Enhancement of Transformation in *Pseudomonas aeruginosa* PAO1 by Mg\(^{2+}\) and Heat

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Genetic exchange in *Pseudomonas aeruginosa* may be accomplished through conjugation, transformation and transduction (6). Standard molecular techniques used in *E. coli* often must be modified for *P. aeruginosa*. Specialized techniques have been developed in *P. aeruginosa* to accomplish genetic exchange more efficiently, which include triparental mating (conjugation) (3), electroporation (1) and chemical and physical cell treatment before transformation (7). Without these specialized techniques, the efficiency of exchange is drastically diminished.

One method to effect transformation in *P. aeruginosa* involves the preparation of competent cells by cold shock of either CaCl\(_2\)- or MgCl\(_2\)-treated cells followed by their transformation at 37°C (4,5,8). In this report, we describe a modification of this competency and transformation procedure, which results in at least a tenfold increase in the number of *P. aeruginosa* PAO1 transformants. The procedure involves MgCl\(_2\) treatment for a competent cell preparation followed by heat pulse at 50°C during transformation. To facilitate its use, the new competency (Table 1) and transformation (Table 2) procedures are given as a step-by-step protocol.

Results obtained from a transformation experiment utilizing MgCl\(_2\) and a transformation experiment using CaCl\(_2\) at 50°C (Figure 1) indicate that when MgCl\(_2\) is used in combination with a 50°C-heat-pulse treatment, a fourfold increase in the number of transformants is obtained (Mann-Whitney U-test, U = 0.0, P = 0.05; Figure 1) (10). Interestingly, colony-forming units (cfu) counts for CaCl\(_2\)-treated cells subjected to a 37°C or a 50°C-heat-pulse treatment were not significantly different (Figure 1). Furthermore, MgCl\(_2\) treatment followed by heat treatment at 37°C had little effect on the resulting transformants (Figure 1). Freezing of the competent cells had no effect on the efficiency of transformation.

Previously reported competency and transformation results using MgCl\(_2\) at 37°C yield transformants of 1.32 × 10\(^5\)/µg of DNA (8), which are comparable to those represented in our controls (1.35 × 10\(^5\)/µg of DNA). In contrast, the new method reported here

![Figure 1. Comparative analyses of *P. aeruginosa* when treated with CaCl\(_2\) and MgCl\(_2\) at 37°C and 50°C. All results represent triplicate measures and are expressed as mean ± S.D. In all cases, number of CFUs for MgCl\(_2\)-treated *P. aeruginosa* cells heat-shocked at 50°C was significantly higher compared to other treatments.](image-url)
Benchmarks

Table 2. Transformation of Competent P. aeruginosa Cells with Plasmid DNA

1. Add 200 µL of competent cells and 10 µL (1 µg) plasmid DNA (in this case, pUCP18 obtained by mini-prep) to a chilled microcentrifuge tube. Mix and incubate the tube on ice for 1 h.
2. Transfer the tubes to a 50°C water bath for 3 min with swirling.
3. Immediately transfer this DNA-cell mixture to ice and incubate for 5 min.
4. Add 1 mL of pre-warmed LB broth to this mixture and incubate the tube in a 37°C water bath for 2 h with gentle swirling. Plasmid pUCP18 is a shuttle vector for gram-negative bacteria and has a marker, which encodes β-lactamase. This allows for selection of ampicillin resistance (Ap') when residing in E. coli and carbenicillin resistance (Cb') when residing in P. aeruginosa (9). In this step, we are expressing the Cb' marker after the heat- and cold-shock treatments (steps 2 and 3).
5. Plate out 0.1 mL of the transformation mixtures on LB/Cb plates (500 µg/mL) and incubate them at 37°C for 24 h.
6. Count the number of transformants obtained after incubation.

yields 1.2 × 10^6/µg of DNA, approximately 10-fold higher. According to Diver et al., optimal conditions for electroporation of P. aeruginosa PAO1 with pUC19 yielded 1.5 × 10^5 transformants/µg of DNA, which is approximately 10 times lower than our procedure (2). Therefore, we believe that this modified procedure should be useful to those performing molecular techniques that involve DNA transfer with P. aeruginosa PAO1.

REFERENCES


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