Biotechnol. Bioprocess Eng. **1998**, 3, 48-49

Efficient Transformation of *Klebsiella oxytoca* by Electroporation

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A protocol for the transformation of *Klebsiella oxytoca* by electroporation was developed. Preparation of competent cells at early exponential phase was most critical to obtain a high transformation efficiency. The highest efficiency of 1.6×10^6 transformants per μg DNA (pBR 322) could be obtained by electroporation of *K. oxytoca* cells prepared at the OD_{600} of 0.2 with 1.25 μg DNA at the filed strength of 2.5 kV, the parallel resistance of 200 Ω and capacitance of 25 μF.

Key words: *Klebsiella oxytoca*, electroporation, efficiency

INTRODUCTION

Since the first development of electroporation technique for the transformation of eucaryotic cells by Zimmerman [1], it has become increasingly popular as a tool for transforming bacterial cells. Although the exact mechanism for the electroporation is not known yet, it is believed that the electric field polarizes the membrane components and results in a voltage potential across the membrane. When the potential exceeds a threshold level, the membrane breaks down, resulting in localized reversible openings. This allows the passage of DNA [2]. This simple technique is especially valuable for the transformation of bacteria, for which existing transformation methods are unreliable, nonexistent, or less efficient. Various cell types differ in the conditions required for efficient uptake of DNA [3]. So, it is necessary to test a range of conditions for a given organism if the procedure is to work with the maximum efficiency.

A new *Klebsiella oxytoca* which shows desulfurization activity was isolated from oil-contaminated soil [4]. To construct a recombinant *Klebsiella oxytoca* strain possessing enhanced desulfurization activity, an efficient transformation method needs to be developed. Several methods have been reported for transferring plasmid DNA into *Klebsiella* by conjugation [5, 6] or by electroporation [7, 8]. However, the transformation efficiencies obtained were rather low at 10^2-10^4 transformants per μg DNA. The objective of this study was to develop an optimal electroporation condition for *Klebsiella oxytoca* to obtain a higher transformation efficiency.

Klebsiella oxytoca A23-3 [4] and pBR322 were used as the host strain and plasmid, respectively. The plasmid pBR322 was prepared from *E. coli* XL1-Blue [*supE*44 *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi relA*1 *lac* F' (*proAB*$^+$ *lacI*^{q} *lacZ*ΔM15 *Tn*10(tet^r))] using QIAGEN Plasmid Maxi Kit (QIAGEN Inc., USA). The fraction of supercoiled form was greater than 90%. Plasmid DNA concentration was determined by measuring the OD_{260} and verified by agarose gel electrophoresis. Cells were cultured in Luria-Bertani medium (1% tryptone, 0.5% yeast extract, 1% NaCl) at 37°C. When necessary, ampicillin (Ap) and kanamycin (Km) were added at the concentrations of 50 and 25 μg/mL, respectively.

K. oxytoca A23-3 cells were grown at 37°C, and were harvested at various phases of growth (OD_{600} of 0.2, 0.3, 0.4 and 0.8) by centrifugation (5500 rpm, 10 min, 4°C). Cell pellet was washed sequentially with 250 mL of ice-cold double-distilled water (ddH_2O) and 20 mL of 10% (w/v) cold glycerol, and was finally resuspended in an appropriate volume of 10% cold glycerol (the final OD_{600} of 100) and stored at − 75°C.

Electroporation was carried out using the BioRad Gene Pulser apparatus (BioRad Laboratories, Richmond, Calif.). Purified plasmid DNA (25, 50, 75 or 100 ng) was mixed with 40 μl of ice-chilled cell suspension, and the mixture was transferred to an ice-chilled electroporation cuvettes (2-mm electrode gap, BioRad). Electroporations were carried out at various conditions: field strength of 8-12.5 kV/cm; parallel resistance of 200 or 400 Ω; capacitance of 25 μF. All experiments were carried out in triplicates. Immediately after the pulse delivery, the cells were resuspended in 1 mL of LB and incubated at 37°C for 1 h with shaking (250 rpm). Dilutions were plated on LB agar plate containing the appropriate antibiotics and incubated at 37°C. The transformation efficiency was expressed as the mumber of transformants per μg DNA.

Using the competent cells prepared at four different growth stages (OD_{600} of 0.2, 0.3, 0.4 and 0.8), electroporation experiments were carried out with 100 ng of plasmid DNA under various conditions (Fig. 1). Under all conditions examined, competent cells prepared at the OD_{600} of 0.2 gave a considerably higher transformation efficiency than the others. A higher transformation efficiency was obtained at the time constant of 4.4 ms (parallel resistance 200 Ω) compared with that obtained at the time constant of 8.5 ms (parallel resistance 400 Ω). Increased cell death was responsible for the lower transformation efficiency at higher time constant (data not shown). Field strength was varied from 8-12.5 kV/cm. The 12.5 kV/cm resulted in the highest transformation efficiency. Transformation was inefficient when the field strength was lower than 11 kV/

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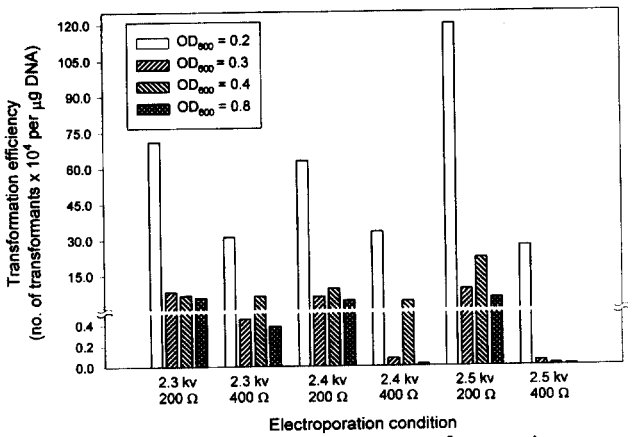

Fig. 1. Transformation efficiency under various conditions.

cm (data not shown). The optimal electroporation condition for the transformation of *Klebsiella* was: competent cell preparation at the OD_{600} of 0.2, field strength of 12.5 kV/cm, parallel resistance of 200 Ω (time constant of 4.4-4.5 ms), capacitance of 25 μF. Under this condition, the transformation efficiency of as high as 1.2×10^6 transformants per μg DNA could be obtained.

The effect of the varying amount of plasmid DNA on transformation efficiency was examined next. Experiments were carried out under the optimal condition described above. The transformation efficiencies obtained with 25, 50, 75 and 100 ng of pBR322 in 40 μL electroporation mixture were 8.2×10^5, 1.6×10^6, 1.1×10^6 and 1.2×10^6 transformants per μg DNA. The highest transformation efficiency was obtained at the DNA concentration of 1.25 μg per mL (50 ng in 40 μL of competent cells).

To examine the general efficiency of electroporation protocol, another plasmid pJKJ101 was used to transform *K. oxytoca* A23-3. The plasmid pJKJ101 is 11.3 kb in size and contains pUC19 backbone and kanamycin (Km) resistance gene. The transformants were selected on LB-Km plate and the efficiency of electroporation was 9.3×10^5 transformants per μg DNA. Since a relatively large plasmid pJKJ101 could transform *K. oxytoca* efficiently, the protocol developed in this study seems to be generally applicable.

The electroporation protocol described in this paper allows transformation of *Klebsiella* with an efficiency of more than 20 fold higher than those reported previously [8], and therfore should be useful for genetically engineering *Klebsiella* strains.

Acknowledgement This work was supported by the R & D Management Center for Energy and Resources of the Korea Ministry of Trade and Industry.

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