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Compatibility and sex specific phage plating characteristics of the TOL and NAH catabolic plasmids

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SUMMARY

The sex specific bacteriophage PR4 has been found to plate on *P. aeruginosa* strains harbouring the TOL catabolic plasmid or the plasmid pND2 derived from TOL. Based on this, attempts were made to place TOL into a *Pseudomonas* plasmid incompatibility group and by showing that pND2 is incompatible with the R plasmid R2, TOL has been placed into the P-9 group. The NAH catabolic plasmid has been reported to be incompatible with TOL, pND2 and a variety of other plasmids derived from TOL. Thus, these plasmids also would appear to belong to the P-9 incompatibility group.

1. INTRODUCTION

One of the approaches commonly employed in studies of the relationship between various plasmids entails a comparative examination of plasmid encoded sensitivity to sex specific phages (Mitsuhashi, 1971; Stanisich, 1974; Falkow, 1975; Olsen & Shipley, 1975). In work oriented around the study of interrelationships between *Pseudomonas* plasmids, a number of phages have been isolated which infect strains harbouring P-1 incompatibility R plasmids. The phage PR4 plates on strains harbouring P-1, P-9 and P-10 (Stanisich, 1974), N (Bradley, 1974), W (Bradley & Rutherford, 1975) and C (Stanisich, 1976) incompatibility group plasmids and on strains harbouring the ungrouped *Pseudomonas* plasmids R18-1, RP1-1 and RP8 (Stanisich, 1974). Actually, RP1-1 is thought to be an alternate designation for R18-1 (Holloway & Richmond, 1973). The naming of the P-1, P-9 and P-10 groupings follows the system recently developed by Jacoby (1978). The phages Pf3 and PRR1 have been reported to plate on strains harbouring P-1 incompatibility group plasmids (Stanisich, 1974; Stanisich, 1976).

Experiments aimed at determining the incompatibility groupings of the catabolic plasmids have shown that CAM is incompatible with P-2 group R plasmids (Jacoby, 1974), that CAM and OCT are incompatible (Chou, Katz & Gunsalus, 1974) and that SAL is incompatible with the P-9 incompatibility group R plasmid R2 (reported in Jacoby, 1978). The nonconjugative XYL plasmid appears to be compatible with both CAM and SAL (Friello *et al.*, 1976). Although NAH and TOL are incompatible with each other (Austen & Dunn, 1977), their incompatibility relationship to other plasmids has not been reported.

This paper demonstrates that certain strains harbouring TOL and a plasmid derived from TOL, pND2 (White & Dunn, 1977), are susceptible to PR4 phage infection and that these plasmids belong to the P-9 plasmid incompatibility group.

2. MATERIALS AND METHODS

The bacterial strains, bacteriophages and plasmids used in this study are detailed in Table 1.

Media, general procedures and techniques for plasmid transfer by the plate mating and tube mating methods have been described previously (White & Dunn, 1977).

Phage propagation in liquid medium. The method used was as described by Stanisich (1974).

Spot test and cross-streak test for lytic activity. For the spot test, approximately 10^8 cells of a log phase culture of the bacterium to be tested was added to a 3 ml nutrient agar layer containing agar at a concentration of 0·6 %. This layer was poured onto the surface of a nutrient agar plate and allowed to set, then a loopfull of phage suspension was spotted onto the surface. Lytic activity was indicated by visible clearing after incubation overnight at temperatures optimum for growth of the test organism. For the cross-streak test, 0·05 ml of a phage preparation at approximately 10^9 phage particles/ml was streaked across a nutrient agar plate which was then allowed to dry. Bacteria to be tested were taken from a nutrient agar plate after incubation overnight and streaked across the phage.

Efficiency of plating (*e.o.p.*). The phage suspension to be tested was assayed on the different bacterial hosts; this e.o.p. was represented as a fraction of the titre measured on the sensitive strain PAO3(RP1).

3. RESULTS

(i) *Plating of PR4, PRR1 and Pf3 on strains harbouring TOL, pND2 or RP1*

The ability of PR4, PRR1 and Pf3 to infect *P. aeruginosa* (PAO1 and its mutant derivative PAO3) and *P. putida* (PP1-2) harbouring TOL, pND2 or RP1 was studied. Strains harbouring RP1 were used as a positive control. PAO3 differs from its parent in that it has a tryptophan requirement and is resistant to the *P. aeruginosa* phage F116, apparently because it lacks the pili believed to be involved in F116 adsorption (Pemberton, 1973).

Sensitivity tests were conducted on solid medium using the cross streak and spot test methods to examine for lytic activity and in liquid medium to test for increase in phage titre. The results are given in Table 2. As expected, both lytic activity on solid medium and an increase in phage titre in liquid medium were observed when PAO strains harbouring RP1 were infected by PR4, PRR1 or Pf3. Unexpectedly these phages were unable to infect PP1-2 hosts harbouring RP1. Cloudy zones of lysis on solid medium and an increase in phage titre in liquid medium resulted when PAO3(TOL), PAO3(pND2) and PAO1(pND2) were infected with PR4. Phage plating on PAO3 harbouring TOL was indistinguishable from

Table 1. *Bacterial strains, bacteriophages and plasmids*

Bacterial strains

Strain no.	Genotype	Reference
PAO1	prototroph, chl2, FP$^-$	Holloway (1969)
PAO3	trp54, FP$^-$	Holloway (1969)
PAO1670	ade136, leu8, rif1, chl3, FP$^-$	Chandler & Krishnapillai (1974*c*)
PP1-2	prototroph	Wong & Dunn (1974)

Bacteriophages

Designation	Characteristics	Reference
PR4	Sex specific phage which plates on strains harbouring P-1, P-9, P-10, N, W and C incompatibility group plasmids and R18-1, RP1-1 and RP8	Stanisich (1974), Bradley (1974), Bradley & Rutherford (1975), Jacoby (1978)
PRR1	Sex specific phage which plates on strains harbouring P-1 group plasmids.	Olsen & Thomas (1973), Stanisich (1976), Jacoby (1978)
Pf3	Sex specific phage which plates on strains harbouring P-1 group plasmids	Stanisich (1974)

Plasmids

Designation	Characteristics	Reference
TOL	Catabolic plasmid which encodes the degradation of the toluates and other aromatic compounds	Williams & Murray (1974), Wong & Dunn (1974), Austen & Dunn (1977)
pND2	*Catabolic plasmid derived from TOL; selected through its stability in PAO3	White & Dunn (1977), White & Dunn (1978)
NAH	Catabolic plasmid which encodes the degradation of naphthalene	Dunn& Gunsalus (1973), Austen & Dunn (1977)
RP1, R68	P-1 incompatibility group R plasmid. Wide transfer range. Cb^r, Tc^r and Nm^r/Km^r	Grinsted *et al.* (1972), Olsen & Shipley (1973), Chandler & Krishnapillai (1974*a*), Jacoby (1978)
R2	P-9 incompatibility group R plasmid. Cb^r, Sm^r and Su^r	Kawakami *et al.* (1972), Jacoby (1978)
R91	P-10 incompatibility group R plasmid. Cb^r only, in *Pseudomonas*	Chandler & Krishnapillai, (1974*b*), Stanisich (1974), Jacoby (1978)
R18-1	Incompatibility group not yet established. Cb^r	Chandler & Krishnapillai (1974*a*), Stanisich (1974), Jacoby (1978)

Abbreviations: ade, adenine requirement; Cb^r, carbenicillin resistant; chl, resistance to chloramphenicol; FP$^-$, absence of any known sex factor; Km^r, kanamycin resistant; leu, leucine requirement; Nm^r, neomycin resistant; PAO, *P. aeruginosa* strain 1; PP1, *P. putida* strain 1; rif, resistance to rifampicin; Sm^r, streptomycin resistant; Su^r, sulphanilamide resistant; Tc^r, tetracycline resistant; trp, tryptophan requirement.

* When TOL is introduced into PA0 host strains it is unstable; the rate of segregation resulting from this instability varies in degree with the particular mutant host. After growth of PAO3(TOL) through approximately 70 generations without selecting for maintenance of TOL, 57% of cells had segregated TOL (White & Dunn, 1977). Variants could be isolated where TOL no longer segregated readily under these experimental conditions (one variant was characterized and called pND2).

plating observed on the same strain harbouring pND2. Because of this, and the problems associated with the stability of TOL in strain PAO, future work in this system was conducted using the stable pND2 plasmid. Neither PRR1 nor Pf3 appeared able to infect or propagate on strains harbouring TOL or pND2. Since PP1-2(RP1) was found to be resistant to the sex specific phages and as strains harbouring RP1 were intended for use as positive controls it was not unexpected to find that PP1-2(TOL) and PP1-2(pND2) were also resistant to infection by PR4, PRR1 and Pf3.

Table 2. *Lytic activity and propagation of PR4, PRR1 and Pf3 on strains harbouring TOL, pND2 and RP1*

	Lytic activity			Increase in phage titre in liquid medium		
Strain	PR4	PRR1	Pf3	PR4	PRR1	Pf3
PAO1(pND2)	(+)	−	−	+	−	−
PAO3 (TOL)	(+)	−	−	+	−	−
PAO3(pND2)	(+)	−	−	+	−	−
PP1-2(TOL)	−	−	−	−	−	−
PP1-2(pND2)	−	−	−	−	−	−
PAO1(RP1)	+	+	+	+	+	+
PP1-2(RP1)	−	−	−	−	−	−
PAO1	−	−	−	−	−	−
PP1-2	−	−	−	−	−	−

The data on phage lytic activity was obtained by using the cross streak and spot test methods: +, very clear and complete lysis; (+), distinct but cloudy lysis; −, no discernible lysis. Increase in phage titre in liquid medium: +, increase in titre of at least 10^3 plaque forming units/ml; −, no detectable increase in phage titre.

(ii) *Efficiency of plating of PR4 on PAO3 harbouring pND2 and various R plasmids*

Stanisich (1974) reported that PAO strains harbouring the R plasmids R91 and R18-1 were usually repressed for functions required for PR4 propagation. This was indicated by insensitivity of these strains to PR4 infection when tested on solid medium, compared to their sensitivity as measured by the ability to propagate the phage in liquid medium. As the lytic zones were cloudy when PR4 was plated on PAO3(pND2), it was decided to compare the e.o.p. of PR4 on PAO3(pND2) to that on PAO3 harbouring RP1, R68, R2, R91 and R18-1. R68 was included in this study as a second example of a P-1 incompatibility group R plasmid (Chandler & Krishnapillai, 1974*a*), R2 as a representative of the P-9 group, R91 representing the P-10 group and R18-1 an ungrouped plasmid (Jacoby, 1978). The results of this experiment are given in Table 3. The e.o.p. of PR4 on PAO3(pND2) was of the same order as that observed for PAO3(R68), PAO3(RP1) and PAO3(R2), but was markedly superior to that on PAO3 (R91) and PAO3(R18-1). The results presented in both Tables 2 and 3, plus the data of Stanisich (1974–1976), imply that there is a close similarity in phage plating properties between PAO strains harbouring TOL or pND2 and strains harbouring R2.

(iii) *Compatibility relationship between pND2 and R plasmids.*

It was demonstrated by Stanisich (1974) that the *Pseudomonas* R plasmids encoding susceptibility to PR4 infection belong to several incompatibility groups. We have previously reported that TOL and pND2 are compatible with R91 of the P-10 incompatibility group (White & Dunn, 1977). We have found, using the same procedures, that the introduction and selection for maintenance of either TOL or pND2 into strains harbouring RP1 or R68 does not force segregation of either of these P-1 incompatibility group plasmids.

Table 3. *The efficiency of plating of PR4 on PAO3 harbouring pND2 and various R plasmids*

Strain	Efficiency of plating
PA03(RP1)	1
PA03(R68)	2×10^{-1}
PA03(pND2)	8×10^{-2}
PA03(R2)	5×10^{-2}
PA03(R91)	1×10^{-6}
PA03(R18-1)	2×10^{-8}
PA03	$< 1 \times 10^{-9}$

The e.o.p. is represented as a fraction of the titre estimated on the host PAO3(RP1). Four individually isolated stabilized transconjugants of PAO3(pND2) were used for the determination of the e.o.p.

To study the relationship between R2 and pND2, the R2 plasmid was transferred by conjugation from PAO1670(R2) into PAO1(pND2) and PAO1 selecting for transfer of the carbenicillin resistance phenotype. To each recipient the transfer frequency was approximately 10^{-5} transconjugants per donor cell. To test for forced segregation of pND2, one hundred transconjugants were streaked twice for single colonies on the selection medium, which selected for maintanance of R2. All transconjugants were found to have lost the ability to grow on *m*-toluate, implying forced segregation of pND2 by R2. Fifty clones of PAO1(pND2) were grown through a similar number of generations on a nutrient agar medium. All fifty retained the ability to grow on *m*-toluate, which illustrates the high stability of pND2 in PAO1. In a reverse cross, pND2 was transferred from PAO1670(pND2) into PAO1(R2) and following selection for growth on *m*-toluate, thus selecting for maintenance of pND2, R2 was found to have been forced to segregate, indicated by loss of the carbenicillin resistance phenotype. These results suggest that pND2 belongs to the P-9 incompatibility group.

4. DISCUSSION

Incompatibility studies conducted in our laboratory have shown that all the catabolic plasmids and their derivatives, which are being studied by our group and which encode for the degradation of aromatic compounds, belong to one incompatibility group. We have found that NAH is incompatible with TOL (Austen &

Dunn, 1977) and with the derivative of TOL, pND2, which was isolated for its stability in PAO host cell strains (White & Dunn, 1977). NAH is also incompatible with pND3, the plasmid constructed by recombination between TOL and the P-10 group R plasmid R91 (White & Dunn, 1977), and with several plasmids derived by apparent transductional shortening of pND3 (White & Dunn, 1978).

The phage plating characteristics exhibited by strains harbouring pND2 directly led to the testing of this plasmid for incompatibility with R2. The plasmids pND2 and R2 were found to be incompatible. This data suggests that all our catabolic plasmids belong to the P-9 incompatibility group.

The observation that PR4 would not plate on PP1-2 harbouring either TOL or RP1 was unexpected. Such a barrier to infection by sex specific phages is not usually encountered, but is possibly due to some peculiarity of the *P. putida* host strain. The NAH plasmid is not transmissible to PAO cell lines (Austen & Dunn, 1977), therefore phage sensitivity could not be readily tested in a host cell which did not interfere with phage plating. A number of other *P. putida* strains harbouring either TOL or NAH were also found resistant to PR4.

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