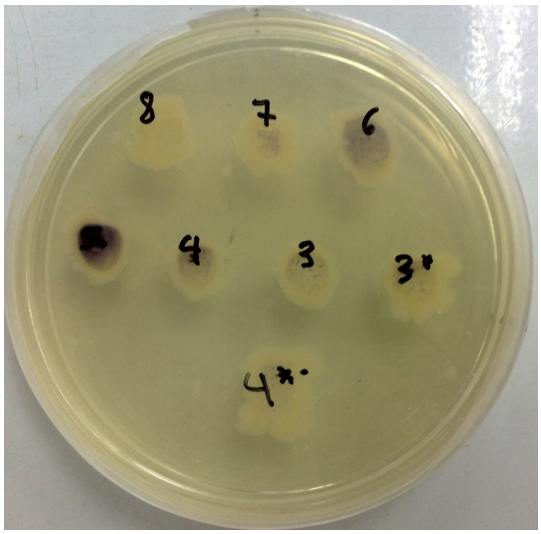

Fig 2. Electroductants of *Chromobacterium violaceum* and donor *Escherichia coli* on LB agar plates contained tetracycline antibiotic.

Table 1. Electroductants of *A. brasilense* colonies obtained from electroduction.

Field strength (kV/cm)	Pulse length (ms)	Total electroductants ($X10^2$), (Mean)
8	7.9	1
7	8.1	4
6	8.6	12
5	8.3	35
4	8.7	17
3	8.6	9
4	18.3	1
3	18.8	1

Table 2. Electroductants of *C. violaceum* colonies obtained from electroduction.

Field strength (kV/cm)	Pulse length (ms)	Total electroductants ($X10^2$), (Mean)
8	8.3	1
7	8.2	3
6	8.2	9
5	8.5	24
4	8.8	7
3	8.7	3
4	18.7	1
3	19.1	1

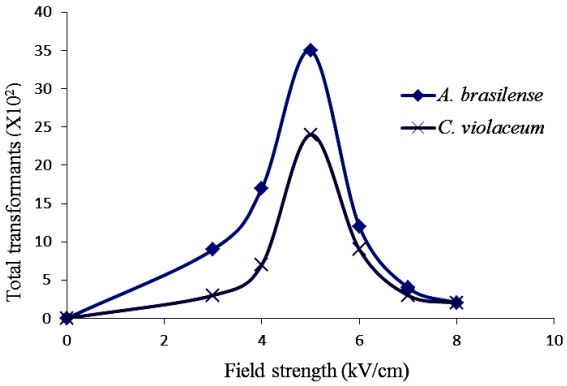

Fig 3. Transformation efficiency for *A. brasilence* and *C. violaceum* at pulse length range from 8.3 to 8.5 milliseconds.

Many of the unsuccessful electroduction researches were reported was using high electric pulse strength with short pulse length as the parameters [9]. From the results we achieved, we believed electroduction by using larger vector like cosmid pLARF1 required a smaller electric field strength and longer pulse length. Smaller electric field strength and longer pulse length will produce a bigger pores and giving a longer time for a bigger size cosmid to entering the recipient bacteria cells from the donor [1,2,4] although the exact mechanism of pore formation was still unknown but were believed it was form in very short time scale and continue to increase in size during the pulse.

However when we doubled the pulse length by setting the capacitor and pulse controller resistance to 25 µF and 800 Ω to obtained an extra-long pulse length range from 18.3 to 19.1 milliseconds with field strength 3 kV/cm and 4 kV/cm, the efficiency of electroduction was decreased dramatically to almost zero electroductant. When the size of pore diameter reached the upper limit threshold or exceed the critical range for the bacteria cell viability, the pore will become too large for the bacteria cell to recover by any spontaneous or biological process and will cause damage to the bacteria cell and affect the transformation efficiency [4,10,11]. These were the reasons why electroduction parameters play a very important role in transformation method.

CONCLUSION

The easy to perform and much more time efficient electroduction method with suitable electroduction parameters had successfully direct transfer a big size cosmid vector pLAFR1 from donor *Escherichia coli* K12 MM294 into the recipients *Azospirillum brasilense* SP7 and *Chromobacterium violaceum* efficiently. The efficiency of this electrical method was basically much better than most of the alternative or old transformation methods. This electroduction methodology will be very helpful in future studies that are related to genetic engineering and molecular biology.

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