Simple and Highly Efficient Transformation Method for *Zymomonas mobilis*: Electroporation

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There are some transformation methods for *Zymomonas mobilis*, a recently reported spheroplast procedure by Yanase et al. and a CaCl₂ procedure by Su et al. The frequency of these methods were about 2 × 10⁶/μg DNA. But these methods were very complicated and time-consuming. We have developed a very easy and rapid transformation method using electroporation, which can give us a high transformation efficiency. The use of an electric field to reversibly permeabilize cells (electroporation) has become an important technique to introduce nucleic acids into eucaryotic and procaryotic cells. Here we report a method to reproducibly electroporate plasmid DNA into *Z. mobilis* based on modification of the protocol developed by Dower et al.

*Z. mobilis* IFO 13756 was grown overnight at 30°C in RM medium (1% yeast extract (Oxoid), 2% glucose, and 0.2% KH₂PO₄, pH 6.0) with stationary culture. A portion (5 ml) of the culture was transferred into 100 ml of RM medium, and incubated at 30°C for 4 hr until early log phase (ABS₆₀₀ 0.3–0.4). The cells were harvested by centrifugation at 1,500 × g for 5 min at 4°C, and resuspended in 2 ml of glycerol sol. containing 10% glycerol and 0.85% NaCl (about 3 × 10⁸ CFU/ml). The cell suspension was chilled on ice for 15 min before electroporation. This cell suspension can be stored at −80°C for more than 1 year. DNA of a shuttle vector, pZA22 was prepared from *E. coli* C600 by the alkaline lysis method of Birnboim and Doly, and dissolved in water at the concentration of 3.0 μg/μl. Exponential decay pulses were generated by a Gene Pulser and Pulser Controller (Bio-Rad Laboratories, Richmond, CA). Two hundred μl samples of cell suspension were mixed with 10 μl of the DNA solution and transferred to chilled 0.2 cm gap cuvettes (Bio-Rad). Following delivery of the electric pulse, the cells were mixed with 1 ml of RM medium and kept for 3 hr at 30°C. The cells were diluted with RM medium and plated on a RM agar plate with tetracycline at the concentration of 30 μg/ml or chloramphenicol at 100 μg/ml. The transformation efficiency was calculated at CFU/μg of plasmid DNA.

Figure 1 shows the effects of field strength on transformation efficiency at an exponential decay constant of 4.5 × 10⁻⁷ sec. The max efficiency (2.5 × 10⁶/μg DNA) was obtained at 10 kV/cm. The reporter genes (β-galactosidase gene, β-galactosidase gene, and CMCase gene*) on pZA22 can transform cells under these conditions. The transformants have been examined for plasmids and for gene expression. The plasmids of transformants can be observed by agarose gel electrophoresis and the gene expression can be detected by a plate assay. The transformants carried the plasmids stably and the reporter genes expressed a high enough level. We have examined the strength of the initial electric field for other *Zymomonas* type strains. The best conditions for *Z. mobilis* ATCC 10988 and *Z. mobilis* NRRL B-14023 are both 7.5 kV/cm. The frequency of ATCC 10988 is 1.0 × 10⁶/μg DNA and NRRL B-14023 is 2.0 × 10⁵/μg DNA.

Fig. 1. The Effects of Field Strength on Transformation Efficiency for *Z. mobilis* IFO 13756.

References