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Detection of FP Plasmids in Hospital Isolates of *Pseudomonas aeruginosa*

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A simple method of detection of FP plasmids with chromosome-mobilizing ability in *Pseudomonas aeruginosa* has been developed.

Plasmids which mobilize the chromosome of *Pseudomonas aeruginosa* (FP plasmids) have been detected in hospital isolates of this species at a frequency of between 15 and 30% (10; P. Royle and B. W. Holloway, unpublished data; R. K. Hindman and A. F. Morgan, unpublished data). The great majority of these FP plasmids mobilize the chromosome from the same origin and in the same direction as FP2. This plasmid has been used extensively in the mapping of the *P. aeruginosa* PAO chromosome, and the FP2 origin of transfer has been arbitrarily chosen as 0 min (3, 5, 12). A notable exception is FP110, which has a major transfer origin located between 25 and 28 min on the *P. aeruginosa* PAO genetic map and which transfers the chromosome in the opposite direction to that of FP2 (P. Royle and B. W. Holloway, manuscript in preparation).

The method used previously for detecting FP plasmids necessitated isolating an auxotroph for each hospital isolate to provide a contraselective marker in plate matings (11) with multiply marked PAO recipients. The streptomycin selection method used for detection of F-factors in *Escherichia coli* (8) is unsuitable due to the high frequency of mutation to streptomycin resistance of many *P. aeruginosa* strains. However, we have never observed mutation to high-level tetracycline resistance in *P. aeruginosa*. This fact has permitted us to increase the rate at which hospital isolates can be screened for FP plasmids by using the following simple procedure. The sources of the hospital isolates and the constituents of growth media have been described elsewhere (2). Minimal medium agar plates supplemented with all but one of the requirements of a multiply auxotrophic recipient and 60 μg of tetracycline (oxytetracycline hydrochloride; Sigma Chemical Co.) per ml were spread with 0.1-ml portions of a saline-washed suspension of a nutrient broth culture (grown overnight at 43°C) of the multiply marked *P. aeruginosa* PAO recipient carrying a Tra^- (transfer-deficient; obtained by selection for resistance to the donor specific phage PRD1 [9]) derivative of the broad-host-range plasmid R18, also termed RP1 (7), which confers resistance to tetracycline, carbenicillin, and neomycin-kanamycin (1). Growth at 43°C renders strain PAO phenotypically restrictionless (4). Preliminary experiments had shown that 60 μg of tetracycline per ml yielded the highest recombinant recovery with hospital isolates known to possess FP plasmids. One of each type of selective plate was then spread with 0.1 ml of a fresh overnight nutrient broth culture of a hospital isolate, and the plates were incubated at 37°C for 2 to 3 days. The substitution of stationary-phase donors for the more usual exponential-phase donors in such matings was found to reduce the recombinant yield somewhat, but this was compensated for by the observation that the use of tetracycline (instead of the lack of a required amino acid) as the contraselective agent greatly increased the recombinant yield for any given hospital isolate. Possibly, this is due to reduced production of bacteriophage and/or aeruginocin by the donors in the presence of tetracycline. Probably, all hospital isolates of *P. aeruginosa* are both lysogenic and aeruginocinogenic (6).

An alternative method for detecting recombinants, by mating in the presence of rifampin with a rifampin-resistant recipient, was tried, but it gave significantly fewer recombinants than the method described above (data not shown).

This procedure was used to screen 454 isolates of *P. aeruginosa*, and 82 (18%) yielded recombinants for at least one of the three markers selected (Table 1). It is possible that transduction rather than conjugation was responsible for some of the recombinants, particularly where the frequencies of recombination were low.

In addition to increasing the rate at which clinical isolates may be screened, this method has the advantage of yielding sufficient recombinants to allow characterization of the chromosome-mobilizing ability (Cma) of the FP plasmids in terms of the predominant site of chromosome transfer. FP2 shows a higher frequency

TABLE 1. *Recombination between PAO222 (R18 Tra⁻) and various hospital isolates of P. aeruginosa*[a]

Hospital isolate designation	No. of recombinants		
	ilv-226⁺	*lys-12*⁺	*pro-82*⁺
Ps861	>500	>500	>500
Ps626	>500	>500	500
Ps898	>500	>500	366
Ps685	>500	>500	294
Ps700	>500	>500	200
Ps756	>500	>500	150
Ps924	>500	420	49
Ps926	366	374	53
Ps927	280	117	12
Ps910	248	356	50
Ps771	230	192	22
Ps911	221	346	55
Ps742	148	250	96
Ps614	145	222	30

[a] PAO222 (13) has requirements for isoleucine–valine (*ilv-226*, 8 min), histidine (*hisII4*, 17 min), lysine (*lys-12*, 20 min), methionine (*met-28*, 30 min), tryptophan (*trp-6*, 35 min), and proline (*pro-82*, 40 min); the map positions given are those of Haas et al. (3). Of the 440 additional isolates, 35 yielded 50 to 200 recombinants for at least one of the three markers, 33 yielded 5 to 50 recombinants for at least one of the three markers, and 372 yielded <5 recombinants for all three markers.

of transfer of markers situated closer to its origin, and hence the relative frequency of transfer for markers situated at different sites on the chromosome gives an indication of the origin of transfer for the new FP plasmid being tested. To characterize such plasmids fully, it was necessary to transfer them to a PAO strain. This was done in the case of 10 FP plasmids which looked as though they might have transfer origins different from FP2, by transfer to a PAO recipient (11) from those PAO222 chromosomal recombinants which had coinherited the FP plasmid, detected by replica plating patched recombinant clones on to a lawn of an appropriately marked PAO recipient with selection for chromosomal recombination. However, when transferred in this way and subsequently characterized by plate matings with various PAO derivatives (data not shown), none of these FP plasmids showed a different origin of transfer from that of FP2. They differed from FP2 in that none of them conferred resistance to mercuric ions.

Thus, FP plasmids with different origins of transfer from FP2 are extremely rare, FP110 being the one exception out of about 150 FP plasmids detected from 750 hospital isolates in this laboratory. The method presented here will be used to screen more isolates, as FP plasmids having origins of chromosome transfer different from that of FP2 would be extremely useful in the mapping of the *P. aeruginosa* chromosome.

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LITERATURE CITED

1. **Chandler, P. M., and V. Krishnapillai.** 1974. Phenotypic properties of R factors of *Pseudomonas aeruginosa*: R factors readily transferable between *Pseudomonas* and the Enterobacteriaceae. Genet. Res. **23**:239–250.
2. **Dean, H. F., A. F. Morgan, L. V. Asche, and B. W. Holloway.** 1977. Isolates of *Pseudomonas aeruginosa* from Australian hospitals having R-plasmid determined antibiotic resistance. Med. J. Aust. **2**:116–119.
3. **Haas, D., B. W. Holloway, A. Schamböck, and T. Leisinger.** 1977. The genetic organization of arginine biosynthesis in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **154**:7–22.
4. **Holloway, B. W.** 1965. Variations in restriction and modification following increase of growth temperature in *Pseudomonas aeruginosa*. Virology **25**:634–642.
5. **Holloway, B. W.** 1975. Genetic organization of *Pseudomonas*, p. 133–161. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons Ltd, London.
6. **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 Ltd, London.
7. **Jacoby, G. A., and J. A. Shapiro.** 1977. Plasmids studied in *Pseudomonas aeruginosa* and other pseudomonads, p. 639–656. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Lederberg, J., E. M. Lederberg, N. D. Zinder, and E. R. Lively.** 1951. Recombination analysis of bacterial heredity. Cold Spring Harbor Symp. Quant. Biol. **16**: 413–443.
9. **Olsen, R. H., J.-S. Siak, and R. H. Gray.** 1974. Characteristics of PRD1, a plasmid-dependent broad host range DNA bacteriophage. J. Virol. **14**:689–699.
10. **Pemberton, J. M., and B. W. Holloway.** 1973. A new sex factor of *Pseudomonas aeruginosa*. Genet. Res. **21**: 263–272.
11. **Stanisich, V. A., and B. W. Holloway.** 1972. A mutant sex factor of *Pseudomonas aeruginosa*. Genet. Res. **19**: 91–108.
12. **Stanisich, V. A., and M. H. Richmond.** 1975. Gene transfer in the genus *Pseudomonas*, p. 163–190. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons Ltd, London.
13. **Watson, J. M., and B. W. Holloway.** 1976. Suppressor mutations in *Pseudomonas aeruginosa*. J. Bacteriol. **125**:780–786.