

Genetic transformation of *Pseudomonas oleovorans* by electroporation

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An electroporation procedure for the transformation of *Pseudomonas oleovorans* was developed using a model plasmid, pCN51. The optimal electrotransformation was achieved with cells harvested at 45 to 60 min of growth and concentrated to a cell density of $5 \text{ OD}_{600\text{nm}}$, plasmid concentration of $6 \mu\text{g}$ per $100 \mu\text{l}$ of cell suspension, and a 0.1-cm gap-width cuvette. Electroporation was performed at the settings of 250Ω , $25 \mu\text{F}$ and 2.5 kV. Transformation yields in the order of 10^3 colony-forming-unit per electroporation sample were obtained. This is a first report of the electroporation of the commercially valuable bacterium *Ps. oleovorans*.

Keywords: *Pseudomonas oleovorans*, electroporation, transformation, poly(β -hydroxyalkanoate), alkane

Introduction

Pseudomonas oleovorans carries out a wide variety of interesting biological activities, such as the synthesis of a class of biodegradable polymers, the medium-chain-length poly(β -hydroxyalkanoates) (de Smet *et al.*, 1983; Gross *et al.*, 1989; Cromwick *et al.*, 1996); the dissimilation of alkane compounds (Chakrabarty *et al.*, 1973); and the ω -oxidation, hydroxylation and epoxidation of various hydrocarbons, alkenes and fatty acids (Peterson *et al.*, 1966; Schwartz, 1973; Abbott and Hou, 1973; Besse and Veschambre, 1994; Archelas and Furstoss, 1997).

Genetic modification could expand the usefulness of *Ps. oleovorans* in various industrial applications. Previous efforts in the genetic engineering of this organism have used the efficient but complicated conjugational transfer procedures that are often species-specific (Chakrabarty *et al.*, 1973; Steinbüchel and Schubert, 1989). Jahng and Wood (1994) reported an unsuccessful electroporation attempt to transform a *Ps. oleovorans* strain. In this communication, the development of an electrotransformation procedure for this pseudomonad species is described. A 5.9-kb shuttle plasmid (pCN51) that contains pseudomonad pPS10 and *E. coli* pMB9 replicons, and a kanamycin-resistance determinant, was used in this study (Nieto *et al.*, 1990). The electroporation procedure presented in this paper provides for a simple means of genetically modifying *Ps. oleovorans*.

Materials and methods

Bacterial strains, plasmids, and growth conditions

Ps. oleovorans NRRL B-14683 and *Escherichia coli* DH5 α were obtained from the NCAUR/ARS/USDA (Peoria, IL) and Life Technologies (Gaithersburg, MD), respectively. Bacteria containing the plasmids pCN51 (Nieto *et al.*, 1990) and pSG312 were purchased from ATCC (Manassas, VA). All microorganisms were grown in Luria medium (1% w/v tryptone; 0.5% w/v yeast extract; 0.5% NaCl). Solid medium was prepared in agar (1.0–1.2%). When needed, ampicillin (100 $\mu\text{g}/\text{ml}$) or kanamycin (30 $\mu\text{g}/\text{ml}$) was included in the culture medium. The pseudomonads and *E. coli* were grown at 30°C and 37°C, respectively.

Molecular biology procedures

Mini-plasmid screening was performed by an alkaline lysis procedure (Sambrook *et al.*, 1989). Large-scale plasmid isolation was carried out by using a Plasmid Midi Kit (Qiagen Inc., Valencia, CA). Restriction enzymes were purchased from Life Technologies and New England Biolabs (Beverly, MA). Agarose gel electrophoresis of DNA was performed in the TBE buffer system (0.089 M Tris base, 0.089 M boric acid, 0.002 M sodium EDTA).

Electroporation

Cells from an overnight culture (50 ml) were harvested by centrifugation ($6,500 \times g$; 15 min; 4°C), washed once in a cold 0.3M sucrose solution (10 ml), and resuspended to a selected cell density in the same solution. The cell suspension (100 μl) was transferred to an electroporation cuvette (Bio-Rad Laboratories, Hercules, CA). A desired amount

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