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AN IMPROVED METHOD OF TRANSFORMATION IN PSEUDOMONADS

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Summary

A protocol for producing competent *Pseudomonas aeruginosa, Pseudomonas putida,* and *Xanthomonas maltophilia* was adapted and modified from existing methods. Cells were incubated on ice for 30 minutes in buffered 100 mM $MgCl_2$ followed by 30 minutes in buffered 100 mM $CaCl_2$ prior to addition of DNA. The $MgCl_2$-$CaCl_2$ incubation increased transformation efficiency two to three times, compared with protocols which use incubation in either Mg^{2+} or Ca^{2+}, but not both.

Introduction

Three methods have been widely adopted for transformation of *Pseudomonas* with plasmid DNA. The first method is triparental filter mating (conjugational mating), the second is direct transformation of competent host cells, and the third is electroporation.

In direct transformation of *Pseudomonas* by plasmid DNA competence is increased through a variety of treatments that alter the permeability of the cell envelope (such as exposure to elevated concentrations of calcium or magnesium salts [Bagdasarian *et al.*, 1979; Mercer and Loutit, 1979; Smith *et al.*, 1981]). We have combined and modified certain of these methods, with the result of a several fold increase in transformation efficiency.

Experimental Methods

Plasmid pSC160 (a 16.0 kb plasmid constructed in our laboratory)(Liu *et al.*, 1995) contains the *Vitreoscilla* hemoglobin gene (Dikshit and Webster, 1988) on a 1.4 kb fragment joined to the *E. coli* vector pUC8 (Messing, 1983) and cloned into the EcoRI site of broad host range vector pKT230 (Bagdasarian *et al.*, 1981). Extraction and purification of pSC160 using alkaline lysis and polyethylene glycol differential precipitation from both Pseudomonads and *E. coli* was a scaled-down version of the method of Sambrook *et al.* (1989). The typical yield of plasmid extracted from Pseudomonads by the above method is about 0.1-0.5 μg of DNA per milliliter of overnight bacterial culture.

The procedure described below was used for transformation of members of the

Pseudomonaceae; it was combined and modified from Bagdasarian *et al.* (1979) and Mercer and Loutit (1979).

1. One ml of an overnight Luria broth (LB) culture is transferred into 50 ml fresh LB and the incubation continued until the OD_{650nm} reaches approximately 0.6 (normally this takes about 4 hours).
2. The cells are collected by centrifugation at 4°C, and the cell pellet resuspended in 10 ml of 100 mM $MgCl_2$, 10 mM PIPES (Piperazine-1,4-bis[2-ethanesulfonic acid]), pH 7.2; the cells are then incubated on ice for 30 minutes.
3. The cells are collected by centrifugation again, and then gently resuspended in 0.5 ml of 100 mM $CaCl_2$, 10 mM PIPES, pH 7.2, 15% (v/v) glycerol, followed by a 30 minute incubation on ice. At this point the competent cells are ready for transformation.
4. To 100 µl of the competent cells from above, 10 µl (approximately 100 ng) of purified plasmid is added, mixed well and the mixture incubated on ice for 30 minutes.
5. The mixture is heat shocked at 42°C for 2 minutes and immediately chilled on ice for 1 hour.
6. One ml of prewarmed (35°C) LB is then added to the mixture and 60 minutes of incubation at 35°C are allowed (with shaking) for the expression of drug resistance.
7. In order to select transformants 50 or 100 µl of each transformation mixture is plated on PIA (*Pseudomonas* Isolation Agar, Difco) agar plates containing amounts of antibiotics that prohibited growth of the untransformed strains (for *P. aeruginosa*, 300 µg kanamycin/ml and 500 µg Na ampicillin/ml; for *P. putida*, 100 µg kanamycin/ml and 200 µg Na ampicillin/ml; for *X. maltophilia*, 80 µg kanamycin/ml and 150 µg Na ampicillin/ml). Both antibiotics were obtained from Sigma Chemical Co.

Results

Three metal ion treatments were used to make competent cells. Two used either calcium or magnesium alone using the protocols and the optimal concentrations of Mercer and Loutit (1979) for $MgCl_2$ (150 mM) and Bagdasarian *et al.* (1979) for $CaCl_2$ (100 mM). The third used our protocol (described above) and successive treatments with $MgCl_2$ and $CaCl_2$ at concentrations at or near these optima (100 mM each). We found, for these three species of the *Pseudomonaceae*, that when the successive treatments with 100 mM $CaCl_2$ and 100 mM $MgCl_2$ were used, the transformation efficiency was two to three times higher than with either metal ion alone (Table 1). Initially, we were able to transform *P. aeruginosa* and *X. maltophilia* with plasmid extracted from *E. coli*. Similar to reports of Bagdasarian *et al.* (1979), and presumably due to possession by *P. putida* of a powerful restriction system, *P. putida* was able to be transformed by plasmid from *P. aeruginosa*, but not by plasmid from *E. coli*.

The initial transformants produced in this way showed some plasmid instability, so plasmid pSC160 was isolated (as described above) from each of the three initial transformants and used to transform each homologous strain (i.e., pSC160 from *X. maltophilia* to transform *X. maltophilia*; pSC160 from *P. putida* to transform *P. putida*; and pSC160 from *P. aeruginosa* to transform *P. aeruginosa*). Again, cells of each species

were made competent using our protocol (successive 100 mM $CaCl_2$ and 100 mM $MgCl_2$ treatments). In this second round of transformation (Table 2) the efficiency increased by about 10-100 times compared with the initial transformation (probably because DNA methylation of pSC160 in the initial transformants protected against restriction). This level is similar to that achieved in *P. aeruginosa* by electroporation (Diver *et al.*, 1990). In addition, pSC160 was stable in all three strains after this second round of transformation.

Table 1. Comparison of transformation efficiencies of competent cells prepared by different chemical treatments. Treatment with both $CaCl_2$ and $MgCl_2$ (as described in the text) was compared with treatment with $MgCl_2$ alone (as described by Mercer and Loutit, 1979) or $CaCl_2$ alone (as described by Bagdasarian *et al.*, 1979). Figures are the averages of 3 independent measurements (standard deviations are in parentheses).

Species	Chemical Treatment	Transformation Efficiency (transformants/μg DNA)
P. aeruginosa	150 mM $MgCl_2$	5.2×10^3 (2.1×10^3)
	100 mM $CaCl_2$	4.6×10^3 (1.7×10^3)
	100 mM $MgCl_2$ and 100 mM $CaCl_2$	1.2×10^4 (4.1×10^3)
P. putida	150 mM $MgCl_2$	2.9×10^2 (1.4×10^2)
	100 mM $CaCl_2$	1.7×10^2 (3.1×10^1)
	100 mM $MgCl_2$ and 100 mM $CaCl_2$	6.1×10^2 (1.1×10^2)
X. maltophilia	150 mM $MgCl_2$	3.3×10^3 (1.2×10^3)
	100 mM $CaCl_2$	4.7×10^3 (1.9×10^3)
	100 mM $MgCl_2$ and 100 mM $CaCl_2$	8.9×10^3 (2.3×10^3)

Table 2. Results of the second round of transformation using, for each species, plasmid prepared from the initial transformant of the same species. Values are the averages of 2 independent measurements (standard deviations are in parentheses).

Species	Chemical Treatment	Transformation Efficiency (transformants/μg DNA)
P. aeruginosa	100 mM $MgCl_2$ and 100 mM $CaCl_2$	1.9×10^5 (4.8×10^4)
P. putida	100 mM $MgCl_2$ and 100 mM $CaCl_2$	5.3×10^4 (2.4×10^4)
X. maltophilia	100 mM $MgCl_2$ and 100 mM $CaCl_2$	1.1×10^5 (4.5×10^4)

Discussion

A combination of Mg^{2+} and Ca^{2+} treatments has been used by other researchers to produce competent Pseudomonads (Sano and Kageyama, 1977; Sinclair and Morgan, 1978; Mercer and Loutit, 1979). In these previously reported protocols, however, the Mg^{2+} treatment is only a wash, as opposed to our 30 min incubation.

Currently, we use the successive metal ion treatment as a standard method for preparation of competent cells of several species of Pseudomonads. We have also found that it increases the efficiency of transformation of *Enterobacter aerogenes* with *E. coli* derived plasmids (L. Colon, personal communication). It is easy, reliable, inexpensive and efficient, thereby offering many advantages over electroporation as well as conventional chemical treatment transformation protocols.

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