TRANSFORMATION OF *PSEUDOMONAS AERUGINOSA* BY PLASMID DNA

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Genetic transformation of *P. aeruginosa* by the drug-resistance factor DNA (RP4) is reported. Transformants retain all the drug-resistance characteristics present in the parent plasmid.

Genetic transformation has proved to be one of valuable techniques in studying genetic fine structures of several microorganisms. KHAN and SEN (1, 2) claimed to have succeeded in genetic transformation with chromosomal DNA in several strains of *Pseudomonas aeruginosa* and *P. fluorescens*. However, this work could not be repeated in our hands nor by BOOKER and LOUTIT (3). Recently it was shown that CaCl_2 treatment was effective for the plasmid-mediated transformation in gram-negative bacteria such as *E. coli*, *S. typhimurium* and *P. putida* (4–6). In this paper, we report the transformation in *P. aeruginosa* with a drug-resistance factor DNA.

MATERIALS AND METHODS

The bacterial strains and plasmids used in these experiments are listed in Table 1. RP4 is a large drug-resistance factor which specifies resistance to carbenicillin, kanamycin, neomycin, and tetracycline, and sensitivity to phage PRR1 (7) and is known to be transmissible between *E. coli* and *P. aeruginosa* by conjugation (7, 8).

The procedure used for the isolation of plasmid DNA was according to that of MATSUBARA, TAKAGI, and MUKAI (9). Concentration of DNA was determined by the absorption at 260 nm. *P. aeruginosa* and *E. coli* were grown in a nutrient broth at 37°. At the late exponential phase, the cells were harvested by centrifugation and washed once with 100 mM MgCl_2 (0.5 volume of the original culture). The cells were centrifuged and resuspended in one-half the original volume of 30 mM CaCl_2 , kept at 0° for 20 min. After centrifugation at 0°, the cells were resuspended in 0.1 volume of 30 mM CaCl_2 . Competent cells (0.2 ml) thus obtained were mixed with 0.1 ml of RP4 DNA solution in TEN buffer (0.02 M Tris(hydroxy-

Table 1. Bacterial strains used.

Bacterial strains	Properties	Source
<i>E. coli</i> C600	thr ⁻ , leu ⁻ , thi ⁻	
<i>E. coli</i> C600 (RP4)	thr ⁻ , leu ⁻ , thi ⁻ , Cb ^r , Km ^r , Nm ^r , Tc ^r	this laboratory
<i>E. coli</i> C600 res ⁻ mod ⁻	thr ⁻ , leu ⁻ , thi ⁻ , res ⁻ , mod ⁻	T. Tanaka
<i>P. aeruginosa</i> P14	prototroph	this laboratory
<i>P. aeruginosa</i> P14 (RP4)	prototroph, Cb ^r , Km ^r , Nm ^r , Tc ^r	this laboratory
<i>P. aeruginosa</i> M12	trp ⁻	this laboratory (PAO subline)
<i>P. aeruginosa</i> PU21 (RP4)	leu ⁻ , ilv ⁻ , rif ^r , Sm ^r , Cb ^r , Km ^r , Nm ^r , Tc ^r	G.A. Jacoby (PAO subline)

Abbreviations used: Cb, carbenicillin; Km, kanamycin; Nm, neomycin; Tc, tetracycline; res, restriction; mod, modification.

methyl)-aminomethane (pH 8.0)—1 mM EDTA—0.02 M NaCl)—30 mM CaCl₂ and incubated at 0°. After 60 min, bacteria were subjected to a heat pulse at 42° for 2–10 min, chilled, and then plated directly onto a nutrient agar containing carbenicillin. When transformants were selected by other antibiotics besides carbenicillin, the mixture was diluted 10 times with a nutrient broth containing 0.4% KNO₃ and incubated overnight at 37° before plating. Transformants were selected on a nutrient agar containing carbenicillin 500 µg/ml, kanamycin 300 µg/ml, or tetracycline 150 µg/ml in the case of *P. aeruginosa* and carbenicillin 500 µg/ml in the case of *E. coli*. Washing cells with 100 mM MgCl₂ was most effective compared to washings with 10 and 100 mM MgCl₂, CaCl₂, or NaCl. The optimum con-

Fig. 1. Effect of concentration of R factor DNA on transformation frequency. Various concentration of RP4 DNA isolated from PU21 (RP4) was added to CaCl₂-treated *P. aeruginosa* M12. The transformation frequency was determined by counting carbenicillin-resistant colonies.

centration of CaCl_2 was 30 mM and could be substituted by the same concentration of MgCl_2 .

RESULTS AND DISCUSSION

A typical result of transformation with this procedure using purified RP4 DNA is shown in Fig. 1. The transformation frequency depended on the DNA concentration and was about 10^{-6} per viable cell at 10 $\mu\text{g/ml}$ DNA. Viability after CaCl_2 treatment was 30–40%. The frequency between different *P. aeruginosa* strains showed somewhat reduced values, possibly because of restriction (Table 2).

Table 2. Comparison of transformation frequency between *P. aeruginosa* strains.

Source of DNA	Recipient cells	
	M12 (PAO)	P14
PU21 (RP4) (PAO)	1*	0.23
P14 (RP4)	0.02	1**

Each recipient was treated with plasmid DNA (23–25 $\mu\text{g/ml}$) from the same or different strain. Transformants were selected with carbenicillin. Ratios of transformation frequency are shown.

* 6.5×10^{-7} /viable cell, ** 2.3×10^{-7} /viable cell.

Table 3. Transformation efficiency between *P. aeruginosa* and *E. coli*.

Source of DNA	Recipient cells		
	<i>P. aeruginosa</i> P14	<i>E. coli</i> C600	<i>E. coli</i> C600 $\text{res}^- \text{mod}^-$
<i>P. aeruginosa</i> P14 (RP4)	1	4×10^{-4}	1
<i>E. coli</i> C600 (RP4)	none	1	1

Recipients were treated with plasmid DNA (45 $\mu\text{g/ml}$) from either *P. aeruginosa* or *E. coli*, and frequencies of Cb^r transformation were compared.

The restriction was much more apparent between *P. aeruginosa* and *E. coli*, while a restriction-negative *E. coli* strain was transformed with a high frequency (Table 3). So far tested, all the transformants selected by carbenicillin showed resistance to other antibiotics as well and in case of PAO strains also showed sensitivity to phage PRR1.

Any transformants of chromosomal markers were not detected by this procedure with the whole bacterial DNA in *P. aeruginosa* PAO, contrary to the report of KHAN and SEN (1, 2).

The procedure for the genetic transformation of *P. aeruginosa* described here suggests a possibility of cloning composite plasmids constructed *in vitro* from genes of *P. aeruginosa* and other species.

This study was presented at the 49th Annual Meeting of the Japanese Biochemical Society, Sept. 2, 1976 (10). While this work was being performed, a report

appeared that referred to the transformability of *P. aeruginosa* with plasmid DNA but no details were given (11).

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