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Exonuclease Activity from *Pseudomonas aeruginosa* Which Is Missing in Phenotypically Restrictionless Mutants

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A phenotypically restrictionless strain of *Pseudomonas aeruginosa* was found to lack a deoxyribonuclease specific for linear duplex DNA. The purified enzyme had an optimum pH of 8.5, required $MgCl_2$ (10 mM) for maximum activity, and did not require ATP. Neither the degradation of heat-denatured DNA nor the degradation of bacteriophage F116 DNA was detected. The genome of bacteriophage F116 was shown to possess single-stranded terminal regions, which account for the resistance to degradation and for the ability of the phage to transfect restriction-proficient strains.

Pseudomonas aeruginosa is capable of genetic recombination through conjugation, transduction, and transformation (8, 16). Mercer and Loutit (14–16) have shown Mg^{2+}-dependent transformation of *P. aeruginosa* with the DNAs of various plasmids and bacteriophages and with chromosomal DNA. When linear duplex chromosomal DNA was used for transformation, a phenotypically restrictionless (Res^-) recipient strain was required, even when the source of DNA was a closely related strain. This observation was puzzling for two reasons. First, bacteriophage F116 linear duplex DNA was capable of transfecting Res^+ cells at a high frequency. Mercer and Loutit (16) proposed that F116 DNA was protected from degradation by a specific mechanism which, for some reason, did not occur with DNA from other bacteriophages. Second, a Res^- phenotype should not have been necessary for transformation with linear DNA from closely related strains if this DNA was degraded by a restriction endonuclease. Therefore, we thought it possible that the Res^- phenotype was brought about by the lack of an exonuclease which was responsible for the degradation of chromosomal DNA in wild-type cells.

Miller and Clark (18) identified three nuclease activities in *P. aeruginosa* and purified two of them. One was similar to exonuclease I of *Escherichia coli*, and the other was similar to exonuclease V, both of which play a role in the transformation process in *E. coli* (3, 21). Lehrbach et al. (10) recently isolated a *P. aeruginosa* mutant lacking the ATP-dependent activity of *Pae*ExoV. A third nuclease, *Pae*ExoIX, active on linear duplex and single-stranded DNA in the presence of EDTA, was identified by Scurlock and Miller (25). This enzyme does not appear to have a functional analog in *E. coli*.

As part of our study on genetic transformation in *P. aeruginosa*, we isolated mutants which were phenotypically restrictionless (16). In this report, we describe the purification and some properties of an exonuclease which is present in wild-type cells but absent in one of our Res^- mutants. In addition, the mechanism of the protection of F116 DNA during the transformation process is described.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *E. coli* strain PB1379 (F^- *thyA deoC2*) was kindly provided by P. L. Bergquist, University of Aukland, New Zealand. With the exception of OT500, all of the other strains are derivatives of *P. aeruginosa* strain 1 and are listed in Table 1. OT500 is a valine-requiring mutant of *P. aeruginosa* strain 78. DNA from the generalized transducing phages F116 (7) and B/78 was used for transfection and as a substrate in nuclease assays. B/78 is a phage obtained from strain B (23).

Media. The growth media used were brain heart infusion broth (Difco Laboratories, Detroit, Mich.), supplemented with 4 g of potassium nitrate per liter, and Luria broth (24). In cases in which solid medium was required, brain heart infusion was solidified with 2% Davis agar. The minimal medium used was that of Davis and Mingioli (4).

Chemicals. [2-^{3}H]adenine and [*methyl*-^{3}H]thymine were obtained from the Radiochemical Centre, Amersham, England. Lysozyme, bovine serum albumin, ribonuclease, and S_1 nuclease were all obtained from Sigma Chemical Company, St. Louis, Mo.; ammonium sulfate, sodium dodecyl sulfate, and Tris buffer were from BDH, Poole, England; and streptomycin sulfate was from Glaxo Laboratories. Yeast nucleic acid was obtained from Hopkins and Williams, and

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TABLE 1. *P. aeruginosa* strains

Strain	Genotype[a]	Source/reference[b]
OT55	*leu-1 lys-1 str*	This laboratory
OT56	*leu-1 lys-1*	This laboratory (11)
OT374	*leu-1 lys-1 str*/F116 lysogen	F116 infection of strain OT55
OT391	RP1$^+$ prototroph	This laboratory
OT500	*val*	(16)
OT684	*leu-1 lys-1 res-4*	Spontaneous temperature-resistant derivative of OT683 (15, 16)

[a] For gene symbols see Bachmann et al. (1).

[b] Strain OT683 (15, 16) was found to carry a mutation rendering it temperature sensitive for growth. Hence, OT684 was isolated as a spontaneous temperature-resistant clone and was used in all transformation experiments. OT683 was isolated as a mutant of *P. aeruginosa* strain 1 on which phage B/78 (grown on *P. aeruginosa* strain 78) could form plaques. We have retained the *res-4* gene symbol until the exonuclease has been further characterized.

phosphocellulose was the kind gift of G. Peterson, University of Otago, New Zealand. Phosphocellulose was prepared for use by washing once with distilled water and once with 0.5 M HCl, followed by repeated washing with water until the pH was 5.0. Potassium hydroxide (0.5 M) was added, and the mixture was left for 30 min, followed by repeated washing with water until the pH was 8.0 to 9.0. KCl (4.0 M) in 50 mM potassium phosphate buffer (pH 7.4) was added, and the phosphocellulose was stored overnight, washed thoroughly with distilled water, and stored in 50 mM potassium phosphate buffer (pH 7.4). Immediately before use, it was washed with potassium phosphate buffer containing 0.1 mM dithiothreitol (buffer A).

DNA. The DNA from bacteriophages F116 and B/78 was labeled with [2-^{3}H]adenine as described by Miller et al. (20) and extracted by the procedure of Hinkle and Miller (6). *P. aeruginosa* and *E. coli* chromosomal DNA were prepared by the method of Marmur (13) after overnight growth in a minimal medium supplemented with [2-^{3}H]adenine for *P. aeruginosa* or [*methyl*-^{3}H]thymine for *E. coli*. Plasmid DNA was isolated from cleared lysates of *P. aeruginosa* by the procedure of Stanisich and Bennet (26). When single-stranded DNA was required, duplex DNA was heated in a boiling water bath for 10 min and immediately placed in an ice water-NaCl bath for 10 min.

Exonuclease assay. The activity of various fractions of the nuclease preparation was determined by measuring the release of acid-soluble radioactivity from the [^{3}H]DNA substrate. The reaction mixture (0.5 ml) was composed of 30 mM potassium phosphate buffer (pH 7.0), 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, and 8 to 10 nmol of [^{3}H]DNA. In some experiments, 50 mM Tris-hydrochloride (pH 8.5) was substituted for potassium phosphate buffer. The specific activity of the DNA was approximately 10,000 cpm/nmol. The reaction was initiated by adding 100 μl of nuclease preparation. After a 30-min incubation at 37°C, reactions were terminated by placing the mixture on ice and adding 0.2 ml of yeast nucleic acid (2 mg/ml in 50 mM Tris-hydrochloride, pH 8.0) followed by 0.7 ml of ice-cold 10% trichloroacetic acid. After 30 min on ice, the acid-insoluble material was collected on a Whatman GF/C filter disk and washed twice with 5% trichloroacetic acid and once with ether. The filter was air dried, and the radioactivity was measured under 5.0 ml of butyl-PBD scintillation fluid (6 g/liter in toluene) in a Packard Tri-Carb liquid scintillation spectrometer, model 2003. One unit of enzyme is defined as the amount which converts 1 nmol of linear duplex DNA to acid-soluble material in 30 min at 37°C.

Endonuclease assay. Endonuclease activity of the nuclease preparation was measured by the method of Scurlock and Miller (25), with the following exceptions. The DNA substrate was covalently closed circular RP1, and samples (30 μl) were subjected to electrophoresis through 0.7% agarose as described by Meyers et al. (17). After being stained with ethidium bromide, the DNA bands were examined on a short-wave transilluminator screen. A decrease in the intensity or relaxation of the closed circular plasmid DNA was indicative of endonuclease activity.

Preparation of cell-free extracts: method 1. Cells were inoculated in 200 ml of nitrate brain heart infusion and were incubated at 37°C until stationary phase had been reached. They were washed twice with 50 mM potassium phosphate buffer (pH 7.0) and suspended in 2.5 ml of 0.85% saline. Cells were disrupted with a Dawe Soniprobe on setting number 4, using 30-s bursts followed by 30 s of cooling for a total of 3 min, and the debris was removed by centrifugation for 10 min at 12,000 × *g*. The supernatant fluid was used immediately to assay nuclease activity.

Preparation of cell-free extracts: method 2. Strains OT56 and OT684 were used to inoculate 20 surgical trays (13 by 19 cm), each containing 200 ml of brain heart agar. The trays were incubated overnight at 37°C, and the cells were removed by scraping the surface of the agar with a microscope slide. The cells were washed three times with 0.85% saline and harvested by centrifugation. The wet weight of the cells was generally 20 to 25 g. They were suspended in 10% sucrose–50 mM Tris-hydrochloride (pH 8.0) at a concentration of 0.5 g/ml and frozen at −20°C. After thawing at room temperature, lysozyme (2 mg/ml in 0.25 M Tris-hydrochloride, pH 8.0) and 1.0 M NaCl were added at a volume of 0.1 ml per 1.0 ml of cell suspension, and the mixture was left on ice for 90 min. Cell debris was removed by centrifugation for 30 min at 30,000 × *g*, and the supernatant fluid was saved (fraction I).

Purification of the enzyme. (i) Streptomycin sulfate precipitation. Fraction I was treated with streptomycin sulfate at a final concentration of 1.0 mg/ml. The solution was slowly stirred at 4°C for 60 min, and the precipitate was removed by centrifugation for 20 min at 25,000 × *g*. The supernatant fluid was saved and dialyzed against 4 liters of 50 mM Tris-hydrochloride (pH 7.4)–0.1 mM dithiothreitol (fraction II).

(ii) Ammonium sulfate precipitation. Solid ammonium sulfate was added to fraction II to a concentration of 35% (wt/vol), and this solution was slowly stirred at 4°C for 60 min. The precipitate was removed by centrifugation for 15 min at 15,000 × *g*, and the supernatant fluid was made 65% (wt/vol) with respect to ammonium sulfate. After being stirred at 4°C for 60 min, the precipitate was removed as described above,

TABLE 2. Purification of the nuclease from OT56[a]

Fraction	Description	Vol (ml)	Total protein (mg)	Sp act (U per mg of protein)	Recovery (%)
I	Cell-free extract	83	547	5.2	100
II	Streptomycin SO_4 supernatant	75	517	4.4	79
III	$(NH_4)SO_4$ precipitate	15	37.5	51.2	67
IV	Phosphocellulose	2	1.2	543	23
V	Glycerol gradient	2	0.038	1,264	1.7

[a] Each fraction was assayed for exonuclease activity, using DNA from bacteriophage B/78 or *E. coli* PB1379 as substrate.

and the supernatant was discarded. The pellet was suspended in 10 ml of buffer A and dialyzed for 24 h against 5 liters of the same buffer (fraction III).

(iii) Phosphocellulose chromatography. A column (20 by 1 cm) containing phosphocellulose in buffer A was washed with 100 ml of buffer A, and fraction III was slowly layered onto the top. This was followed by a 100-ml wash with buffer A. A 150-ml linear gradient of 0 to 1.2 M KCl in buffer A followed, and 5.0-ml fractions were collected. The flow rate was maintained at 10 ml/h. Each fraction was checked for nuclease activity on linear duplex DNA, and active fractions were pooled and concentrated with an Amicon pressure concentrator to produce fraction IV.

(iv) Glycerol gradient. Fraction IV was layered onto a 20 to 40% (wt/vol) linear glycerol gradient and centrifuged for 18 h in the SW41 rotor of a Beckman L5-65 ultracentrifuge. The gradient was fractionated by puncturing the bottom of the centrifuge tube and collecting 0.5- to 1.0-ml samples. Active samples were pooled to make fraction V.

S_1 nuclease digestion. S_1 nuclease (26 U) and 1 to 5 μg of DNA were mixed in 10 mM Tris-hydrochloride (pH 8.0)–1.0 mM EDTA–0.12 mM $ZnCl_2$ (250-μl volume). The mixture was incubated at 37°C for 1.0 h and the DNA was precipitated in ethanol and extracted once each with phenol and chloroform.

Other methods. Protein determinations were made by the procedure of Lowry et al. (12), using bovine serum albumin as a standard. Transfection of *P. aeruginosa* with the DNA of bacteriophages F116 and B/78 was carried out by the procedure of Mercer and Loutit (16), except the heat-pulse was for 1.0 min at 55°C.

RESULTS

Nuclease activity in crude extracts of Res$^+$ and Res$^-$ cells. Cell-free extracts of OT56 and OT684 were prepared (method 1) and assayed to detect nuclease activity on bacteriophage B/78 DNA. The extract of the Res$^+$ strain OT56 exhibited 100-fold greater degradation of B/78 DNA than that of OT684 (data not shown). Neither extract showed any activity in the absence of $MgCl_2$ or on a single-stranded DNA substrate, as one would have expected from the data of Miller and Clark (18). The difference in activity on B/78 DNA between Res$^+$ and Res$^-$ extracts encouraged us to purify the nuclease in OT56 since it was apparently absent in OT684.

Purification of the nuclease. Lysates of *P. aeruginosa* strains OT56 and OT684 were prepared (method 2) and subjected to a number of purification steps (Table 2). The enzyme activity at each step was determined by measuring the degradation of linear duplex DNA from phage B/78 or the chromosome of *E. coli* PB1379. During phosphocellulose chromatography, a peak of nuclease activity was eluted with 0.6 M KCl in the OT56 preparation, and a similar peak was shown to be absent in the preparation from OT684 (Fig. 1). This was the only difference detected in nuclease activity between the two strains, and this was confirmed in four separate experiments. The optical absorbance profiles were identical for both strains in each experiment (data not shown). The pool of phosphocellulose fractions showing this nuclease activity was therefore purified further on a glycerol gradient. The complete purification procedure resulted in a 243-fold purification of the nuclease, with 1.7% recovery.

Properties of the nuclease in fraction V. The purified nuclease was found to be EDTA sensitive and was partially dependent upon $MgCl_2$. In the absence of $MgCl_2$, the enzyme retained 17% of its maximal activity. The addition of ATP to the reaction mixtures had no effect.

The optimum pH of the nuclease was approximately 8.5, and it retained 22% of its maximal

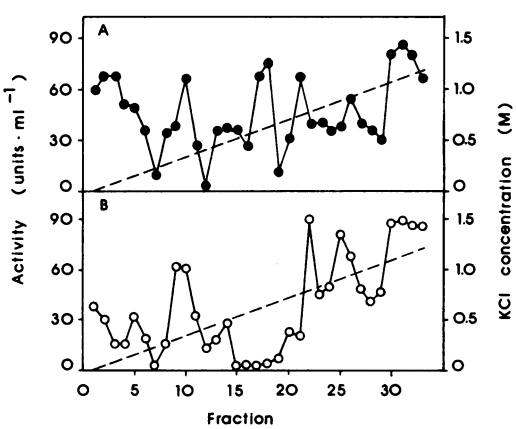

FIG. 1. Phosphocellulose chromatography of *P. aeruginosa* Res$^+$ and Res$^-$ lysates (fraction III). (A) OT56 (Res$^+$); (B) OT684 (Res$^-$). Chromatography and assay procedures are described in the text. The broken line represents the KCl concentration. Optical absorbance profiles were identical in each case (data not shown).

TABLE 3. Substrate specificity of the nuclease in fraction V[a]

DNA substrate	nmol solubilized	
	Native DNA	Heat-denatured DNA
P. aeruginosa chromosome	2.10	0
E. coli chromosome	1.73	0
Phage B/78	2.65	0
Phage F116	0	0

[a] Nuclease assays were carried out as described in the text. The above values are corrected by subtraction of blanks containing no enzyme.

activity at pH 8.0 and 12% at pH 9.0. At pH 6.0, 29% was retained. Most of the initial experiments were carried out at pH 7.0, but similar results were obtained when the pH was raised to 8.5.

The nuclease was active against linear duplex DNA from *P. aeruginosa* strains 1 and 78, *E. coli* PB1379, and bacteriophage B/78 (propagated on strains 1 and 78) (Table 3). When DNA from these sources was heat denatured, no degradation was detected. The DNA from bacteriophage F116 was not degraded under any conditions, which was a curious result considering that the F116 genome has been reported to be linear duplex DNA (7). A possible explanation for this observation is given below.

The plasmid RP1 was not degraded in the presence of as much as 10 U of enzyme, indicating that the enzyme possessed little or no endonuclease activity (data not shown). Therefore, the only substrate which was susceptible to degradation appeared to be linear duplex DNA, with the exception of that from bacteriophage F116. In reactions which were allowed to run to completion, the enzyme rendered 100% of native DNA to acid-soluble products.

Bacteriophage F116 DNA as substrate. F116 DNA is a linear duplex molecule (7, 20) and can be maintained as a plasmid in lysogenic strains (19). The latter was suggested as a result of isopycnic centrifugation studies (19) and was confirmed in this study (not shown). The linear form was not degraded by the nuclease purified from strain OT56, whereas other molecules of this type, such as B/78 and chromosomal DNA, are subject to degradation. In light of the observation that F116 DNA can exist in both linear and covalently closed circular forms, we thought it possible that the linear form may possess single-stranded terminal regions, thus protecting it from Res^+ exonuclease degradation. To test this hypothesis, [2-^{3}H]adenine-labeled F116 DNA was treated with S_1 nuclease, which is specific for single-stranded DNA (27). The S_1-treated DNA was then used as a substrate for the Res^+ exonuclease (Table 4). Under these

TABLE 4. Activity of the nuclease purified from strain OT56 on F116 and S_1-treated F116 DNA

Sample[a]	nmols solubilized[b]
F116 DNA	0
F116 DNA treated with S_1 nuclease	1.48
DNA control	0

[a] F116 DNA was treated with S_1 nuclease by mixing 26 U of enzyme with 10 nmol of DNA in TE buffer and 0.12 mM $ZnCl_2$ and then incubating the mixture for 1.0 h at 37°C. The DNA was ethanol precipitated and extracted once with phenol and chloroform before use.

[b] The specific activity of the DNA was 10,000 cpm/nmol.

conditions, the Res^+ exonuclease was able to degrade F116 DNA, with an activity comparable to that found when a chromosomal DNA substrate is used. Nearly 15% of the [^{3}H]DNA was solubilized in 30 min, compared with 26.5% when phage B/78 was used (Table 3). Also, when S_1-treated F116 DNA was used to transfect OT56 and OT684 recipients, no protection of F116 was observed in OT56, as measured by plaque formation (Table 5). OT684 cells were still efficient recipients. These results suggested that single-stranded regions do exist at the ends of the F116 DNA molecule.

DISCUSSION

A new exonuclease has been identified in *P. aeruginosa* strain 1. The enzyme was active against linear duplex DNA and could not degrade heat-denatured linear DNA or circular plasmid DNA. Exonuclease activity was independent of ATP and was stimulated by the presence of Mg^{2+}. A Res^- mutant of *P. aeruginosa*, OT684, was shown to lack this exonuclease activity.

Previous reports (15, 16) have indicated that during the process of genetic transformation in *P. aeruginosa*, linear duplex DNA is degraded,

TABLE 5. The effect of treatment with S_1 nuclease on transfection with the DNA from bacteriophages F116 and B/78

DNA[a]	No. of plaques[b]	
	OT684	OT56
F116	240	640
S_1-treated F116	190	0
B/78	572	0
S_1-treated B/78	468	0

[a] F116 and B/78 DNA were treated with S_1 nuclease as described in the legend to Table 3.

[b] Transfection was carried out as described by Mercer and Loutit (16), except that the heat pulse was for 1.0 min at 55°C.

whereas it is not degraded in the Res^- strain. Plasmid DNA transforms Res^+ and Res^- strains with equal efficiency. The results presented in this report support the in vivo studies and suggest that the exonuclease present in wild-type strains is responsible for the degradation of linear duplex DNA during transformation.

Miller and Clark (18) carried out a survey of major nucleases in *P. aeruginosa* strain 1 and identified three activities. One was similar to exonuclease V of *E. coli*, and the other was similar to exonuclease I of *E. coli* (2, 9, 22). The third activity, which was ATP independent and degraded linear duplex DNA only, was not purified since it was very unstable. The exonuclease identified in this report was also unstable, losing activity after approximately 2 weeks at −20°C. It is possible that the Res^+ exonuclease was identified by Miller and Clark (18) and that our purification procedure allowed for greater stability. We did not detect any activity specific for single-stranded DNA in crude lysates from Res^+ and Res^- strains, as was reported by Miller and Clark (18). We cannot account for this observation, although the methods of cell growth and breakage were different in each case. A possible *E. coli* counterpart for this enzyme is exonuclease VIII (5), the *recE* gene product, but it is premature to draw any conclusions. Hence, we have retained the gene symbol *res*.

The bacteriophage F116 was shown to possess DNA with single-stranded terminal regions, thus providing an explanation for its resistance to degradation by the Res^- exonuclease. The end regions are not required for normal phage infection and propagation, since plaques are produced after transfection of a Res^- strain with S_1 nuclease-treated F116 DNA. We do not know whether or not these terminal regions play a role in the establishment of lysogeny.

Although the exonuclease identified in this report may play a role in genetic recombination in *P. aeruginosa*, it is not possible to draw any conclusions regarding the nature of the recombination pathways operating in this organism. From the studies of Miller and Clark (18), Scurlock and Miller (25), and Lehrbach et al. (10) and this report, it is becoming clear that *P. aeruginosa* possesses nucleases both unique and similar to those found in *E. coli*.

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LITERATURE CITED

1. **Bachmann, B. J., D. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
2. **Clark, A. J.** 1974. Progress toward a metabolic interpretation of genetic recombination of *Escherichia coli* and bacteriophage lambda. Genetics **78**:259–271.
3. **Cosloy, S. D., and M. Oishi.** 1973. The nature of the transformation process in *Escherichia coli* K-12. Mol. Gen. Genet. **124**:1–10.
4. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B12. J. Bacteriol. **60**:17–28.
5. **Gillen, J. R., and A. J. Clark.** 1974. The *recE* pathway of bacterial recombination, p. 123–136. *In* R. F. Grell (ed.), Mechanisms in recombination. Plenum Publishing Corp., New York.
6. **Hinkle, N. F., and R. V. Miller.** 1979. pMG7-mediated restriction of *Pseudomonas aeruginosa* phage DNA is determined by a class II restriction endonuclease. Plasmid **2**:387–393.
7. **Holloway, B. W., and V. Krishnapillai.** 1975. Bacteriophages and bacteriocins, p. 99–132. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, Inc., New York.
8. **Holloway, B. W., V. Krishnapillai, and A. F. Morgan.** 1979. Chromosomal genetics of *Pseudomonas*. Microbiol. Rev. **43**:73–102.
9. **Kushner, S. R., H. Nagaishi, and A. J. Clark.** 1974. Isolation of the enzyme associated with the *sbcA* indirect suppressor, p. 137–143. *In* R. F. Grell (ed.), Mechanisms in recombination. Plenum Publishing Corp., New York.
10. **Lehrbach, P. R., C. D. Dirckze, and B. T. O. Lee.** 1980. A mutant of *Pseudomonas aeruginosa* deficient in an ATP-dependent deoxyribonuclease. J. Gen. Microbiol. **120**:377–384.
11. **Loutit, J. S.** 1969. Investigation of the mating system of *Pseudomonas aeruginosa* strain 1. V. The effect of N-methyl-N'-nitro-N-nitrosoguanidine on a donor strain. Genet. Res. **14**:103–109.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Marmur, J.** 1961. A procedure for the isolation of DNA from microorganisms. J. Mol. Biol. **3**:208–218.
14. **Mercer, A. A., and J. S. Loutit.** 1978. Transformation of *Pseudomonas aeruginosa* by DNA of the conjugative plasmid FP2. Proc. Univ. Otago Med. Sch. **56**:19–20.
15. **Mercer, A. A., and J. S. Loutit.** 1978. Transformation and transfection of *Pseudomonas aeruginosa* with linear DNA. Proc. Univ. Otago Med. Sch. **56**:56–57.
16. **Mercer, A. A., and J. S. Loutit.** 1979. Transformation and transfection of *Pseudomonas aeruginosa*: effects of metal ions. J. Bacteriol. **140**:37–42.
17. **Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529–1537.
18. **Miller, R. V., and A. J. Clark.** 1976. Purification and properties of two deoxyribonucleases of *Pseudomonas aeruginosa*. J. Bacteriol. **127**:794–802.
19. **Miller, R. V., J. M. Pemberton, and A. J. Clark.** 1977. Prophage F116: evidence for extrachromosomal location in *Pseudomonas aeruginosa* strain PAO. J. Virol. **22**:844–847.
20. **Miller, R. V., J. M. Pemberton, and K. E. Richards.** 1974. F116, D3 and G101: temperate bacteriophages of *Pseudomonas aeruginosa*. Virology **59**:566–569.
21. **Oishi, M., and S. D. Cosloy.** 1972. The genetic and biochemical basis of the transformability of *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **49**:1568–1572.
22. **Oishi, M., S. D. Cosloy, and S. K. Batu.** 1974. Transformation-mediated recombination in *Escherichia coli*, p. 145–154. *In* R. F. Grell (ed.), Mechanisms in recombination. Plenum Publishing Corp., New York.
23. **Pearce, L. E., and J. S. Loutit.** 1965. Biochemical and genetic grouping of isoleucine-valine mutants of *Pseudomonas aeruginosa*. J. Bacteriol. **89**:58–63.

24. **Pemberton, J. M., and A. J. Clark.** 1973. Detection and characterization of plasmids in *Pseudomonas aeruginosa* PAO. J. Bacteriol. **114**:424–433.
25. **Scurlock, T. R., and R. V. Miller.** 1979. *Pae*Exo IX: a unique deoxyribonuclease from *Pseudomonas aeruginosa* active in the presence of EDTA. Nucleic Acids Res. **7**:167–177.
26. **Stanisich, V. A., and P. M. Bennett.** 1976. The properties of hybrids gormed between the P-group plasmid RP1 and various plasmids of *Pseudomonas aeruginosa*. Mol. Gen. Genet. **149**:217–223.
27. **Vogt, V. H.** 1973. Purification and further properties of single-strand-specific nuclease from *Aspergillus oryzae*. Eur. J. Biochem. **33**:192–200.