Bacteriophage D: an IncD Group Plasmid-specific Phage

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The existence of the plasmid incompatibility group D was reaffirmed as a result of compatibility experiments done on plasmids R687, R711b, R778b and R840 which were previously tentatively accepted as constituting the group. The group was further delineated by the isolation of a phage, phage D, which adsorbed specifically to IncD plasmid-encoded pili produced by *Escherichia coli* K12 strains and strains of *Salmonella typhimurium*, *Proteus morganii* and *Klebsiella oxytoca* harbouring one of these plasmids. Plaque formation, like that of phage pilH α , was temperature sensitive in that plaques formed at 26 °C but not at 37 °C. Plaques were fairly clear, regular in outline and varied from pinpoint to about 1.5 mm in diameter on *E. coli* hosts where plaques were detected, but on the other hosts the plaques were more turbid and often irregular in outline. The phage did not plate (or propagate) on IncD plasmid-carrying strains of *Providencia alcalifaciens*, *Providencia stuartii* or *Serratia marcescens*. The phage had an isometric hexagonal outline with a diameter of about 27 nm. It contained RNA and resembled two other RNA-containing phages, M and pilH α , by being sensitive to chloroform. It adsorbed to the sides of the very distal ends of the shafts of IncD plasmid-coded pili.

INTRODUCTION

The plasmid incompatibility (Inc) group D has not been formally described. Datta (1979) mentioned it for the first time merely as an unpublished observation.

Plasmids R687, R711b, R778b and R840, which now constitute the group, were present in strains of *Providencia* (Hedges, 1974). On the basis of incompatibility reactions, R711b and R778b were at the time assigned to the IncX plasmid group. Plasmids R687 and R840 were members of a group of 15 plasmids which all coded for resistance to ampicillin and kanamycin but were heterogenous in other respects. Lack of suitable markers limited incompatibility tests and these two plasmids, as well as a number of others, were not allocated to particular incompatibility groups although the suggestion was made that the collection could represent several undescribed groups. By 1977 (Bradley, 1977) there was some doubt about the classification of R711b and R778b as experiments had indicated that R711b and the IncX plasmid R6K were not incompatible.

Bradley (1977) found that pili produced by plasmids R711b and R778b were serologically related. These pili resembled those produced by IncFI and F_olac plasmids morphologically (thick, flexible) but differed serologically from pili specified by the latter plasmids. He also determined that although the RNA-containing phage R17 adsorbed to the shafts of only the IncFI pili, the filamentous phage fd adsorbed to the tips of all the above pili and lysed the corresponding *Escherichia coli* host strains.

Bradley & Meynell (1978) failed to propagate phage MS2 on strains of *E. coli* carrying plasmid R711b. In electron microscopic specimens of these bacteria exposed to high concentrations of the phage and fixed with formalin, they demonstrated adsorption of phage MS2 along the entire length of R711b-coded pili. Without this fixation the pili did not show adsorbed virions. They concluded that adsorption of phage MS2 to R711b pili was a reversible event not leading to any significant level of infection, as no plaques were found, and that formalin apparently stabilized the adsorption.

Bradley (1980) listed the plasmids R711b and R778b as belonging to Inc group D and demonstrated a serological relationship between pili determined by the then unclassified plasmid R687 and those encoded by R711b. Subsequently Sirgel *et al.* (1981) and Bradley *et al.* (1982) added plasmids R840 and R687 respectively to the IncD group on the grounds of unpublished experiments of R. W. Hedges.

Phage J (Bradley *et al.*, 1982), apart from plating (or propagating) on IncJ and some IncC plasmid-carrying strains of *E. coli*, also plated on strains of the latter organisms harbouring any of the IncD plasmids. This DNA-containing phage which resembles phage T3 (Bradley, 1967) but is significantly smaller, adsorbed to the shafts of the pili coded for by these plasmids. The lysis observed served to confirm the phage fd plating experiments and morphological findings (Bradley, 1977, 1980) on strains carrying R711b, R778b and R687, that all these plasmids synthesized pili constitutively.

To help in classifying the as yet poorly described IncD group, it was decided to investigate the incompatibility arrangement of the four plasmids mentioned above. Also in order to have a rapid means of identifying bacteria harbouring these plasmids, we attempted the isolation of a phage from sewage, specifically lytic for these strains. Since the plasmids code for constitutive production of pili (see above) this was considered to be a feasible proposition (Meynell *et al.*, 1968; Meynell & Lawn, 1968). Here properties of such an RNA-containing phage are described.

METHODS

Bacteria, bacteriophages and plasmids. Bacteria and bacteriophages are listed in Table 1. Species identification of the original IncD plasmid-harbouring *Providencia* strains was not made (Hedges, 1974) and the strains are no longer available. Plasmids used for incompatibility testing and host range experiments originated from the collection of Naomi Datta (Datta, 1977, 1979). Apart from plasmids belonging to the IncD incompatibility group (Tables 2 and 3), only some of those which encoded constitutive pilus synthesis (as judged by plaque formation by appropriate plasmid-specific phages) were used in host range experiments (Coetzee *et al.*, 1985). Thus one or more plasmids belonging to the following Inc groups were used: C (Sirgel *et al.*, 1981), F subgroups I–IV (Jacob *et al.*, 1977), HI and HII (Coetzee *et al.*, 1985), I₁ and I₂ (Coetzee *et al.*, 1982), J (Bradley *et al.*, 1982), M (Coetzee *et al.*, 1983), N, P and W (Coetzee *et al.*, 1979), X (Bradley *et al.*, 1981*b*), and plasmid EDP208 (*F₀lac drd*) (Bradley *et al.*, 1981*a*). Plasmid-carrying strains were maintained on selective antibacterial drug-containing media to minimize plasmid segregation (Bradley, 1977).

Media, antibacterial agents, general phage techniques, transfer of plasmids by conjugation, organic solvent and RNAase sensitivity of phage, temperature sensitivity of plaque formation and production of phage lysates by a soft agar layer technique. These were as described and referred to by Adams (1950), Coetzee *et al.* (1979, 1985), Bradley *et al.* (1980, 1981*b*), Ackermann *et al.* (1978) and Hershey *et al.* (1943). The minimal glucose agar was as described in Clowes & Hayes (1968). As all IncD plasmids are unstable in *E. coli* and in *Salmonella typhimurium* strains (see Results; Bradley, 1977), the broth and soft top-layer agar used in experiments with these plasmid-carrying strains contained appropriate plasmid-selective antibacterial agents. Phage isolation experiments and all other experiments were done at an incubation temperature of 26 °C unless otherwise stated. This was done to avoid the initial confusion resulting from the isolation of the temperature-sensitive phage pilH_z using a temperature of 37 °C (Coetzee *et al.*, 1985). The 26 °C incubation temperature was considered appropriate in view of the fact that the non-temperature sensitive RNA-containing phages C-1 (Sirgel *et al.*, 1981) and M (Coetzee *et al.*, 1983) plated on their respective hosts at the latter temperature (J. N. Coetzee, unpublished).

Classification of plasmids by incompatibility. This was based on the principles outlined by Datta (1979) using the methods of Coetzee *et al.* (1972). Briefly, R⁺ *E. coli* cultures were grown in media selective for the plasmid carried, washed three times in drug-free broth and then allowed to conjugate for 4 h in that medium. Mating mixtures were plated on medium selective for transconjugants and on non-selective medium to provide colonies in which retention of the resident plasmid could be determined. All crosses were done at least three times.

Phage isolation. Preliminary experiments had indicated that phage fd only plated on *E. coli* K12 strains carrying the plasmids R687, R711b, R778b and R840. The phage did not plate on *S. typhimurium* M827-2 or *Providencia*

Table 1. *Bacteria and bacteriophages*

Bacteria	Properties*	Reference
<i>Escherichia coli</i> K12		
J53	Pro ⁻ Met ⁻ (λ)	Bachmann (1972)
J53-2	Pro ⁻ Met ⁻ Rif ^R (λ)	
J62	Lac ⁻ Pro ⁻ His ⁻ Trp ⁻ (λ)	Datta <i>et al.</i> (1980)
J62-1	Lac ⁻ Pro ⁻ His ⁻ Trp ⁻ Nal ^R (λ)	
<i>Salmonella typhimurium</i>		
LT2trpA8-1	Spontaneous Nal ^R mutant of the tryptophan A auxotroph of LT2	Coetzee <i>et al.</i> (1979); J. N. Coetzee (unpublished)
<i>Serratia marcescens</i>		
SM89-1	Spontaneous Nal ^R mutant of SM89	Bradley <i>et al.</i> (1982)
<i>Klebsiella oxytoca</i>		
G147		Coetzee <i>et al.</i> (1983)
<i>Proteus morganii</i>		
2815nal-r		Coetzee (1966); Coetzee <i>et al.</i> (1966)
2815-2	Spontaneous Rif ^R mutant of 2815	J. N. Coetzee (unpublished)
<i>Providencia alcalifaciens</i>		
P29nal-r		Coetzee <i>et al.</i> (1966); Coetzee & Kruger (1980)
P29-2	Spontaneous Rif ^R mutant of P29	J. N. Coetzee (unpublished)
<i>Providencia stuartii</i>		
NCTC 9297-2	Spontaneous Nal ^R mutant of NCTC 9297	Coetzee (1963)
Bacteriophages		
fd	Filamentous phage adsorbing to tips of IncF subgroups I-IV, IncD and F ₀ lac plasmid-coded pili	Marvin & Hoffmann-Berling (1963); Bradley (1977)
F ₀ lac	RNA-containing phage adsorbing only to the sides of the distal ends of shafts of F ₀ lac plasmid-coded pili	Bradley <i>et al.</i> (1981a)
J	Resembles phage T3 but is significantly smaller. DNA-containing and adsorbs to shafts of IncJ, C and D plasmid-coded pili	Bradley (1967); Bradley <i>et al.</i> (1982)
M	RNA-containing phage adsorbing uniformly to the sides of shafts of IncM-coded pili. Sensitive to chloroform	Coetzee <i>et al.</i> (1983)
MS2	RNA-containing phage adsorbing uniformly along the sides of shafts of IncF subgroups I-IV plasmid-coded pili. The phage was also shown to adsorb to sides of IncD-coded pili in formalin-fixed preparations but does not form plaques on strains carrying IncD plasmids	Davis <i>et al.</i> (1961); Bradley & Meynell (1978)
pilH α	RNA-containing phage of adsorbing uniformly to the sides of shafts of IncHI and IncHII-coded pili. Sensitive to chloroform and temperature-sensitive for plaque formation	Coetzee <i>et al.</i> (1985)
t	RNA-containing phage adsorbing only to the sides of the distal ends of shafts of IncT-coded pili	Bradley <i>et al.</i> (1981c)

* Abbreviations: Lac⁻, inability to use lactose as carbon source; λ , lysogenic for phage lambda; Nal^R, resistant to nalidixic acid; Rif^R, resistant to rifampicin.

alcalifaciens P29nal-r harbouring these plasmids. This possibly meant that the corresponding pili were not being properly expressed by these strains (see Results). These results ruled out the advantageous selective plating method devised by Meynell & Lawn (1968). Sewage samples (obtained from various sewage works in the Pretoria-Johannesburg area) were accordingly simply enriched with one of the IncD plasmid-bearing *E. coli* strains (Bradley *et al.*, 1981b) in the presence of 20 μ g nalidixic acid ml⁻¹ to inhibit DNA-containing phages (Watanabe & August, 1967). After overnight incubation the enriched sewage filtrates were spotted on lawns of the same strain used for enrichment, growing on nutrient agar and on this agar containing 25 μ g RNAase ml⁻¹ (BDH). Filtrates

were also spotted on growing lawns of the isogenic strains lacking the particular plasmid and incubated overnight. By noting the sensitivity of prospective plasmid-specific phage to RNAase, this approach excluded the unnecessary re-isolation of broad host range DNA-containing filamentous phages.

Concentration of phage. Final concentrations of polyethylene glycol (mol. wt 6000) of 8% (w/v) (Bradley *et al.*, 1981 c), 10% (Bradley *et al.*, 1981 a) and 11% (Coetzee *et al.*, 1985) with 0.5 M-NaCl were assessed (not shown) for concentrating phage D (see Results) by the method of Yamamoto *et al.* (1970). The optimal concentration proved to be 11% (w/v). Pellets were finally suspended in small volumes of distilled water.

Bacteriophage propagation. The method was that of Coetzee *et al.* (1985) using phage D (see Results).

Preparation of phage antiserum. Eight injections (1.0 ml, 1×10^{10} p.f.u. ml⁻¹) of phage D.J53(R778b) (see Results) were given intravenously at 3 d intervals to two rabbits. After resting the animals for 15 d, blood was collected and the serum was heated at 56 °C for 30 min to inactivate complement. The serum was then absorbed with strain J53(R778b) to remove somatic, flagellar and pili antibodies.

Electron microscopy. A large loopful of J53-2(R711b) from an overnight culture on solid medium was thoroughly mixed with a 0.1 ml sample of phage D in broth (titre 1×10^9 p.f.u. ml⁻¹). Suspensions of bacteria from such cultures yielded many detached pili (Bradley *et al.*, 1981 c). After incubation at 26 °C for 30 min, a carbon-coated electron microscope specimen support grid was touched on the surface. Excess bacteria were removed by dabbing the grid on the surface of a 0.1 M-ammonium acetate solution. Negative staining was with a mixture of equal volumes of 0.1 M-ammonium acetate and 2% (w/v) sodium phosphotungstate solutions (pH 7.4).

RESULTS

Transfer of plasmids by conjugation

Plasmids R687, R711b, R778b and R840 transferred by intraspecific surface mating in *E. coli* at frequencies of 3×10^{-3} per donor h⁻¹. This is a frequency about four times higher than that reported for R711b by Bradley *et al.* (1980). The plasmids transferred from the *E. coli* hosts to the *Salmonella*, *Providencia*, *Proteus*, *Klebsiella* and *Serratia* hosts at frequencies of about 1×10^{-5} per donor h⁻¹. The plasmids transferred intraspecifically between strains of *Proteus morganii* or *Providencia alcalifaciens* (Table 1) at frequencies of about 1×10^{-3} per donor h⁻¹ (not shown). These results may be taken to mean that, while restriction possibly limited intergenus transfer of the plasmids, intraspecies transfer proceeded at levels which could be indicative of constitutive pilus synthesis (Coetzee *et al.*, 1983).

Incompatibility grouping of plasmids

Overnight cultures of strains of *E. coli* or *S. typhimurium* carrying plasmids R687, R711b, R778b or R840 in drug-free broth usually contained >50% clone-forming cells that were drug-sensitive whereas similar cultures of strains of *Providencia alcalifaciens* or *Providencia stuartii* yielded >99% of drug-resistant clones (not shown). This instability of the plasmid in strains of *E. coli* complicated the determination of plasmid incompatibility specificities as strains of the latter organism were the only hosts used for these tests. The presence of kanamycin in the medium ensured that *E. coli* strains carrying plasmids R687, R711b or R840 were purely R⁺. However, 6 h cultures of strains carrying plasmid R778b in the presence of sulphonamide had about 60% of clones sensitive to this bacteriostatic (Gale *et al.*, 1972) drug that had presumably lost plasmid R778b. For this reason R778b was used in donor strains and not in recipient strains as testing for displacement of this unstable plasmid in a resident capacity would be unreliable (see Methods and Table 2).

No clone composed of cells resistant to both sulphonamide and kanamycin was ever observed in the only crosses possible under the above limitations (Table 2 and not shown) and it was concluded that the plasmids were incompatible with one another. The plasmids were however compatible with representatives of all other incompatibility groups (not shown), including IncX plasmids with which some were incorrectly described as being incompatible (Hedges, 1974). On these grounds the four plasmids were allocated to the D incompatibility group, an arrangement which previously had only been tentatively accepted.

Table 2. *Incompatibility of plasmids R687, R711b, R778b and R840*

Incompatibility experiments were done as referred to in Methods. Plasmid R687 determined resistance to ampicillin and kanamycin, R711b to kanamycin, R778b to sulphadimidine and R840 to ampicillin and kanamycin. Colonies were isolated on minimal glucose medium supplemented with proline, histidine and tryptophan, either drug-free to allow growth of the J62 strain or containing 100 µg sulphadimidine (Sul) ml⁻¹ to select for transconjugants carrying R778b. Kan^R, kanamycin resistant; Kan^S, kanamycin sensitive.

Donor strain	Recipient strain	Isolation medium	No. of colonies	
			Kan ^R	Kan ^S
J53-2(R778b)	J62(R711b)	+ Sul	0	20
J53-2(R778b)	J62(R711b)	Drug-free	17	3
J53-2(R778b)	J62(R687)	+ Sul	0	20
J53-2(R778b)	J62(R687)	Drug-free	15	5
J53-2(R778b)	J62(R840)	+ Sul	0	20
J53-2(R778b)	J62(R840)	Drug-free	18	2

Isolation of a J53(R778b)-dependent bacteriophage

From the tenth sample of sewage enriched with strain J53(R778b) a phage was isolated which plated on the above strain but neither on this strain growing in the presence of RNAase nor on a culture of J53. The phage was named phage D and was routinely propagated on J53(R778b).

Properties of phage D

Plaque formation and morphology. Phage D was isolated (see Methods) by using an incubation temperature of 26 °C. Temperature sensitivity tests revealed that plaque formation by phage D proceeded on the IncD plasmid-carrying strains of *E. coli*, *S. typhimurium*, *K. oxytoca* and *Proteus morganii* at temperatures up to only 32 °C (not shown). Phage fd plated on the same *E. coli* strains at temperatures of at least 37 °C (not shown). A temperature of 26 °C was then adopted for all other experiments with phage D. Plaques on lawns of *E. coli* which had been incubated overnight were fairly clear, regular in outline and varied from about 1.5 mm in diameter to pinpoint. On all other hosts on which the phage plated, plaques were more turbid and many of the larger plaques were irregular in outline. This could imply that phage adsorption sites were not as freely available as on the *E. coli* strains (Meynell, 1978).

Phage morphology and adsorption site. Phage D had the semi-transparent appearance and isometric hexagonal outline (Fig. 1c) typical of RNA-containing phages (Coetzee *et al.*, 1985). It has a diameter of about 27 nm. Preliminary experiments indicated that phage D adsorbed to pili in small numbers only. Therefore adsorption was demonstrated using a mixture of virions with an excess of D pili so that there were relatively few unadsorbed phage particles. This method revealed that the virions adsorbed only to the sides of one of the ends of pilus shafts either singly (Fig. 1a) or in pairs (Fig. 1b). Adsorption was to the distal tapered end of the pilus (Fig. 1a) (Bradley *et al.*, 1981c). A distal adsorption site on the pilus was also found for the RNA-containing phages t (Bradley *et al.*, 1981c) and F₀lac (Bradley *et al.*, 1981a).

Sensitivity to organic solvents or RNAase. The plaque-forming titre of a phage D suspension was reduced by about 75% as a result of chloroform treatment, whereas the titre of phage MS2 was unaffected. The titre of phage fd was reduced by more than 99% following similar exposure. Titres of phage D and the latter two phages were not reduced by diethyl ether treatment. Plaque formation by D and MS2 was totally inhibited by RNAase whereas that of phage fd was unaffected.

Host range. Although all the plasmids encoded constitutive synthesis of sex pili in particular hosts (see Introduction and Methods), phage D only lysed strains of *E. coli*, *S. typhimurium*, *K. oxytoca* and *Proteus morganii* carrying IncD plasmids (not shown and Table 3). It did not plate or propagate on the *Providencia* or the *Serratia* strains carrying IncD plasmids. Also the plating activity of phages fd and J, on IncD carrying strains, was limited to *E. coli* hosts (Table 3). Phage

Fig. 1. Phage D virions adsorbed to IncD pili synthesized by strain J53-2(R711b). (a) Single virion adsorbed near tapered pilus tip. (b) Two virions adsorbed near pilus tip. (c) Two loose-lying virions showing hexagonal isometric outline. Bar markers, 100 nm.

D did not plate or propagate on strains of *E. coli* carrying plasmids belonging to Inc groups C, F subgroups I–IV, HI and HII, I₁ and I₂, J, M, N, P, W, X or EDP208. It thus differed in specificity from phages MS2, fd, F₀lac and J. Plating results (Table 3) of the latter two phages confirmed previous results (Bradley *et al.*, 1981*a*, 1982).

Serology. Two rabbit sera raised against phage D.J53(R778b) had homologous *K* values of 280 and 320 min⁻¹ respectively. The sera (used in dilutions of 1:100 and 1:50) had no neutralizing activity (*K* = 0) against phages MS2, t, F₀lac, M or pilH α . Apart from containing RNA these phages have one or other property similar to phage D (Table 1).

DISCUSSION

The compatibility properties of the four plasmids investigated (see Results) reaffirmed the existence of the previously poorly described incompatibility group D (see Introduction). These plasmids originated in *Providencia* isolates from patients in hospitals of four cities in England and Canada (Hedges, 1974) whilst none have been reported in natural isolates of any other bacterial species. In that genus they appear to be inherited stably (see Results) but when transferred to strains of *E. coli* or *S. typhimurium* their instability is so marked as to suggest that their replication (or partition) mechanisms are unable to function efficiently in the alien hosts.

The host range of phage D serves to delineate strains of *E. coli* bearing plasmids R687, R711b, R788b or R840 as a group from those of IncF subgroups I–IV, F₀lac, IncC and J plasmids, carried by the same hosts. All these plasmids code for thick flexible pili (Bradley, 1977, 1980). The host range thus confirms results of incompatibility experiments mentioned above.

It was not entirely unexpected that phage D did not plate or propagate on the *Providencia* or *Serratia* strains carrying IncD plasmids despite the fact that the strains possessed intact transfer systems (see Results). Similar situations have been repeatedly encountered (Bradley *et al.*, 1981*b, c*; Coetzee *et al.*, 1979). Hua *et al.* (1981) investigated a *Caulobacter* system where

Table 3. *Host range of phage D compared to that of phages MS2, fd, F₀lac and J*

Dilutions of phage suspensions (about 1×10^9 p.f.u. ml⁻¹) were dropped on lawns of organisms contained in top-layer agar. Plates were incubated at 26 °C for 18 h. Phage propagation was assessed as referred to in Methods.

Host	Plasmid Inc group	Phage action*					Plasmid reference
		D	MS2	fd	F ₀ lac	J	
J62-1(RA1)	C	—	—	—	—	+	Jacob <i>et al.</i> (1977)
J62-1(PHH1184)	C	—	—	—	—	+	Datta <i>et al.</i> (1980)
J62-1(R711b)	D	+	—	+	—	+	Hedges (1974); Bradley <i>et al.</i> (1982)
J62-1(R840)	D	+	—	+	—	+	
J53(R687)	D	+	—	+	—	+	
J53(R778b)	D	+	—	+	—	+	
LT2trpA8-1(R687)	D	+	—	—	—	—	
LT2trpA8-1(R840)	D	+	—	—	—	—	Hedges (1974); Bradley <i>et al.</i> (1972)
SM89-1(R711b)	D	—(-)	—	—	—	—	
G147(R687)	D	+	—	—	—	—	
G147(R711b)	D	+	—	—	—	—	
G147(R778b)	D	+	—	—	—	—	
2815nal-r(R778b)	D	+	—	—	—	—	
2815-2(R840)	D	+	—	—	—	—	
P29-2(R687)	D	—(-)	—	—	—	—	
P29-2(R711b)	D	—(-)	—	—	—	—	
P29-2(R778b)	D	—(-)	—	—	—	—	
P29nal-r(R840)	D	—(-)	—	—	—	—	
NTTC 9297-1(R687)	D	—(-)	—	—	—	—	
NCTC 9297-1(R711b)	D	—(-)	—	—	—	—	
NCTC 9297-1(R840)	D	—(-)	—	—	—	—	
J53-2(R386)	FI	—	+	+	—	—	Dennison (1972)
J53-2(R1drd19)	FII	—	+	+	—	—	Jacob <i>et al.</i> (1977)
J53(ColB-K98)	FIII	—	+	+	—	—	Frydman & Meynell (1969)
J62-1(R124)	FIV	—	+	+	—	—	Hedges & Datta (1972)
J62-1(EDP208)	F ₀ lac	—	—	+	+	—	Bradley & Meynell (1978)
J62-1(R997)	J	—	—	—	—	+	Matthew <i>et al.</i> (1979); Bradley (1980)

* +, Plaque formation; —, no visible action; (-), no titre increase in two propagation experiments.

† Plaques more turbid and irregular in outline.

the pili coded for by the IncP plasmid RP1 were functionally changed in the latter host. The pili still provided adequate conjugative functions, but now lacked receptor sites for phage PRR1. The selectivity of phage fd for *E. coli* hosts (Table 3) also has a precedent: phage f1 which resembles the former phage (Denhardt, 1975; Hill & Petersen, 1982) only plates on *E. coli* strains carrying IncF plasmids and not on strains of *S. typhimurium* with the same plasmids (Loeb, 1960). Nevertheless, the fact that the Providencia strains carrying the plasmids were not lysed by phage D is surprising considering the derivation of these plasmids (see above).

No serological relationship could be demonstrated between phages D and MS2 (see Results) despite the normally fleeting association detected by Bradley & Meynell (1978) between the latter phage and IncD plasmid-coded pili. A possible explanation could be that whereas formalin-fixed preparations show phage MS2 adsorbed uniformly along the length of the shafts of R711b pili, phage D only adsorbed to the very distal sides of the shafts. These sites could differ antigenically from the rest of the pilus shaft.

Apart from the particular adsorption site on pili (see Results), phage D differs from most other RNA-containing phages (Fiers, 1979) in two further respects: chloroform sensitivity of the virions and the temperature sensitivity of plaque formation (see Results). The former property is shared with phages M and pilH α (Coetzee *et al.*, 1983, 1985). The latter feature was considered a unique property of phage pilH α until the present phage was isolated. The restricted development temperature of these wild phages has not been investigated further but certainly justified the lower temperature used for phage isolation.

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