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Transformation of *Pseudomonas aeruginosa* Strain PAO with Bacteriophage and Plasmid DNA

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Abstract

A procedure has been developed which allows transformation of *P. aeruginosa* strain PAO with plasmid and bacteriophage DNA at a frequency of 10^{-6} per recipient cell. The method is similar in outline to that developed for *Escherichia coli*. It involves growing the recipient cells to 3–5 × 10^8 per ml in nutrient broth, washing the cells with 0·1 M $MgCl_2$, resuspending in 0·175 M $CaCl_2$ for 20 min, exposing to DNA for 1 h and then heat pulsing at 42°C for 1 min. Some plasmid markers are expressed immediately, whereas others require time for phenotypic expression.

Introduction

Bacterial transformation is a mechanism of genetic exchange involving the uptake of naked, exogenous DNA by a recipient bacterial cell, and the subsequent persistence of this genetic information within that cell and its descendants. Cells that are able to take up DNA are said to be competent. In a number of bacterial species including *Bacillus subtilis*, *Haemophilus influenzae* and *Streptococcus* spp. transformation occurs naturally, and cells may be rendered competent by manipulation of the growth conditions (Tomasz 1969).

Recently techniques have been developed for the induction of competence in other bacterial species such as *Escherichia coli* (Cohen *et al.* 1972), *Staphylococcus aureus* (Lindberg and Novick 1973), and *Pseudomonas putida* (Chakrabarty *et al.* 1975). Uptake of DNA does not necessarily ensure recombination of chromosomal DNA. In *E. coli*, for example, a *recB21 recC22 sbcB15* (or *sbcA*6) * recipient genotype is required to prevent degradation of the incoming DNA by the *recB* and *recC* endonuclease (Oishi and Cosloy 1972; Cosloy and Oishi 1973). However, the establishment of plasmid DNA is not affected by the *recB* and *recC* endonuclease.

The most topical use of plasmid transformation is in the cloning of DNA following *in vitro* construction of plasmid chimeras (Cohen and Chang 1974). It has also been used in the isolation of plasmid mutants following *in vitro* mutagenesis (Hashimoto and Sekiguchi 1976). A more general use is as a biological assay of plasmid DNA. This would prove to be a valuable adjunct to physical studies of plasmids in *Pseudomonas aeruginosa* strain PAO, particularly as this strain has been shown to harbour a number of cryptic plasmids (Pemberton and Clark 1973). It would also be invaluable

* Genotype symbols are those used on the standard map of *E. coli* (Bachmann *et al.* 1976). The phenotype associated with *recB* and *recC* mutations is recombination deficiency and this is suppressed by *sbcA* and *sbcB* mutations.

in the characterization of plasmids suspected of carrying regions of the bacterial chromosome, particularly in organisms other than *Escherichia coli* which are not so well characterized genetically. For such studies it is necessary that the transformation system be reliable, so that some credence may be placed on negative results. Olsen and Hansen (1976) have transformed *P. aeruginosa* with plasmid DNA using a method developed for *Salmonella typhimurium* (Leberberg and Cohen 1974), but they do not report whether the method could be used routinely. M. Kageyama (personal communication) has also achieved plasmid transformation of *P. aeruginosa*. However, as different strains of *P. aeruginosa* show considerable genetic variation (Holloway 1975), it is quite likely that the optimum conditions will vary for different strains. As strain PAO (Holloway 1969) and its derivatives are widely used by *Pseudomonas* workers, we report here a series of experiments which define the optimum conditions for both bacteriophage and plasmid transformation of *P. aeruginosa* strain PAO.

Table 1. Bacterial strains and plasmids

Gene symbols stand for genes determining and controlling methionine biosynthesis (*met*), isoleucine and valine biosynthesis (*ilv*), leucine biosynthesis (*leu*), and arginine biosynthesis (*arg*). Chromosomal genes determining streptomycin and chloramphenicol sensitivity are symbolized *str* and *chl* respectively. Plasmid phenotype symbols are used according to the proposals of Novick *et al.* (1976). Aer = tolerance to aeruginocin 41, Cb = carbenicillin resistance, Hg = mercuric ion resistance, Gm = gentamycin resistance, Km/Nm = kanamycin and neomycin resistance, Sm = streptomycin resistance, Su = sulfonamide resistance, Tc = tetracycline resistance, Tra = ability to mediate conjugation

Strain or plasmid	Genotype or phenotype	Molecular weight	References
Strain			
PAO1	Prototroph, *chl*-2	—	Holloway (1969)
PAO8	*met*-28, *ilv*-202, *str*-1	—	Isaac and Holloway (1968)
PAO25	*leu*-10, *argF*10	—	Haas and Holloway (1976)
PAO38	*leu*-38	—	Stanisich and Holloway (1969)
Plasmid			
R68	Cb, Km/Nm, Tc, Aer, Tra	36×10^6	Holloway and Richmond (1973), Jacob *et al.* (1977)
pMG2	Sm, Su, Gm, Hg, Tra	23×10^6	Shahrabadi *et al.* (1975), Jacoby and Shapiro (1977)

Materials and Methods

The bacterial strains and plasmids used in this study are listed in Table 1.

Media

Nutrient broth (NB), nutrient agar (NA), minimal medium (MM), and TNM buffer (0·01 M tris, 0·15 M NaCl, 0·01 M $MgSO_4$, pH 7·4) have been described elsewhere (Stanisich and Holloway 1972). The tris–EDTA buffer used is 0·03 M tris, 0·005 M EDTA, 0·05 M NaCl (pH 8·0).

Chemicals

All chemicals used were reagent grade or better.

Bacteriophage

B3c is a clear plaque mutant of the temperate bacteriophage B3 (Holloway *et al.* 1960). It has a molecular weight of 25×10^6 (Holloway and Krishnapillai 1975).

General phage methods are described in Adams (1959). Large scale B3c lysates were prepared as described by Davison *et al.* (1964). After low speed centrifugation to remove bacterial debris

the phage lysates were concentrated by centrifugation at 35 000 *g* for 2 h, and resuspension of the resulting phage pellet was carried out in 1% of the original volume of TNM buffer by standing at 4°C for 48 h.

Characterization of Transformants

Putative transformants were partially purified by patching on to selective plates, which after incubation were replicated to appropriately supplemented MM or NA plates. Inheritance of aeruginocin resistance was determined by replicating onto an NA plate spread with 0·3 ml of the aeruginocin AR41, prepared as described by Holloway *et al.* (1974). Transmissibility (Tra^+) of antibiotic resistance was tested by replication onto an antibiotic supplemented MM plate freshly spread with 0·1 ml of an overnight NB culture of *P. aeruginosa* strain PAO1. Chromosome mobilization ability (Cma^+) was tested by replication onto an unsupplemented MM plate spread with PAO38.

Extraction and Storage of Phage B3c DNA

Phage DNA was extracted from 7·5 ml of a phage suspension, titre 7×10^{11} plaque forming units per millilitre, by mixing with an equal volume of freshly distilled phenol saturated with 0·1 M disodium tetraborate. After centrifugation the aqueous layer was exhaustively dialysed against 0·14 M NaCl–0·001 M EDTA (pH 8·0). The DNA was precipitated with ethanol, spooled onto a glass rod, and finally dissolved in 2 ml of the tris–EDTA buffer. Sealed tubes of DNA in solution were stored at 4°C for up to 4 months without detectable decrease in transformation efficiency.

Extraction of Plasmid DNA

Plasmid DNA was extracted using a procedure similar to that described by Freifelder (1976). A 500-ml NB culture of stationary phase cells was harvested, washed three times in saline and resuspended in 3 ml of 25% (w/v) sucrose–2×10^{-5} M n-dodecylamine in tris–EDTA buffer. Meynell (1971) showed that n-dodecylamine sensitizes gram-negative cells to lysozyme. To this was added 0·2 ml of 0·25 M EDTA (pH 8·0) and 0·2 ml of lysozyme (10 mg/ml) in tris–EDTA buffer and after incubation at 37°C for 20 min 0·4 ml of 2% (w/v) sarcosyl (Ciba-Geigy, as Sarkosyl NL30) in tris–EDTA buffer was gently mixed in with a glass rod until viscosity effectively prevented further mixing. The resulting gel was centrifuged at 130 000 *g* for 90 min, yielding a supernatant that contained covalently closed and circular DNA and protein (Freifelder 1976). Protein was removed by passage through small columns of Sepharose 2B (Pharmacia Fine Chemicals) using 0·5 M ammonium acetate (pH 8·0) as the eluting fluid. Plasmid DNA came off just after the void volume, and was usually collected as a single fraction equal to 50% of the void volume. Plasmid extracts were stored frozen at −20°C.

DNA Assay

DNA extracts were routinely assayed by spectrophotometric analysis at 254 nm in a Zeiss PMQII spectrophotometer, using calf thymus DNA (Sigma) solutions of known concentration as standards. Protein contamination was routinely checked for by determining the ratio of absorbance at 254 nm and 280 nm. Absence of protein contamination was confirmed using the Folin–Ciocalteau reagent (Lowry *et al.* 1951) with bovine serum albumin (Sigma) as a standard.

Preparation of Competent Cells

Initially the method used was that described by Chakrabarty *et al.* (1975) for *P. putida* except that harvested cells were washed with 0·1 M $MgCl_2$ instead of 0·1 M NaCl. Cell growth was monitored using a Klett–Summerson photoelectric colorimeter. All glassware involved in preparing competent cells for transformation was washed in chromic acid and stored in a cold room with an ambient temperature of *c.* 4°C. All procedures were carried out in the cold room, tubes being held at 0°C in an ice bath.

Transformation Procedure

DNA solutions were dispensed into Wassermann tubes, and made up to the appropriate $CaCl_2$ concentration by addition of 1/10 volume of $CaCl_2$. Two volumes of competent cells were added and the contents gently mixed. Control tubes containing DNA only and cells only were always included. Cells were heat pulsed by partially immersing in water at 60°C for 10–15 s until the tube temperature reached 42°C (a thermometer in a temperature control tube was always included)

followed by transfer to a 42°C water bath for the appropriate time. After the heat pulse step the tubes were returned to the ice bath. Phage DNA-mediated transformation was detected by assaying for infectious centres on NA using an overnight NB culture of PAO1 as indicator. Plasmid DNA-mediated transformation was detected by assaying for transformants on NA supplemented with the appropriate antibiotic. Recipient cell survival was determined from the cells-only control tube. Unless otherwise stated transformation frequencies are calculated as infectious centres or transformants per surviving recipient cell.

Enzymic controls

Deoxyribonuclease I (Sigma Chemical Co.), ribonuclease A (type 1-A, Sigma Chemical Co.) and protease (type VI, Sigma Chemical Co.) were used as described by Cohen *et al.* (1972).

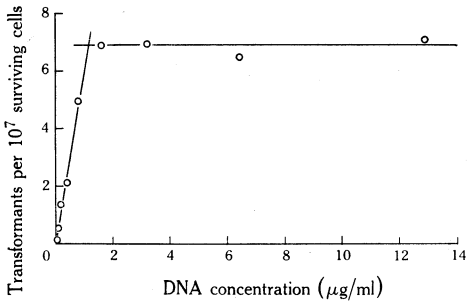

Fig. 1. Effect of phage DNA concentration on transformation frequency. 0·2-ml aliquots of serial two-fold dilutions of bacteriophage B3c DNA were added to tubes each containing 0·4 ml of PAO1 cells which had been rendered competent as described by Chakrabarty *et al.* (1975) for *P. putida*. The tubes were held at 0°C for 60 min, and heat pulsed for 2 min. Duplicate 0·1-ml aliquots were assayed for infectious centres as described in Methods. Cell survival was 50%. The intersect of the two lines occurs at a DNA concentration of 1·15 μg/ml.

Results

Determination of Optimum Conditions

The primary aim of this study was to develop a reliable system for plasmid transformation in *P. aeruginosa*. However, initial experiments were carried out using B3c bacteriophage because of the ease with which large quantities of pure phage DNA can be obtained. B3c was chosen because of its molecular weight of 25×10^6. This is small enough to avoid serious problems of DNA shearing while pipetting, but large enough to reduce the risk of developing a system which could not accommodate the larger plasmids. PAO1 was used as the recipient for these experiments. Each parameter was varied in turn, and as each optimum value was determined, subsequent experimental conditions were altered to accommodate this information. Fig. 1 shows that as the DNA concentration is raised the transformation frequency increases linearly, indicating first-order kinetics. Saturation occurs at 1·15 μg/ml, and the input cell titre was $1{\cdot}85 \times 10^9$ per ml. Assuming that non-viable cells adsorb DNA just as efficiently as surviving cells, this corresponds to 15·3 phage genomes per cell. In subsequent experiments a DNA concentration of 3·2 μg/ml was used to ensure saturation and allow for variation in input cell numbers.

The next parameter to be varied was $CaCl_2$ concentration. Fig. 2 shows that as the concentration is raised transformation frequency per surviving recipient cell increases, but that cell survival decreases markedly at high concentrations. This finding is quite repeatable and was the same when PAO8 was used as the recipient. As 175 mM $CaCl_2$ gave the greatest number of infectious centres it was chosen as

the optimum concentration. Subsequent attempts to increase cell survival at high $CaCl_2$ concentrations by resuspending cells at 100 mM $CaCl_2$ (which gave the highest survival) followed by dialysis against 200 mM $CaCl_2$ for varying lengths of time were not effective (C. H. Hamann and A. F. Morgan, unpublished data). The mechanism by which $CaCl_2$ induces competence is not understood (Taketo 1974) but it is clearly not simply a matter of the concentration at the time of exposure to DNA.

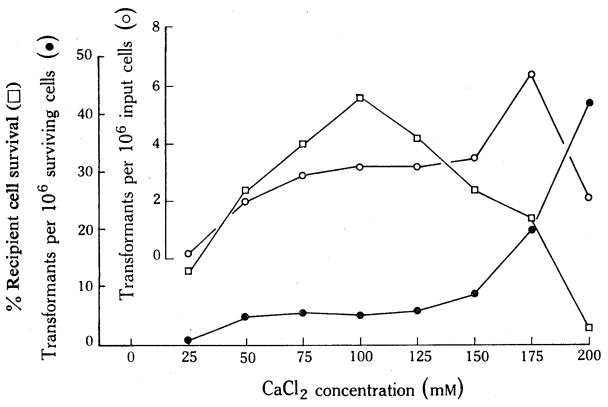

Fig. 2. Effect of $CaCl_2$ concentration on transformation frequency. 0·2-ml aliquots of bacteriophage B3c DNA at a concentration of 9·6 μg/ml were added to tubes containing PAO1 cells which had been treated as in Fig. 1 except that the final $CaCl_2$ concentration was varied. The tubes were held at 0°C for 60 min, heat pulsed for 2 min and then assayed for infectious centres as described in Methods. A parallel series of cells was used to determine recipient cell survival.

The effects of altering the duration of $CaCl_2$ treatment, and the time of exposure to DNA are presented in Table 2.

Table 2. Effects of alterations to duration of (*a*) heat pulse, (*b*) $CaCl_2$ treatment, and (*c*) exposure to phage DNA

The three parameters were varied in the order indicated, with the value in italics taken as the optimum value. For (*a*) cells were treated with 100 mM $CaCl_2$ for 20 min, and for both (*a*) and (*b*) cells were exposed to phage DNA for 60 min

(*a*) Heat pulse (s)	Transformants per 10^6 surviving cells	(*b*) $CaCl_2$ treatment (min)	Transformants per 10^6 surviving cells	(*c*) DNA exposure (min)	Transformants per 10^6 surviving cells
15	0·54	0	n.t.d.[A]	10	0·7
30	0·88	5	0·58	20	1·3
60	*1·2*	10	1·2	40	1·8
90	1·0	*20*	*1·5*	*60*	*2·1*
120	1·2	30	1·5	90	1·9

[A] No transformation detected.

Recipient Cell Growth Stage

In order to determine whether the growth regime of the recipient cells had a significant effect samples were taken at various times from an exponentially growing broth culture, the cells were treated to induce competence, and the transformation frequency was determined. Both cell survival and transformation frequency fell slightly as the cells approached stationary phase, the best results being obtained when the culture had a viable count of between 3 and 5 × 10^8 per ml.

Duration of Competence

E. coli cells treated with $CaCl_2$ can be maintained at 0°C for 10 days without significant loss of competence or cell viability (Taketo 1972). This has obvious logistical advantages when transformation is to be used as a routine assay. Accordingly, competent PAO1 cells were prepared and held at 0°C. B3c DNA was added to samples at various times. Transformation frequency per surviving cell rose slightly over the first 6 h, but after 24 h it had fallen to less than 10% of the initial frequency, even though cell viability remained unchanged. Consequently competent PAO cells need not be prepared immediately prior to use, but must be prepared fresh each day.

Summary of optimum transformation conditions

Initial cell density 3–5 $\times$ 10^8 per ml; 0·1 M $MgCl_2$ wash; 175 mM $CaCl_2$ treatment for 20 min; 60 min exposure to DNA, followed by a 1-min heat pulse.

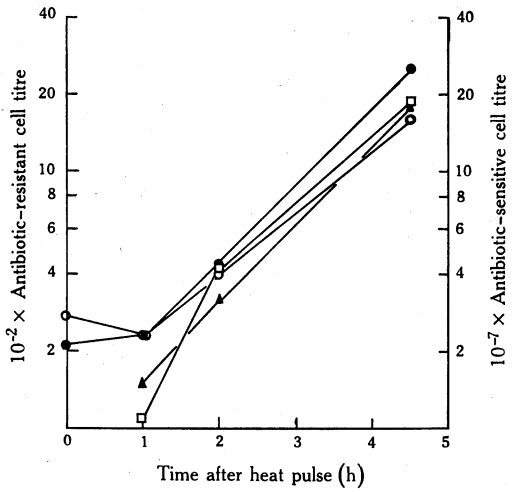

Fig. 3. Transformation of PAO25 with R68 DNA. After the void volume had passed through the column, 0·3 ml of eluate was collected directly into a tube containing 0·6 ml of competent cells, prepared as described on p. 684. After heat pulsing, the cells were diluted 10-fold into prewarmed broth and shaken at 37°C. At intervals the viable count was determined on NA (●), and on NA supplemented with 500 μg/ml carbenicillin (○), 250 μg/ml tetracycline (▲), or 400 μg/ml neomycin (□). No transformants were seen at zero time when selection was made for tetracycline or neomycin resistance.

Enzymic Controls

As was to be expected, transformation was unaffected by treating the B3c DNA with RNAase or protease or DNAase after the heat pulse, but DNAase treatment of the DNA prior to addition of competent PAO1 cells abolished transformation.

Transformation with Plasmid DNA

For these experiments PAO25 was used as the recipient and plasmid DNA was extracted from PAO8 derivatives harbouring the appropriate plasmid. Chromosomally marked strains allow distinction between transformed cells and donor cells which have survived the plasmid extraction procedure. A preliminary experiment showed that transformation of PAO25 with phage B3c is the same as for PAO1.

The first plasmid extracted was R68. The Sepharose column eluate was used undiluted. As the eluate contained 18·5 μg/ml DNA, it was assumed that saturation of the competent cells was achieved. Fig. 3 shows that of the antibiotic resistance

markers carried by R68, only carbenicillin resistance can be selected for without allowing time for phenotypic expression. The β-lactamase gene carried by R68 is known to be expressed constitutively (Fullbrook *et al.* 1970). Fifty clones from each type of media and each time were tested for unselected markers. All had the nutritional requirements of the recipient and all were Cb^r Nm^r Tc^r Aer^r Tra^+. Thus all transformants had apparently received complete plasmids. When the transformation procedure was repeated using DNA that had been exposed to mild shearing by means of pipetting several times, the transformation frequency dropped to 30%, but once again all transformants exhibited all the plasmid markers. Chakrabarty *et al.* (1975) reported that shearing of the closely related plasmid RP1 (Grinsted *et al.* 1972) resulted in *P. putida* transformants 40% of which had lost plasmid markers and which presumably carried deletions. We have never observed such deletions in transformants of *P. aeruginosa* strain PAO, irrespective of the plasmid used. This may reflect differences in nuclease activity between the two species.

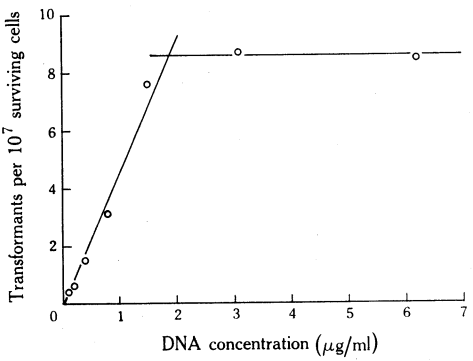

Fig. 4. Effect of plasmid DNA concentration on transformation frequency. Plasmid pMG2 was isolated from a PAO8 derivative harbouring the plasmid. The Sepharose column eluate was assayed for DNA as described in Methods. 0·2-ml aliquots of the eluate, and of a series of two-fold dilutions, were added to tubes containing 0·4 ml of competent PAO25 cells. After adsorption and heat pulsing, quadruplicate 0·1-ml aliquots were plated from each tube onto NA supplemented with 500 μg/ml streptomycin. The intersect of the two lines occurs at a DNA concentration of 1·9 μg/ml. Input cell titre was $3{\cdot}13 \times 10^9$ per ml, and cell survival was 50%.

Effect of DNA Concentration on Transformation Frequency

Because R68 is large enough to be sheared even by pipetting, the smaller plasmid pMG2 was used. Fig. 4 shows a very similar pattern to that obtained with B3c transfection. The saturating DNA concentration of 1·95 μg/ml corresponds to 16·4 plasmid molecules per cell. Both this value and the transformation frequency obtained are very close to the values obtained for transformation with B3c DNA.

Discussion

The protocol presented here can be used to transform *P. aeruginosa* strain PAO with phage or plasmid DNA at a frequency of 10^{-6} per surviving recipient cell. As is the case for *E. coli* the process has first-order kinetics. Cell survival is lower because of the higher concentrations of $CaCl_2$ required to render recipient cells competent. The frequencies obtained are quite reproducible but are 100-fold less than those reported for *E. coli* (Cohen *et al.* 1972; Taketo 1972), and 10–100-fold lower than those claimed for *P. putida* (Chakrabarty *et al.* 1975). However, in the latter instance it is not clear from the results whether the authors are observing a high frequency

of transformation or a low frequency followed by subsequent conjugal transmission of the plasmid. The reproducibility of the procedure reported here has allowed its use in the isolation of transfer-deficient mutants of the plasmid R91-5 following *in vitro* mutagenesis of the plasmid DNA (J. M. Carrigan, Z. M. Helman and V. Krishnapillai, personal communication).

The plasmid extraction method used here is simple, but is not applicable to all plasmids. We have made many attempts to isolate plasmid DNA from strains carrying the R68 derivative R68-45 (Haas and Holloway 1976), but only once was transformation achieved (data not shown). On the one successful occasion, however, all transformants that were tested carried apparently normal plasmids that exhibited all the R68-45 markers. Just why R68 should be easy and R68-45 difficult to isolate by this method is not clear. R68-45 is reported to be slightly larger than R68—$37{\cdot}4 \times 10^6$ compared to 36×10^6 (Jacob *et al.* 1977)—but the nature of the additional DNA is unknown. The plasmid extraction method used here would probably be unsuitable for plasmids that exist intracellularly as relaxation complexes, as sarcosyl is one of a number of agents known to destroy such complexes (Clewell and Helinski 1970; Helinski and Clewell 1971).

Attempts to apply this system to transformation of chromosomal markers were unsuccessful (data not shown). This is presumably due to exonuclease degradation of incoming linear DNA. Miller and Clark (1976) have shown that *P. aeruginosa* contains an exonuclease that is very similar to ExoV of *E. coli*, which is thought to be the product of the *recB* and *recC* genes (Tomizawa and Ogawa 1972). This exonuclease is possibly the cause of our failure to detect transformants that had lost unselected plasmid markers, due to degradation of any sheared plasmid DNA. Chromosomal transformation of *P. aeruginosa* probably awaits the isolation of the equivalent of the *recB21 recC22 sbcB15* (or *sbcA6*) mutants of *E. coli*.

Acknowledgments

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References

Adams, M. H. (1959). 'Bacteriophages'. (Interscience: New York.)

Bachmann, B. J., Low, K. B., and Taylor, A. L. (1976). Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriol. Rev.* **40**, 116–67.

Chakrabarty, A. M., Mylroie, J. R., Friello, D. A., and Vacca, J. G. (1975). Transformation of *Pseudomonas putida* and *Escherichia coli* with plasmid-linked drug-resistance factor DNA. *Proc. Natl Acad. Sci. U.S.A.* **72**, 3647–51.

Clewell, D. B., and Helinski, D. R. (1970). Properties of a deoxyribonucleic acid–protein relaxation complex and strand specificity of the relaxation event. *Biochemistry* **9**, 4428–40.

Cohen, S. N., and Chang, A. C. Y. (1974). Transformation of *Escherichia coli* by plasmid chimeras constructed *in vitro;* a review. In 'Microbiology 1974'. (Ed. D. Schlessinger.) pp. 66–75. (American Society for Microbiology.)

Cohen, S. N., Chang, A. C. Y., and Hsu, L. (1972). Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. *Proc. Natl Acad. Sci. U.S.A.* **69**, 2110–14.

Cosloy, S. D., and Oishi, M. (1973). The nature of the transformation process in *Escherichia coli* K12. *Mol. Gen. Genet.* **124**, 1–10.

Davison, P. F., Freifelder, D., and Holloway, B. W. (1964). Interruptions in the polynucleotide strands in bacteriophage DNA. *J. Mol. Biol.* **8**, 1–10.

Freifelder, D. (1976). Preparation of covalently closed and open circular DNA molecules of Phage λ. *Biochem. Biophys. Acta* **432**, 113–17.

Fullbrook, P. D., Elson, S. W., and Slocombe, B. (1970). R-factor mediated β-lactamase in *Pseudomonas aeruginosa*. *Nature (London)* **226**, 1054–6.

Grinsted, J., Saunders, J. R., Ingram, R. C., Sykes, R. B., and Richmond, M. H. (1972). Properties of an R factor which originated in *Pseudomonas aeruginosa* 1822. *J. Bacteriol.* **110**, 529–39.

Haas, D., and Holloway, B. W. (1976). R factor variants with enhanced sex factor activity in *Pseudomonas aeruginosa*. *Mol. Gen. Genet.* **144**, 243–51.

Hashimoto, T., and Sekiguchi, M. (1976). Isolation of temperature-sensitive mutants of R plasmids by *in vitro* mutagenesis with hydroxylamine. *J. Bacteriol.* **127**, 1561–3.

Helinski, D. R., and Clewell, R. B. (1971). Circular DNA. *Annu. Rev. Biochem.* **40**, 899–942.

Holloway, B. W. (1969). Genetics of *Pseudomonas*. *Bacteriol. Rev.* **33**, 419–43.

Holloway, B. W. (1975). Genetic organization of *Pseudomonas*. In 'Genetics and Biochemistry of *Pseudomonas*'. (Eds P. H. Clarke and M. H. Richmond.) pp. 133–62. (John Wiley: London.)

Holloway, B. W., Egan, J. B., and Monk, M. (1960). Lysogeny in *Pseudomonas aseruginosa*. *Aust. J. Exp. Biol. Med. Sci.* **38**, 321–30.

Holloway, B. W., and Krishnapillai, V. (1975). Bacteriophages and bacteriocins. In 'Genetics and Biochemistry of *Pseudomonas*'. (Eds P. H. Clarke and M. H. Richmond.) pp. 99–132. (John Wiley: London.)

Holloway, B. W., and Richmond, M. H. (1973). R factors used for genetic studies in strains of *Pseudomonas aeruginosa* and their origin. *Genet. Res.* **21**, 103–6.

Holloway, B. W., Rossiter, H., Burgess, D., and Dodge, J. (1974). Aeruginocin tolerant mutants of *Pseudomonas aeruginosa*. *Genet. Res.* **22**, 239–53.

Isaac, J. H., and Holloway, B. W. (1968). Control of pyrimidine biosynthesis in *Pseudomonas aeruginosa*. *J. Bacteriol.* **96**, 1732–41.

Jacob, A. E., Cresswell, J. M., and Hedges, R. W. (1977). Molecular characterisation of the P group plasmid R68 and variants with enhanced chromosome mobilizing ability. *FEMS Microbiol. Lett.* **1**, 71–4.

Jacoby, G. A., and Shapiro, J. A. (1977). Plasmids studied in *Pseudomonas aeruginosa* and other pseudomonads. In 'DNA Insertion Elements, Plasmids, and Episomes'. (Eds A. I. Bukhari, J. A. Shapiro, and S. L. Adhya.) pp. 639–56. (Cold Spring Harbor Laboratory: New York.)

Lederberg, E. M., and Cohen, S. N. (1974). Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. *J. Bacteriol.* **119**, 1072–4.

Lindberg, M., and Novick, R. P. (1973). Plasmid-specific transformation in *Staphylococcus aureus*. *J. Bacteriol.* **115**, 139–45.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–75.

Meynell, G. G. (1971). Dodecylamine in the isolation of bacterial DNA. *Biochem. Biophys. Acta* **240**, 37–8.

Miller, R. V., and Clark, A. J. (1976). Purification and properties of two deoxyribonucleases of *Pseudomonas aeruginosa*. *J. Bacteriol.* **127**, 794–802.

Novick, R. P., Clowes, R. C., Cohen, S. N., Curtiss III, R., Datta, N., and Falkow, S. (1976). Uniform nomenclature for bacterial plasmids: a proposal. *Bacteriol. Rev.* **40**, 168–89.

Oishi, M., and Cosloy, S. D. (1972). The genetic and biochemical basis of the transformability of *Escherichia coli* K12. *Biochem. Biophys. Res. Commun.* **49**, 1568–72.

Olsen, R. H., and Hansen, J. (1976). Evolution and utility of a *Pseudomonas aeruginosa* drug resistance factor. *J. Bacteriol.* **125**, 837–44.

Pemberton, J. M., and Clark, A. J. (1973). Detection and characterization of plasmids in *Pseudomonas aeruginosa* strain PAO. *J. Bacteriol.* **144**, 424–33.

Shahrabadi, M. S., Bryan, L. E., and Van Den Elzen, H. M. (1975). Further properties of P-2 R-factors of *Pseudomonas aeruginosa* and their relationship to other plasmid groups. *Can. J. Microbiol.* **21**, 592–605.

Stanisich, V., and Holloway, B. W. (1969). Conjugation in *Pseudomonas aeruginosa*. *Genetics* **61**, 327–39.

Stanisich, V. A., and Holloway, B. W. (1972). A mutant sex factor of *Pseudomonas aeruginosa*. *Genet. Res.* **19**, 91–108.

Taketo, A. (1972). Sensitivity of *Escherichia coli* to viral nucleic acid. V. Competence of calcium-treated cells. *J. Biochem.* (*Tokyo*) **75**, 973–9.

Taketo, A. (1974). Sensitivity of *Escherichia coli* to viral nucleic acid. VIII. Idiosyncrasy of Ca^{2+}-dependent competence for DNA. *J. Biochem.* (*Tokyo*) **75**, 895–904.

Tomasz, A. (1969). Some aspects of the competent state in genetic transformation. *Annu. Rev. Genet.* **3**, 217–32.

Tomizawa, J., and Ogawa, H. (1972). Structural genes of ATP-dependent deoxyribonuclease of *Escherichia coli*. *Nature* (*London*) *New Biol.* **239**, 14–16.

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Note Added in Proof

Since submission of this manuscript the authors have had their attention drawn to the following publication: Sano, Y., and Kageyama, M. Transformation of *Pseudomonas aeruginosa* by plasmid DNA. *J. Gen. Appl. Microbiol.*, 1977, **23**, 183–6. That paper reports a similar transformation frequency to that presented here, but describes different optimum conditions.