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Transformation of *Pseudomonas aeruginosa* with Chromosomal DNA into *Bacillus subtilis*

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Successful transformation of gram-negative and gram-positive bacteria was achieved. Genetic transformation of ***Pseudomonas aeruginosa*** KYU-1 to ***Bacillus subtilis*** Marburg 168 was performed with linear chromosamal DNA, provided the cells were treated with cold calcium chloride and a heat pulse. The molecular weight of transforming DNA of the donor was markedly dependent on the transformation frequency. At 6.2×10^6 daltons, transformation frequency with chromosomal DNA of ***P. aeruginosa*** into ***B. subtilis*** was obtained at 2.0x10-5. The divalent ions such as Ca^{2+} and Mg^{2+} enhanced transformation frequency.

INTRODUCTION

Genetic transformation of ***Pseudomonas aeruginosa*** with linear chromosomal DNA has been reported (Khan and Sen, 1967), but this work has been proved difficult to repeat (Booker and Loutit, 1974; Sano and Kageyama, 1977). Sano and Kageyama (1977), however, reported that the Ca^{2+} technique was adopted to ***P. aeruginosa so*** as to allow transformation by the DNA of the plasmid RP1 and it was also observed that $MgCl_2$ could be used in place of $CaCl_2$ to allow such transformation. Mercer and Loutit (1978a,b) have shown Ca^{2+}-dependent transformation of ***P. aeruginosa*** strain 1 by the plasmid RP1 and also by DNA of the conjugative plasmid FP2. They also observed that treatment with $MgCl_2$ allowed a restrictionless mutant of ***P. aeruginosa*** strain 1 to be transfected by the DNA of phage F116 and transferred by purified chromosomal DNA (Mercer and Loutit, 1979).

In our previous paper (Ueda and Hara, 1981), we reported that ***P. aeruginosa*** KYU-1 produced high molecular weight DNA extracellularly. Furthermore, successful transformation of ***P. aeruginosa*** by intra- and extracellular DNA was achieved by using $CaCl_2$ treatment in order to elucidate the nature of the extracellular DNA produced by strain KYU-1 (Hara et al., 1981).

While gene exchange appears to be common among both gram-positive and gram-negative bacteria, transformation appears to be the principal mode of gene exchange among gram-positive bacteria, such as Bacillus (Young and Wilson, 1975), and rare among the gram-negative bacteria.

We have tried to establish transformation technique between gram-positive and gram-negative bacteria. The present paper described some impor-

tant findings.

MATERIALS AND METHODS

Microorganisms

The microorganisms used were *Pseudomonas aeruginosa* KYU-1 (prototroph) and *Bacillus subtilis* Marburg 168 (*trp*⁻).

Medium and culture conditions

The composition of bouillon-yeast extract (BY) medium and of minimal medium were reported by Yamaguchi *et al.* (1974). Cells were grown in BY-medium with shaking at 30°C or 37°C as indicated.

Transformation procedure

Transforming DNA from exponentially growing cells of strain KW-1 was prepared by the method of Saito and Miura (1963). DNA was used at a saturating concentration except in those experiments when it was specifically indicated. Concentration of DNA was determined by measuring absorbance at 260nm.

Transformation procedure described by Cohen *et al.* (1972) was used. The cells of *B. subtilis 168* were grown in BY-medium overnight at 30°C on a shaker. A portion (0.2 ml) of such a culture was reinoculated in 10 ml of a modified Spizizen's minimal medium containing L-tryptophan (50 μg/ml) and grown for 2.5 to 3 hr. At the late exponential phase, the cells were harvested by centrifugation and washed with 5 ml of 10 mM NaCl. The cells were again centrifuged and resuspended in 5 ml of 0.1 M $CaCl_2$. The cells were kept at 0°C for 20 min, harvested and resuspended in 1 ml of 0.1 M $CaCl_2$. An aliquot (0.2 ml) of these cells was afterwards mixed with 0.1 ml of DNA solution and 0.2 ml of $CaCl_2$ solution and kept at 0°C for 60 min. The cell-DNA mixture was then subjected to a heat pulse at 42°C for 2 min and chilled in ice for 5min. It was then diluted 10 times with fresh medium and allowed to grow overnight at 37°C on a shaker. 0.1 ml of aliquot was plated on minimal medium agar plate. The plates were usually incubated for 2 to 3 days at 37°C before scoring the number of transformants. Transformation frequencies were calculated by the quotient of the numbers of transformants and the numbers of colony-forming units.

Sucrose density gradient centrifugation

DNA prepared by the method of Saito and Miura (1963) was centrifuged in neutral 5 to 20 % linear sucrose gradient in a buffer consisting of 0.03 M Tris-HCl (pH 8.0), 0.005 M EDTA and 1 M NaCl. The sample was spun at 20°C and 84,000~ g for 4 hr in Hitachi RPS-50 rotor. The molecular weight of the DNA at the peak of absorbance was determined according to the method of van der Schans *et al.* (1969).

RESULTS

Effect of DNA size on transformation frequency

The DNA samples were prepared from 5 to 20 % sucrose density gradient. As shown in Table 1, transformation frequency with chromosomal DNA of *P. aeruginosa* into *B. subtilis* was markedly dependent on the molecular weight of DNA used and the frequency of transformation was 2.0×10^{-5} when the molecular weight of DNA was 6.2~ 10^6 daltons.

Table 1. Effect of DNA size on transformation frequency. Transformation was carried out as described in Materials and Methods. DNA of different molecular weight used was prepared by 5 to 20 % sucrose density gradient at $84,000\times g$ for 4 hr. DNA concentration used was 1.0 μg/ml.

Molecular weight	Transformation frequency
3.7×10^6	7.4×10^{-7}
6.2×10^6	2.0×10^{-5}
1.8×10^6	4.4×10^{-7}

Therefore, chromosomal DNA of molecular weight of 6.2~ 10^6 daltons was used in this study.

Effect of different concentrations of $CaCl_2$ on transformation frequency

Transformation of *P. aeruginosa* was achieved with chromosomal DNA by treatment with $CaCl_2$. The optimum concentration of $CaCl_2$ required to obtain a maximal number of transformants was determined. As shown in Fig. 1, 60mM $CaCl_2$ was optimum for outerspecific transformation. No such transfor-

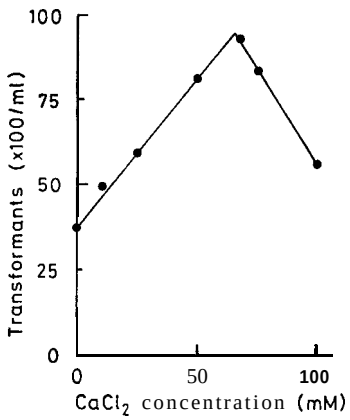

Fig. 1. Effect of various concentrations of $CaCl_2$ *on* transformation frequency. $CaCl_2$-treated cells of B. *subtilis 168 were* exposed with chromosomal DNA of *P.* aeruginosa KYU-1 (1.0 μg/ml) for 60min at 0°C. Samples were plated on minimal agar plates to select transformants for *trp*$^+$ and all the plates were kept overnight at 37°C. Transformation frequency was determined by counting *trp*$^+$ colonies.

mation was, of course, observed either in the absence of DNA or in the absence of $CaCl_2$ treatment of the cells.

Henceforth, the concentration of $CaCl_2$ used was 60 mM.

Effect of DNA concentration on transformation frequency

When the DNA concentration was varied, it was observed that transformation frequency increased with increasing concentration of DNA upto a certain limit, above which no further increase in the frequency was detected. Fig. 2 shows the response of the recipient strain ***B. subtilis*** 168 to DNA preparations from the donor strain ***P. aeruginosa*** KYU-1. The frequency of transformation increased linearly with the increase of concentration of DNA added, and the saturating concentration of transforming DNA was reached at about 0.05 μg/ml.

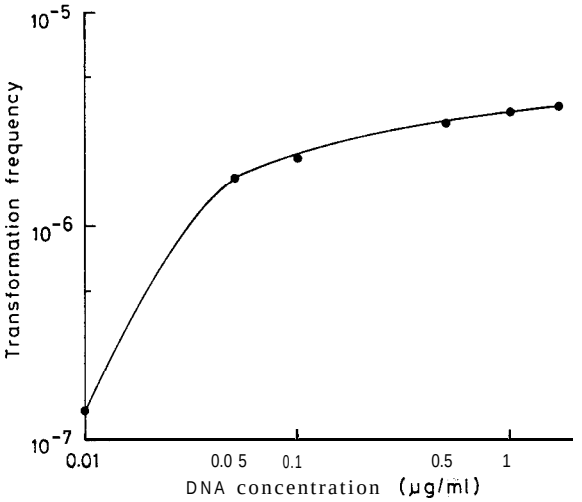

Fig. 2. Effect of DNA concentration on transformation frequency. Various concentrations of chromosomal DNA of P. ***aeruginosa*** KYU-1 were added to $CaCl_2$-treated ***B. subtilis 168***. Samples were plated on minimal agar plates and all the plates were kept overnight at 37°C. Transformation frequency was determined by counting ***trp***$^+$ colonies.

Table 2. Effect of temperature on a heat pulse. $CaCl_2$-treated cells were treated with DNA of strain KYU-1 (10 μg/ml) and then subjected to a heat pulse for 2 min at various temperature. Transformants were plated on minimal agar plates to select for ***trp***$^+$ transformants and incubated overnight at 37°C.

Temp. (°C)	Transformation frequency
37	*1.5 x* 10^{-8}
40	9.3 2.4 x x 10^{-8} 10^{-6}
42	
45	*1.9 x 10-5*
	10^{-8}

Table 3. Effect of various periods of a heat pulse. $CaCl_2$-treated cells were exposed with DNA (1.0 μg/ml) and subjected to a heat pulse at 42°C for various periods as indicated. Transformants were spread on minimal agar plates to select for *trp*+ transformants and incubated at 37°C.

Period (min)	Transformation frequency
0	3.4×10^{-7}
1	5.7×10^{-6}
2	2.0×10^{-5}
3	7.7×10^{-6}
5	6.8×10^{-6}

Effect of heat pulse on transformation frequency

Effects of a heat pulse on transformation frequency were examined as shown in Tables 2 and 3. Two minutes were found to be enough for the period of a heat pulse and the optimum temperature for a heat pulse (2 min) was at 42°C.

Effect of metal ion on transformation frequency

It was observed that the addition of $CaCl_2$ and $MgCl_2$ resulted in transformants of only a small fraction of the recipient cells as shown in Table 4, and the considerably higher frequency of transformation was achieved after treatment with divalent metal ions such as Ca^{2+} and Mg^{2+}, compared with that with other metal ions. But $MgSO_4$ had no beneficial effect and even a single transformant was not detected.

Table 4. Effect of metal ions on transformation frequency. Cells were treated with various metal ions (each concentration 60mM) as described in the text and exposed to DNA (1.0 μg/ml). Transformants were spread on minimal agar plates to select for *trp*+ transformants and incubated overnight at 37°C.

Metal ion	Transformation frequency
No salt	10^{-8}
$CaCl_2 \cdot 2H_2O$	2.0×10^{-5}
$MgCl_2 \cdot 6H_2O$	1.5×10^{-5}
$MgSO_4 \cdot 7H_2O$	10^{-8}
$MnCl_2$	4.6×10^{-7}
KCl	2.4×10^{-7}

DISCUSSION

Successful transformation of gram-negative bacteria to gram-positive bacteria was achieved. One of the techniques was that of Mandel and Higa (1970), who showed that treatment with cold calcium chloride enabled *Escherichia coli* to take up the purified DNA of phages lambda and P2 and to produce viable phage. Recent work has shown that purified plasmid DNA and chromosomal DNA will also transform *E. coli* after cold $CaCl_2$ treatment (Cohen

et *al.*, 1972; Cosloy and Oishi, 1973).

Genetic transformation of *E. coli* and *Salmonella typhimurium* has been demonstrated with plasmid DNA, provided the cells were treated with $CaCl_2$ to make them permeable to the naked DNA (Cohen *et al.*, 1972: Lederberg and Cohen, 1974) and also with chromosomal DNA, provided the *E. coli* cells additionally lacked ATP-dependent DNase and exonuclease I (Oishi and Cosloy, 1972).

Pseudomonas putida (Chakrabarty *et al., 1975;* Johnston and Gunsalus, 1977) is a gram-negative bacterium, which has been demonstrated to undergo both transductional and conjugational gene exchange with other members of the species. In a recent report (Sano and Kageyama, 1977), the Ca^{2+} technique was adopted to *P. aeruginosa so* as to allow transformation by the DNA of the plasmid RP1. Furthermore, Ca^{2+}-dependent transformation of *P. aeruginosa* by purified chromosomal DNA was performed (Mercer and Loutit, 1978a; Mylroie *et al., 1978).*

We performed the DNA-mediated transformation of *P. aeruginosa* and *B. subtilis,* provided the cells were treated with $CaCl_2$ and a heat pulse. At this time, the molecular weight of DNA of the donor was greatly dependent on the frequency of transformation. When the molecular weight of transforming DNA was 6.2~ 10^6 daltons.

Mercer and Loutit (197813) observed that treatment with $MgCl_2$ allowed a restrictionless mutant of *P. aeruginosa* strain 1 to be transformed by purified chromosomal DNA. In a subsequent report (Mercer and Loutit, 1979), they reported that $CaCl_2$, $MgCl_2$ and $MnCl_2$ were found to promote transformation of *P.* aeruginosa.

In the case of *P. aeruginosa* KYU-1, transformation was achieved with chromosomal DNA after treated with $CaCl_2$ or $MgCl_2$, but no such transformation was observed when $MnCl_2$ was used. At that time, $CaCl_2$ treatment and a heat pulse were necessary for the transformation of *Pseudomonas,* but if any of these procedures was omitted, no transformant could be detected.

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