Bacteriophages *F₀lac h*, SR, SF: Phages Which Adsorb to Pili Encoded by Plasmids of the S-Complex

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Phage *F₀lac* is an RNA-containing phage which plates only on strains carrying the plasmid EDP208, a pilus derepressed derivative of the unique incompatibility plasmid *F₀lac*. A host range mutant, phage *F₀lac h*, was selected which plated on strains carrying the ungrouped plasmid pPLS::Tn5 and lysed strains carrying another ungrouped plasmid TP224::Tn10 or the Com9 plasmid R71. An RNA-containing phage, SR, was isolated from sewage on bacteria harbouring plasmid pPLS::Tn5. It was antigenically distinct from the above two phages but had the same host range as phage *F₀lac h*. Phages *F₀lac h* and SR adsorbed unevenly to the shafts of the conjugative pili. Another phage, SF, was filamentous and plated or propagated on strains carrying any of the above plasmids as well as on strains harbouring IncD or F-complex plasmids. Plasmids TP224::Tn10 and pPLS::Tn5 were compatible with representative plasmids of all Inc groups also encoding thick flexible pili. The four plasmids EDP208, R71, TP224::Tn10 and pPLS::Tn5 were compatible with one another except for the reaction of TP224::Tn10 in the presence of pPLS::Tn5 which was slightly ambiguous. The host ranges of the bacteriophages, together with the serological relatedness of the thick flexible pili determined by these four compatible plasmids, suggested that they constitute a new complex, here designated S.

INTRODUCTION

Plasmid *F₀lac* (Falkow & Baron, 1962; Table 1) was formerly designated as the sole member of group FV of the F-complex of plasmids (Datta, 1975) on grounds of morphological similarity of the thick flexible pili (Bradley, 1980) encoded by the plasmid to those of other F-complex plasmids (Lawn *et al.*, 1967), the alleged serological cross reactions with other F-like pili and the fact that the tips of *F₀lac* pili adsorbed filamentous phages like M13 (Lawn *et al.*, 1967) and fd (Datta, 1979). Plasmid *F₀lac* pili do not adsorb RNA-containing phages like f2, MS2 or Qβ which adsorb specifically to shafts of F-like pili (Lawn *et al.*, 1967). Bradley (1978) found that *F₀lac* pili were serologically unrelated to those of F-like plasmids and plasmid *F₀lac* has been removed from the F-complex and simply classified as unique (Datta, 1979).

Using EDP208, a variant of plasmid *F₀lac* which produces pili constitutively, two closely related RNA-containing phages designated UA6 and *F₀lac* were isolated. These adsorbed specifically to the distal sides of the tips of *F₀lac* pili (Armstrong *et al.*, 1980; Bradley *et al.*, 1981a).

Three other plasmids encoding thick flexible pili serologically related to those of plasmid EDP208 have been described. The R factor R71 (Chabbert *et al.*, 1972) was described as the sole representative of compatibility group 9, and the enterotoxin plasmid TP224 and its transposon-bearing derivative (Table 1; Scotland *et al.*, 1979) were compatible with a set of reference plasmids (see Bradley, 1981). Plasmid TP224::Tn10 was also compatible with plasmids EDP208 and R71 (Bradley *et al.*, 1981*a*) and like plasmid F₀lac (see above) it was suggested that TP224 be given unique incompatibility status (Bradley, 1981). Like F₀lac these two plasmids produce very low levels of piliation on host bacteria; the pili are serologically related (Bradley, 1980) and tests for phage F₀lac propagation on such strains yielded inconclusive results (Bradley *et al.*, 1981*a*).

The third plasmid, pPLS (Table 1; Bradley, 1985), is present in *Escherichia coli* strain CG140 (Shipley *et al.*, 1978). It codes for the constitutive production of large numbers of thick flexible pili which are morphologically and serologically related to those determined by plasmid EDP208 (Bradley, 1985). *E. coli* K12 strains harbouring pPLS are not lysed by phage F₀lac and plasmid pPLS has not been classified by incompatibility reactions (Bradley, 1985).

Here we describe the isolation of a mutant of phage F₀lac which formed plaques on bacteria harbouring plasmid pPLS::Tn5, and the identification of phages from sewage which plated specifically on strains carrying the latter plasmid. The host range of these phages has been investigated. The compatibility of the ungrouped plasmids TP224::Tn10 and pPLS::Tn5 was also tested with plasmids of other incompatibility groups encoding thick flexible pili, and the compatibility of plasmids EDP208, R71, TP224::Tn10 and pPLS::Tn5 was assessed. This limited range of testing is justified by the fact that no plasmids encoding thick flexible pili have been found to be incompatible with plasmids encoding thin flexible pili or rigid pili (Bradley, 1980).

METHODS

Bacteria. *Escherichia coli* K12 strains used were J53 (Lac⁺F⁻Pro⁻Met⁻) and a rifampicin resistant (Rif^R) mutant J53-2 (Coetzee *et al.*, 1985*a*); J62 (Lac⁻F⁻Pro⁻His⁻Trp⁻); a nalidixic acid resistant (Nal^R) mutant J62-1 and the Rif^R mutant J62-2 (Datta *et al.*, 1980); JE2571 (F⁻Leu⁻Thr⁻Str^RFla⁻Pil⁻) and the corresponding Nal^R and Rif^R mutants JE2571-1 and -2 respectively (Bradley, 1985). The *Klebsiella pneumoniae* strain used was G162 (Coetzee *et al.*, 1983). The *Salmonella typhimurium* strain used was M827-2, a spontaneous Rif^R mutant of LT2 strain M827 (Bradley *et al.*, 1982*b*). Sr41-2 is a spontaneous Rif^R mutant of *Serratia marcescens* strain 41 (Coetzee *et al.*, 1983).

Plasmids and bacteriophages. Plasmids were from the collections of Naomi Datta (Datta, 1979) and D. E. Bradley. Those used for compatibility testing and/or phage host range experiments are listed in Table 1. Other plasmids belonging to Inc groups which encoded constitutive pilus synthesis (as judged by plaque-formation by appropriate plasmid-specific phages), namely I₁ and I₂ (Coetzee *et al.*, 1982), M (Coetzee *et al.*, 1983) and N, P and W (Coetzee *et al.*, 1979), were also used in phage host range experiments. In addition plasmids R1, RA1, R15, R40a, R721, RP4 and Sa of known molecular mass (Jacob *et al.*, 1977) were used to calibrate gel electrophoresis columns. Phages used in host range experiments were phages F₀lac (Bradley *et al.*, 1981*a*), fd (Marvin & Hoffmann-Berling, 1963), D (Coetzee *et al.*, 1985*b*) and MS2 (Davis *et al.*, 1961). The latter two phages are serologically distinct as judged by neutralization tests (Coetzee *et al.*, 1985*b*).

Media, antibacterial agents, general phage techniques, transfer of plasmids by plate mating, organic solvents and ribonuclease sensitivity of phage, temperature sensitivity of plaque formation and production of phage lysates by a soft agar layer technique. These were as described by Adams (1950) and Coetzee *et al.* (1979, 1985*a*). Since two phages recently isolated from sewage (Coetzee *et al.*, 1985*a*, *b*) were temperature-sensitive for plaque formation, isolation of phage was done in duplicate at 26 and 37 °C (Coetzee *et al.*, 1985*a*). Plates spotted with phage D (Coetzee *et al.*, 1985*b*) were incubated at 26 °C and the same temperature was used for the conjugative transfer of IncHI plasmids (Coetzee *et al.*, 1985*a*) and work with IncT plasmids (Bradley *et al.*, 1981*b*). For all other experiments the temperature was 37 °C unless otherwise stated. All plates were incubated overnight.

Phage isolation. The method of Coetzee *et al.* (1985*a*) was used. The partner strains were JE2571 (pPLS::Tn5) and M827-2 (pPLS::Tn5). Sewage specimens were obtained from various sewage works in the Pretoria and Johannesburg areas. The isogenic strains lacking the plasmid served as controls.

Concentration of bacteriophages. Phage lysates of the RNA-containing phages F₀lac, F₀lac *h* and SR (see Results) were concentrated by the method previously used (Coetzee *et al.*, 1983) with the reagent concentrations used for phage F₀lac (Bradley *et al.*, 1981*a*).

Phage growth on bacterial strains harbouring plasmids repressed for pilus synthesis. Two types of experiments were

used. Both were based on observations that plasmids may be temporarily derepressed for pilus synthesis immediately after transfer to a new bacterial host (Meynell *et al.*, 1968). The first entailed the addition of phage to newly formed plasmid transconjugants and demonstrating an increase in phage titre by the method of Coetzee *et al.* (1985a). A phage titre increase of at least 10-fold that of the negative control, in two separate experiments, was taken as a positive result. With the second method, drops of phage were spotted on nutrient agar plates carrying sheets of newly-formed transconjugants; phage development was noted by the appearance of areas of clearing after incubation (Bradley & Fleming, 1983).

Phage antiserum. Rabbit antisera against individual phages *F₀lac h*, SR and SF all grown on J53(pPLS::Tn5) (see Results) were prepared as described by Coetzee *et al.* (1985b) using strain J53(pPLS::Tn5) for adsorption of corresponding antibodies. Phage *F₀lac* antiserum (Bradley *et al.*, 1981a) with a homologous *K* value of 345 min⁻¹ was also used.

Plasmid compatibility tests. The construction of the *E. coli* K12 strains carrying markers of both plasmids under test ('doubles'), the determination of surface exclusion, elimination of markers of the resident plasmid by the incoming plasmid and stability of markers of two plasmids under non-selective conditions in the same cell during a growth period of at least 25 generations were done by the method of Datta *et al.* (1980). The only difference was that conjugal transfer of plasmids was done by plate mating for 1 h (see above) instead of using broth. The procedure for demonstrating the separate existence of the plasmids in a host by conjugal transfer with selection for markers of only one of the pair at a time (Coetzee *et al.*, 1972) was replaced, in the case of the intercompatibility testing of plasmids EDP208, R71, TP224::Tn10 and pPLS::Tn5, by the following method. This entailed demonstrating the presence on agar gels of the two separate plasmids in samples taken from a plate selective for markers of both plasmids. Plasmid DNA was extracted from the latter cultures followed by agarose gel electrophoresis (Datta *et al.*, 1980; Bradley, 1985) to indicate the presence of DNA bands of the two plasmids occupying their separate characteristic positions. The approximate molecular mass of the plasmids was calculated according to Meyers *et al.* (1976) from simultaneous calibrations with plasmids of known mass (see above).

Electron microscopy. This was done as described by Bradley *et al.* (1981a).

RESULTS

Selection of mutant phage F₀lac h

Concentrated suspensions of phage *F₀lac* (1×10^{11} p.f.u. ml⁻¹), when dropped on lawns of J53(pPLS::Tn5) and incubated, formed 0–3 plaques per drop on the latter strain. After repeated single-plaque isolations from platings of phage from one of the above plaques, the phage responsible, with the extended host range (see below), was named phage *F₀lac h*.

Isolation of phages SR and SF from sewage

With the eighth sewage specimen examined, two phages were isolated from different plaques appearing on the same plates of JE2571(pPLS::Tn5) incubated at the two temperatures used. The phages from these plaques did not plate on control strains JE2571 or M827-2. The one which was sensitive to RNAase was named phage SR; the other was named phage SF (see below). The phages were routinely cultivated on strain JE2571(pPLS::Tn5).

Properties of phages F₀lac h, SR and SF

Plaque formation and morphology. The three phages plated with equal efficiency on strain J62(EDP208) at an incubation temperature of 26 or 37 °C but phage *F₀lac h* was unique in that its plaques were much smaller (pin-point) at the higher temperature. This temperature-dependence of plaque-size was also noted on strains of other genera on which phage *F₀lac h* plated and was linked with adsorption efficiency (see below). Plaques of this phage on the above-mentioned strains at 26 °C were relatively clear and uniform at about 2 mm in diameter. Phage SR also formed relatively clear uniform plaques of about 2 mm in diameter, while phage SF formed turbid plaques which varied from pin-point to larger than those of the other two phages, and were usually irregular in outline.

Phage morphology and adsorption sites. Phages *F₀lac h* and SR had the semi-transparent appearance and hexagonal outline (Figs 1a, b, 2a) typical of RNA-containing phages (Coetzee *et al.*, 1983). Both had a diameter of about 28 nm.

At 37 °C phage *F₀lac h* adsorbed unevenly in large numbers along the length of shafts of detached pPLS pili (Fig. 1a). The average number of virions attached per pilus was 23 in a

Table 1. *Plasmids used for compatibility testing and/or phage host range experiments*

Plasmid	Grouping	Relevant properties*	References
<i>F₀lac</i>	Unique	A transmissible plasmid <i>F₀</i> carrying <i>lac</i> genes. The name of the plasmid is meant to imply that it does not confer chromosomal donor ability or affect the pre-existing mating polarity of the carrier cell. Encodes only thick flexible pili. Pilus synthesis repressed. Phages <i>F₀lac</i> and fd lyse bacteria harbouring this plasmid. Lac ⁺	Falkow & Baron (1962) Datta (1979) Bradley <i>et al.</i> (1981a)
EDP208	Unique	Constitutive pilus producing derivative (= <i>F₀lac drd</i>) of <i>F₀lac</i> constructed by N. Willetts. Produces >10 < 100 pili per cell in <i>E. coli</i> K12 strains. Phage <i>F₀lac</i> adsorbs to distal ends of shafts of the thick flexible pili. Phage fd adsorbs to pilus tips. Lac ⁺	Armstrong <i>et al.</i> (1980) Bradley (1980)
R71	Com9	Pilus synthesis repressed. Thick flexible pili. Pili serologically related to those of <i>F₀lac</i> . Determinants A, C, S, T, Su	Chabbert <i>et al.</i> (1972)
R71-1	Com9	A deletion mutant of R71 lacking determinant T. Determinants A, C, S, Su	Scavizzi (1973)
TP224::Tn10	Unique	Conjugative plasmid determining heat stable enterotoxin labelled with resistance determinant T by integration of Tn10. Derived from <i>E. coli</i> strain E7476. Pilus synthesis repressed. Pili serologically related to EDP208 pili. Determinant T	Scotland <i>et al.</i> (1979) Bradley (1980)
pPLS::Tn5	ND	Constitutive production of very large numbers (about 100) of thick flexible pili per cell in <i>E. coli</i> K12 strains. Pili serologically related to plasmid <i>F₀lac</i> -coded pili. Mobilizes the K88 colonization antigen. Labelled with determinant K by integration of Tn5.	Shipley <i>et al.</i> (1978) Bradley (1985)
R57b-1	IncC	Deletion mutant of R57b. Forms thick flexible pili constitutively. Determinants C, Su	Datta & Hedges (1972)
R711b	IncD	Constitutive pilus synthesis. Thick flexible pili serologically related to all other IncD plasmids but not to IncFI or <i>F₀lac</i> pili. Pilus tips adsorb phage fd. Distal ends of pilus shafts adsorb phage D. Determinant K	Coetzee <i>et al.</i> (1985b)
R778b	IncD	Constitutive thick flexible pilus synthesis. Pilus tips adsorb phage fd. Distal ends of pilus shafts adsorb phage D. Determinant Su	Coetzee <i>et al.</i> (1985b)
R687	IncD	Constitutive thick flexible pilus synthesis. Pilus tips adsorb phage fd. Distal ends of pilus shafts adsorb phage D. Determinants A, K	Coetzee <i>et al.</i> (1985b)
<i>Flac</i>	IncFI	Constitutive synthesis of thick flexible pili. Lac ⁺	Jacob & Adelberg (1959)
R386	IncFI	Constitutive pilus synthesis. Thick flexible pili serologically related to other IncF pili. Pili adsorb phages MS2 (shafts) and fd (tips). Determinant T	Dennison (1972)
R1-1	IncFII	Spontaneous segregant of R1 lacking determinant K. Encodes thick flexible pili. Determinants A, C, S, Su	Hedges & Datta (1972)
R1drd16	IncFII	Mutant of R1. Constitutive thick flexible pilus synthesis; lacks all resistance determinants except K. Pili adsorb phages MS2 (shafts) and fd (tips). Determinant K	Frydman & Meynell (1969)
R124	IncFIV	Constitutive thick flexible pilus synthesis. Pili adsorb phages MS2 (shafts) and fd (tips). Determinant T	Hedges & Datta (1972)
R27	IncHI1	Repressed synthesis of thick flexible pili. Determinant T	Coetzee <i>et al.</i> (1985a)
pHH1508a	IncHII	Constitutive synthesis of thick flexible pili. Determinants S, Te, Tp	Bradley <i>et al.</i> (1982a) Coetzee <i>et al.</i> (1985a)

Table 1 (continued)

Plasmid	Grouping	Relevant properties*	References
R997	IncJ	Constitutive synthesis of thick flexible pili. Determinants A, S, Su	Bradley <i>et al.</i> (1982 <i>b</i>)
R402	IncT	Constitutive synthesis of thick flexible pili. Determinants A, S	Coetzee <i>et al.</i> (1972)
R753	IncV	Repressed synthesis of thick flexible pili. Determinants A, C, S, Su	Jacob <i>et al.</i> (1977)
R6K	IncX	Constitutive synthesis of thick flexible pili. Determinants A, S	Bradley (1980)

ND, Not determined.

* Resistance determinants: A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamide; T, tetracycline; Te, potassium tellurite; Tp, trimethoprim.

Fig. 1. Electron micrographs of negatively stained phage preparations. (a) Phage *F₀lac h* adsorbed to pPLS pili at 37 °C; (b) the same at 26 °C. (c) Phage *F₀lac* adsorbed to aggregated pPLS pili at 37 °C. Bar, 100 nm.

sample of 22 detached pili of about equal length. Similar adsorption also occurred to pili of plasmid EDP208 (not shown). At 26 °C, adsorption was much less efficient with an average of 1.6 virions adsorbed per pilus in a sample of 18 detached pili of roughly the same length as those mentioned above (Fig. 1*b*). This difference in adsorption of phage *F₀lac h* was linked with a

Fig. 2. Electron micrographs of negatively stained phage preparations. (a) Phage SR adsorbed to pPLS pili at 37 °C. (b), (c) Phage SF adsorbed to pPLS pilus tips; the pili are thicker than the virions and points of attachment are arrowed. Bar, 100 nm.

difference in plaque morphology at these temperatures (see above). Since sensitivity to the *F₀lac h* parent phage, phage *F₀lac*, was not specified by plasmid pPLS (Table 2), attempts were made to determine whether this was due to lack of adsorption to pPLS pili. When incubated at 37 °C with pPLS pili under similar conditions to those used for phage *F₀lac h*, phage *F₀lac* did not adsorb to single detached pPLS pili (not shown) but did appear to adsorb to these pili when the latter were close together or tangled (Fig. 1c). No adsorption to pPLS pili could be demonstrated, under similar conditions, with the serologically unrelated phage MS2 (see below).

Phage SR adsorption to pPLS pili at 37 °C for 1 h was similar to that of phage *F₀lac h* (Fig. 2a). Adsorption at 26 °C was only slightly less efficient (not illustrated) with no marked difference as with phage *F₀lac h*.

Phage SF was filamentous with a length of about 925 nm (Fig. 2b). A number of virions twice this length were also present. The tips of the phage were slightly tapered and had a similar morphology (see Bradley *et al.*, 1982b). It adsorbed inefficiently to the tips of pPLS pili and phage-pilus complexes such as shown (Fig. 2b, c) were difficult to find. This poor adsorption could be a cause of the characteristic heterogeneity of the plaque morphology (see above; Meynell, 1978).

Sensitivity to organic solvents or RNAase. The plaque-forming titres of phage *F₀lac h* and SR were reduced by a factor of about 10 after exposure to chloroform for 1 h but were unaffected by

Table 2. Host range of phages *F₀lac*, *F₀lac h*, SR, D, MS2, SF and fd

Dilutions of phage suspensions (about 5×10^8 p.f.u. ml⁻¹) were dropped on lawns of organisms contained in top-layer agar. Plates were incubated overnight at 37 °C with the exception of plates of phage D where the incubation temperature was 26 °C. Phage propagation and temporary derepression spot tests were done as described in Methods. Phage action: +, plaque formation; -, no visible action; (+), positive phage propagation and temporary derepression spot tests obtained in at least two experiments; (-), negative phage propagation and temporary derepression spot tests obtained in three experiments.

Host	Plasmid group*	Phage action						
		<i>F₀lac</i>	<i>F₀lac h</i>	SR	D	MS2	SF	fd
J62(<i>F₀lac</i>)	SI	- (+)	- (+)	- (+)	- (-)	- (-)	- (+)	- (+)
M827-2(<i>F₀lac</i>)	SI	- (+)	- (+)	- (+)	- (-)	- (-)	- (+)	- (-)
J62-1(EDP208)	SI	+	+	+	-	-	+	+
M827-2(EDP208)	SI	+	+	+	-	-	+	-
JE2571-2(R71)	SII	- (-)	- (+)	- (+)	- (-)	- (-)	- (+)	- (-)
JE2571(R71-1)	SII	- (-)	- (+)	- (+)	- (-)	- (-)	- (+)	- (-)
JE2571-2(TP224::Tn10)	SIII	- (-)	- (+)	- (+)	- (-)	- (-)	- (+)	- (-)
JE2571(pPLS::Tn5)	SIV	-	+	+	-	-	+	-
M827-2(pPLS::Tn5)	SIV	-	+	+	-	-	+	-
G162(pPLS::Tn5)	SIV	- (-)	+	+	-	-	+	-
Sr41-2(pPLS::Tn5)	SIV	- (-)	+	+	-	-	+	-
J53(R687)	D	-	-	-	+	-	+	+
J53(R711b)	D	-	-	-	+	-	+	+
J62-2(R386)	FI	-	-	-	-	+	+	+
J53-2(R1drd19)	FII	-	-	-	-	+	+	+
J53-2(R124)	FIV	-	-	-	-	+	+	+

* For plasmid reference see Table 1; for plasmid group see Discussion.

diethyl ether. The phage *F₀lac* control was perfectly stable under these conditions. Phage SF underwent >99.9% inactivation as a result of exposure to chloroform but was stable in the presence of diethyl ether.

Plaque formation by phages *F₀lac h* and SR was totally inhibited by the presence of RNAase in plating media whereas that of phage SF was unaffected.

Serology. Rabbit sera raised against phages *F₀lac h*, SR and SF (see Methods) had homologous *K* values of 295 min⁻¹, 310 min⁻¹ and 825 min⁻¹ respectively.

Phage *F₀lac* antiserum (see Methods) neutralized phage *F₀lac h* at a frequency of 320 min⁻¹ but had no neutralizing activity (*K* = 0) against phage SR. Phage *F₀lac h* antiserum neutralized phage *F₀lac* at a frequency of 280 min⁻¹ but also had no inhibitory effect on plaque formation by phage SR. The antiserum prepared against the latter phage had no neutralizing activity against phages *F₀lac* or *F₀lac h*. None of the above sera had any activity against phages MS2 or D respectively nor against the filamentous phage SF. The phage SF antiserum had no neutralizing activity (*K* = 0) on phage fd. It was thus concluded that phage *F₀lac* and its mutant *F₀lac h* are related serologically but are distinct from phages SR, MS2 and D, each of which is serologically unique (Coetzee *et al.*, 1985*b*). Also, phage SF is antigenically distinct from all above-mentioned phages.

Host range. Phages *F₀lac h* and SR had similar host ranges forming plaques only upon strains carrying plasmids EDP208 or pPLS::Tn5, and showing an increase in titre also with strains carrying the repressed plasmids *F₀lac*, R71 or TP224::Tn10 using the propagation and temporary derepression techniques (Table 2). The phages plated with similar efficiencies on the *E. coli*, *Salmonella*, *Klebsiella* and *Serratia* plasmid-harbouring strains. Use of the propagation and temporary derepression techniques also finally established (see Bradley *et al.*, 1981*a*) that the host range of phage *F₀lac* was limited to strains carrying plasmids *F₀lac* or EDP208 and not plasmids R71 or TP224::Tn10. Phages MS2 and D lysed only strains harbouring plasmids belonging to the F-complex and group D respectively. Phage SF formed plaques on strains of *E. coli* carrying any of the plasmids which confer sensitivity to phage fd (F-complex, EDP208,

IncD plasmids) but whereas fd was confined to *E. coli* hosts, phage SF formed plaques on plasmid-carrying *Salmonella*, *Klebsiella* and *Serratia* strains. Another difference in host range is that phage SF, but not phage fd, can form plaques upon strains carrying pPLS and can propagate on strains harbouring TP224 or R71 (Table 2). The phages did not plate on bacteria carrying plasmids coding for constitutive pilus production belonging to any of the other Inc groups tested (see Methods; not shown).

Compatibility of plasmids. Plasmids TP224::Tn10 and pPLS::Tn5 were separately tested for compatibility with one representative plasmid (Table 1) of Inc groups C, D, F-complex, H-complex, J, T, V and X which also encode thick flexible pili (Bradley, 1980). Due to marker overlap, TP224::Tn10 could not be tested with the FIV plasmid R124 and the HII plasmid R27 (Table 1). In all other instances *E. coli* K12 strains were constructed which bore markers of both plasmids under test. No surface exclusion was encountered and dislodgement of resident plasmids was not detected. In all instances apart from two, the different pairs of plasmids co-existed stably during growth in drug-free broth and their separate identity was shown by their individual transmissibility to other K12 strains (not shown). The two exceptions were in pairs having the IncD plasmid R778b or IncHII plasmid pHH1508a as one of the partners. In the former instance the marker of plasmid R778b was lost in about 50% of clone-forming cells after overnight growth in drug-free broth whereas in the latter case the corresponding figure was about 6%. However in no instance was the loss greater than that which occurred in control cultures lacking plasmids TP224::Tn10 or pPLS::Tn5. Instability of plasmids of Inc groups D and HII has been recorded (Bradley *et al.*, 1982a; Coetzee *et al.*, 1985b) and it is concluded that, with the limitations mentioned above, plasmids TP224::Tn10 and pPLS::Tn5 are compatible with plasmids of all Inc groups encoding thick flexible pili.

The approximate molecular masses of the plasmids EDP208, R71, TP224::Tn10 and pPLS::Tn5, as determined by gel electrophoresis, were 57 MDa, 51 MDa, 83 MDa and 43 MDa respectively.

Strains of JE2571 were constructed which carried pairwise markers of the six possible combinations of the latter four plasmids (not shown). Because of overlap of markers of R71 and TP224::Tn10 the mutant R71-1 (Table 1) was used for construction of this pair of plasmids. In all cases except pPLS::Tn5 with TP224::Tn10, the 'doubles' were perfectly stable and the four clones of each studied, bearing markers of both plasmids, produced gel patterns on electrophoresis corresponding to the DNA of the separate plasmids (not shown). None of the plasmids exhibited surface exclusion towards any of the others (not shown). When pPLS::Tn5 was introduced into JE2571-1(TP224::Tn10) or TP224::Tn10 into JE2571-2(pPLS::Tn5) the resident plasmid was not eliminated and again no surface exclusion was detected. However, there was some instability of the tetracycline resistance marker: it was lost from three of these clones in 3 out of 50, 16 out of 50 and 11 out of 50 colonies respectively. When some of these deficient clones were tested by electrophoresis (see Methods) only pPLS::Tn5 registered on the gels indicating that loss of tetracycline resistance corresponded to loss of the entire TP224::Tn10 plasmid. When plasmid TP224::Tn10 was tested alone for stability under similar conditions it was perfectly stable. Plasmid DNA from three of the stable 'doubles' of this combination yielded bands corresponding to each separate plasmid on electrophoresis. It is concluded that all the plasmids are compatible with one another except the slightly ambiguous behaviour of TP224::Tn10 in the presence of pPLS::Tn5. Both these plasmids carry transposons at unspecified locations and some interference with the expression of genes involved in compatibility cannot be ruled out.

DISCUSSION

Amongst properties studied, the group of plasmids EDP208 (= *F₀lac drd*; unique grouping), R71 (Com9 grouping), TP224::Tn10 and pPLS::Tn5 resemble one another only in that they encode conjugative pili which are morphologically and serologically similar (see Introduction) and act as receptors for phages *F₀lac h*, SR and SF (Table 2). They differ in phenotype but all the plasmids are mutually compatible apart from the slightly ambiguous behaviour which

TP224::Tn10 and pPLS::Tn5 display towards one another. Also plasmids TP224::Tn10 and pPLS::Tn5 which have not been grouped (see Introduction) are compatible with plasmids of all Inc groups encoding thick flexible pili (see Results).

Where plasmids with distinct compatibility specificities determine serologically related pili, it has been the practice to describe them as constituting a complex encompassing several incompatibility groups, e.g. the F-complex (Hedges & Datta, 1972), the H-complex (Bradley *et al.*, 1982*a*) and the I-complex (Bradley, 1984). For these reasons it is proposed to re-group plasmids EDP208 and R71 with the addition of plasmids TP224::Tn10 and pPLS::Tn5 to constitute an S-complex of plasmids (for Sheena Dennison): SI with plasmid *F₀lac* as the prototype, SII represented by R71, SIII by TP224::Tn10 and SIV which accommodates pPLS::Tn5 as the only known member. Only R71 is an R factor (Table 1) but members of the S-complex must clearly be abundant to support the phage populations described here.

Some plasmids from *Serratia marcescens* were described as constituting a group IncS but these have been shown to belong in group IncHI2 (Taylor & Grant, 1977) so there is little room for confusion.

Although polyclonal antisera may not distinguish between pili of plasmids of the S-complex (see Bradley, 1985), phage *F₀lac* clearly discriminates between isolated pili of plasmids EDP208 (SI) and pPLS (SIV) (see Results; Table 2). This distinction occurs despite the fact that specific adsorption does apparently occur to aggregated pili of the latter plasmid (Fig. 1*c*). If phage *F₀lac* resembles other RNA-containing phages like MS2 (Davis *et al.*, 1961) or Q β (Watanabe *et al.*, 1967) each virion should contain a single molecule of protein A responsible for adsorption to the appropriate pilus (Fiers, 1979). A single particle of phage *F₀lac* should adsorb to only one pilus and the above observation cannot be explained without further knowledge, particularly regarding the surface electrical charges involved. However, this adsorption may constitute an inefficient form of attachment which could represent the infective route of a virus whose progeny might have the extended host range of the mutant phage *F₀lac h* described here.

The discovery of host range mutants of RNA-containing phages is a rare event. By 1965 (Zinder, 1965) none had been reported. Dennison & Hedges (1972) discovered such mutants of phage SP which had an extended host range on bacteria carrying plasmids of the F-complex. A study of the physical basis of the change in host range was not attempted.

The fact that phages *F₀lac h* and SR adsorb along the length of EDP208 pilus shafts (see Results), whereas phage *F₀lac* attaches mainly to the sides of the very tips of these shafts (Bradley *et al.*, 1981*a*), may be an indication of a difference existing between the distal and more proximal areas of the shafts of the EDP208 pili. This raises the possibility that other phages exist which may adsorb to the more proximal areas of shafts of pili for which only distal-adsorbing RNA-containing phages have been discovered to date (Bradley *et al.*, 1981*b*; Coetzee *et al.*, 1985*b*).

There is a precedent for the finding that serologically distinct phages like *F₀lac h* and SR (see Results) adsorb to shafts of apparently homogenous pili like those encoded by plasmids EDP208 or pPLS::Tn5 (Table 1). Phages MS2 and Q β , which are serologically virtually unrelated (Watanabe *et al.*, 1967), also adsorb to shafts of pili encoded by some plasmids of the F-complex resulting in equal efficiency of plating of the latter two phages on a strain carrying one of these plasmids (Dennison & Hedges, 1972). Silverman *et al.* (1968) have postulated that the F-pilus contains at least one chemical group necessary for the common adsorption of the serologically unrelated phages f2 and Q β .

The diminished plaque size produced by phage *F₀lac h* on the plasmid-bearing strains at 37 °C compared with that at 26 °C (see Results) could possibly be explained by the much greater adsorption at 37 °C (Fig. 1*a, b*). Compared to derepressed F-complex plasmids (Lawn *et al.*, 1967), some plasmids of the S-complex produce large numbers of pili (Table 1; Bradley, 1985). If large numbers of virions are adsorbed by numerous pili, free phage available for extending the infection may rapidly become exhausted as a plaque forms from a single infective unit. If adsorption is reduced as at 26 °C, there may be more virions available for a longer period to infect the growing bacteria, resulting in larger plaques. However RNA-containing phages usually have burst-sizes of thousands of particles and even if many are non-viable (Cramer &

Sinsheimer, 1971) it is difficult to envisage excessive adsorption to pili as the sole reason for lack of infective phage at 37 °C. The situation differs from that of two wild-type temperature-sensitive RNA-containing phages, pilH α and D (Coetzee *et al.*, 1985*a, b*), neither of which formed plaques at 37 °C.

Phage SF, the filamentous phage which adsorbs to tips of pPLS::Tn5-coded pili (Fig. 2*b, c*) has a broader host range than the RNA-containing phages mentioned above (Table 2). This is the usual finding for tip-adsorbing phages (see Coetzee *et al.*, 1979).

RNA-containing phages are regarded as chloroform-resistant (Coetzee *et al.*, 1982). Recently RNA-containing phages have been described (Coetzee *et al.*, 1983, 1985*a, b*) suspensions of which are partially inactivated by chloroform. Phages F₀lac h and SR are also sensitive to a degree (see Results) and it is interesting that phage F₀lac, the parent of the former phage, is resistant to chloroform (Bradley *et al.*, 1981*a*). The sensitivity of phage SF to chloroform (see Results) is characteristic of other filamentous phages (Bradley *et al.*, 1982*b*).

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