Note

Application of Electroporation for Transformation in *Erwinia carotovora*

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*E. carotovora* is a phytopathogenic bacteria causing soft-rot disease, due to pectinolytic enzymes that degrade plant cell walls. In order to clarify the regulation-mechanism for the multiple pectinase production and the relationship between the pathogenicity and pectinase production, detailed analyses of these phenomena are required on the gene level. Although conjugation and transduction in *E. carotovora* have been used for genetic analysis, they are not enough for genetic research on this bacterium. Therefore, it is very important to establish an effective transformation system in *E. carotovora*. Reverchon and Robert-Braudouy reported the establishment of a transformation system in *E. chrysanthemi*, having used the transformation procedure involving CaCl2 for *E. coli*. The efficiency of their transformation system was about $6 \times 10^2$ transformant cells per /ig DNA. When we used their protocol for the transformation of plasmid pBR 329 in *E. carotovora* Er, the efficiency was about 50 transformant cells per /ig DNA, which was too low for utilization of this procedure for self-cloning in *E. carotovora* Er. The ampicillin gene on pBR329 could be expressed in *E. carotovora* although the efficiency of transformation was lower than that in *E. coli*. Next, we tried to use the electroporation method, which is known to be useful for introducing DNA into animal or plant cells, for the transformation of the plasmid in *E. carotovora* Er. The basic methods used were those in the manual of Gene Pulser (Bio-Rad Lab). Cells of *E. carotovora* Er were grown in LB medium containing 1% polypeptone, 0.5% yeast extract and 0.5% NaCl, pH 7.0, at 30°C with shaking. The cells were harvested at the middle of the logarithmic growth phase, OD600~0.5, by centrifugation, resuspended in the transformation buffer (1/20 volume of the culture) containing 272 mM sucrose, 1 mM MgCl2 and 7 mM sodium phosphate, pH 7.4, and then washed once with the same buffer. One-tenth /ig of pBR329 was added to 0.8 ml of the cell suspension obtained as above, followed by incubation on ice for 20 min. The cell suspension containing plasmid DNA was transferred to cuvettes and then pulsed at various voltages, at 25 /ig/ml capacity. After pulsing, the cell suspension was incubated on ice for 20 min. Five ml of fresh LB medium was added to the cell suspension, followed by incubation for 1 hr at 30°C with shaking. The incubated cell suspension was plated out on LB agar medium containing 20 /ig/ml ampicillin for selecting transformant cells. The results are shown in Fig. 1. The transformation efficiency increased with increasing voltage, while the survival had decreased to 24% at the voltage of 2.5 kV. The maximum efficiency of transformation was about $2 \times 10^7$ transformant cells/ /ig DNA at 2.5 kV. When plasmid pNN101 containing the pectate lyase I gene from *E. carotovora* Er was used, almost the same efficiency was obtained (data not shown). The presence of pBR329 or pNN101 in the

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transformant cells was confirmed by the miniscreening method for plasmid DNA detection. These results indicate that this transformation method is very effective and might be useful for various genetic studies on *E. carotovora* Er. However, the effectiveness of this transformation method was limited to this strain, and we could not detect any transformant cells when other *E. carotovora* strains, T 29 and Ar, were used as recipient strains. *E. carotovora* T 29 and Ar were kindly donated by Dr. H. Tsuyama. We are now trying to improve this system as a general transformation method for *E. carotovora*.

REFERENCES