

Host specificities of RNA phages

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SUMMARY

Host ranges of members of four groups of male-specific RNA coliphages were determined by plating on hosts carrying various derepressed plasmids. An RNA phage originally isolated on *Pseudomonas aeruginosa* failed to form plaques on any of the strains of *Escherichia coli*.

INTRODUCTION

The RNA phages of *Escherichia coli* adsorb to pili whose protein (pilin) is determined by a gene of the F (fertility) factor (Brinton, Gemski & Carnahan, 1964). The F factor and the col V factor (Kahn & Helinski, 1964; MacFarren & Clowes, 1967; Lawn & Meynell, 1970) are naturally occurring plasmids which synthesize pili constitutively. Many other plasmids, e.g. many of the antibiotic resistance (R) factors, carry genes specifying the formation of pili similar to those determined by the F factor (F-like pili) but the pilus genes are repressed and only a small proportion of host cells produce pili. Mutants constitutive for pilus production have been obtained from several of these plasmids (Meynell & Datta, 1967; Meynell, Meynell & Datta, 1968).

Although the pili produced by bacteria carrying derepressed F-like R factors are closely similar to those of bacteria carrying the F factor they are not identical. Lawn & Meynell (1970) showed that four classes of F-like pili could be distinguished serologically. For the purpose of this paper we have called the classes A, B, C and D. F and col V-K94 produced indistinguishable pili (class A). Pili determined by R 1 were unique (class B) as were those produced by R 538.1 (class C). Pili produced by cells carrying R 100, R 136 or R 192 constituted a fourth group (Class D).

Male-specific RNA phages fall into four groups distinguishable by a variety of physicochemical techniques (Watanabe, Miyake *et al.* 1967; Watanabe, Nishihara *et al.* 1967; Sakurai, Miyake, Shiba & Watanabe, 1968; Miyake, Shiba, Sakurai & Watanabe, 1969). The differences between these groups, in particular the antigenic differences, which imply differences in the conformation of the coat proteins, suggest that the adsorption specificities of the phages might not be identical. Silverman, Mobach & Valentine (1967) had previously shown that a small minority of the MS2^R mutants of an Hfr strain of *E. coli* remained sensitive to phage Q β . We therefore decided to test the ability of representative phages from each of the four

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classes to form plaques on bacteria carrying plasmids specifying distinguishable F-like pili.

Two RNA phages infecting *Pseudomonas aeruginosa* have been reported (Feary, Fisher & Fisher, 1963; Bradley, 1966). These are very closely related (D. E. Bradley, personal communication). They adsorb upon pili produced by *Ps. aeruginosa* (Bradley, 1966).

MATERIALS AND METHODS

Phages

These are shown in Table 1.

Bacteria

J5.3 (*E. coli* K 12 met F, proA, F⁻) (Clowes & Hayes, 1968).

Plasmids

These are shown in Table 2.

Media

Nutrient broth, nutrient agar and top agar (Clowes & Hayes, 1968).

Table 1. *Phages used in experiments*

Phage male-specific	Group	References
MS 2	I	Davis, Strauss & Sinsheimer (1961)
GA	II	Watanabe, Miyake <i>et al.</i> (1967); Sakurai, Watanabe & Ohno (1967)
QB	III	Watanabe, Miyake <i>et al.</i> (1967) Sakurai <i>et al.</i> (1967)
SP	IV	Sakurai <i>et al.</i> (1968) Miyake <i>et al.</i> (1969)
<i>Pseudomonas</i> Pp 7		Bradley (1966)

Table 2. *Plasmids used in experiments*

Plasmids	Serological group	Resistances	References
F-like			
F	A	None	Cavalli, Lederberg & Lederberg (1953) Hayes (1953)
R 1.19	B	kan*	Meynell & Datta (1967)
R 538.1 drd	C	str, chl, sul	Cooke & Meynell (1969)
R 192.7	D	str, tet, chl, sul	Cooke & Meynell (1969)
R 136.8	D	tet	Cooke & Meynell (1969)
R 384 drd	?D†	tet	S. Dennison, unpublished
R 386 drd	?A?C†	tet	S. Dennison (1972)
I-like			
R 64.11	—	str, tet	Cooke & Meynell (1969)
R 144.3	—	kan	Cooke & Meynell (1969)

* A segregant of R 1.19 carrying resistance to kanamycin only.

† Evidence presented in this paper.

Resistances: chl = chloramphenicol; kan = kanamycin; str = streptomycin;
sul = sulphonamides; tet = tetracycline.

Phage adsorption

Host bacteria were grown overnight in broth, diluted tenfold in fresh broth and incubated without shaking for 2 hr. at 37° C. Unshaken cultures were used to favour pilus formation. Of this culture 0.25 ml. was added to 2.5 ml. of molten top agar at 46° C.; 0.2 ml. of the appropriate dilution of phage suspension was added and, after 10 min. for adsorption, the contents of the tube were poured onto a nutrient agar plate. Plates were incubated overnight at 37° C.

RESULTS

Efficiency of plating

Suspensions of the four phages were diluted to give approximately equal titres (10^9 plaque forming units per ml.) on J5.3 (F). Relative titres of these suspensions on various hosts are recorded in Table 3.

Three host range mutants of phage SP (SP_{h1}, h₂ and h₃) isolated from plaques on J5.3 (R1.19) are included in Table 3. These show a new host range.

Plaque morphology

On J5.3 (F), phage GA produced large plaques with well-marked fuzzy halos. Within the halos, 'satellite' plaques were seen (Pl. 1, fig. 1). On J5.3 (R538.1 drd) GA formed plaques with less well developed 'satellite' systems (Pl. 1, fig. 2). Plaques with well-marked, rather fuzzy halos seem to be the norm for all the phages tested, but on J5.3 (R538.1 drd) phage SP formed plaques with sharp edges (Pl. 2, fig. 3). On J5.3 (R386 drd) all four phages plated with high efficiency but produced very turbid plaques, rather difficult to count (Pl. 2, fig. 4).

On J5.3 (R1.19) phage Q β produced plaques with low efficiency. The plaques were very variable in size and, probably, many were too small and faint to be counted. Such a result is predictable when the receptors are poorly adapted for the phage.

Phage Pp7 did not form plaques upon any of the tested strains of *E. coli* carrying derepressed R factors of F or I type.

Table 3. *Relative efficiencies of plating of phages on various hosts*

Phage	Host							
	J5.3	J5.3 (F)	J5.3 (R1.19)	J5.3 (R538.1 drd)	J5.3 (R192.7)	J5.3 (R136.8)	J5.3 (R384 drd)	J5.3 (R386 drd)
S2	0	1	1.5×10^{-1}	1.9×10^{-1}	3.5×10^{-4}	2.5×10^{-4}	1.2×10^{-4}	8×10^{-1}
A	0	1	0	2.9	0	0	0	2×10^{-1}
β	0	1	2.5×10^{-5}	2.0	5.6×10^{-5}	4.0×10^{-5}	4.3×10^{-5}	3×10^{-1}
P	0	1	2×10^{-7}	0.9	0	0	0	10^{-1}
Ph ₁	0	1	1	1.9	n.t.	0	n.t.	10^{-1}
Ph ₂	0	1	1	1.2	n.t.	0	n.t.	4×10^{-1}
Ph ₃	0	1	8×10^{-2}	5×10^{-1}	n.t.	0	n.t.	2×10^{-1}

n.t. = Not tested.

DISCUSSION

All RNA phages so far described adsorb to pili. The genetic determinants responsible for the receptor pili of *Pseudomonas aeruginosa* (Bradley, 1966) and *Caulobacter* sp. (Schmidt, 1966) are unknown, but all the RNA phages of *Escherichia coli* adsorb to F-like pili.

In nature, F-pili are produced either constitutively as with F or col V factors, or by cells carrying plasmids whose pilin-producing genes have escaped repression by some physiological peculiarity, e.g. by cells which have recently accepted the plasmid (Watanabe, 1963). So far as is known, the only naturally occurring plasmids which produce F-pili constitutively form pili of serological class A. All the phages tested adsorb efficiently to pili of this type. This may be because all isolations of male-specific phages have been made using bacteria carrying the F factor. Alternatively the selective advantage of being able to adsorb to constitutively produced pili may be so great that no phages have been able to evolve exclusively an adsorption specificity for pili whose synthesis is normally repressed. The point could be settled by making a survey of male specific phages isolated by adsorption to pili distinct from those determined by F (such as those specified by R 1, R 136 or R 192).

Pili produced by J 5.3 (R 386 drd) seem to have a specificity of adsorption similar to those of J 5.3 (F) and J 5.3 (R 538. 1). Compatibility studies and inhibition of plaque formation by phage T 7 on R 386 suggest that this plasmid resembles F more closely than do other F-like R factors (Dennison, 1972).

The adsorption specificities of the various RNA phages may throw light on the selective pressures that have led to the evolutionary diversification of sex pilus specificity. Most of the R factors tested produce pili less efficient as phage receptors than those encoded by F (R 538 is the only exception). The widespread prevalence of RNA phages capable of adsorbing to sex pili must favour mutant plasmids, coding for pili that are not efficient phage receptors (though still efficient for plasmid transfer).

Phage adsorption specificities of the various pili may be compared with the serological relationships (Lawn & Meynell, 1970). Serologically R 136 and R 192 pili are indistinguishable and it is satisfying to find that their phage adsorption properties are closely similar. R 384 determines very similar pili judging by phage adsorption specificity.

R 538. 1 pili are serologically intermediate between those encoded by F (or col V-K 94) and those of R 1. Thus, anti R 538. 1 pilus serum adsorbed with R 1 pili, still reacted with F (or col V-K 94) pili and serum adsorbed with F (or col V-K 94) pili could still react with R 1 pili (Lawn & Meynell, 1970). Phage specificities of F and R 538. 1 pili are very similar, confirming the serological relationship. On the other hand R 538. 1 pili and R 1 pili have very different phage receptor properties. Perhaps the R 1 pilus specificity (class B) arose as the result of a mutation to resistance towards phages of groups II and IV in a plasmid previously determining a pilus similar to that of R 538. 1. This hypothesis is open to experimental test.

Serological comparisons of RNA phages have led Miyake *et al.* (1969) to postulate that groups I (e.g. MS 2) and II (e.g. GA) are related and that groups III (e.g.

$Q\beta$) and IV (e.g. SP) are also related. However, phages GA and SP show similar host ranges differing from those of MS2 and $Q\beta$. (This is especially marked on hosts producing pili of class D.) Thus the host range patterns do not support the proposed relationships.

Plaque formation by phage $Q\beta$ on J5.3 (R1.19) is a very inefficient process. Only a small proportion of the phage particles form countable plaques. These plaques are heterogeneous in size and when the plates are inspected carefully one gets the impression that there are numerous plaques too small or too turbid to be counted. This is what might be expected for plaque formation by a phage poorly adapted for adsorption to the receptors on a host. Probably among the phage particles from the largest plaques one would find host range mutants better adapted to adsorption on the host. We looked at phage from plaques formed by phage SP on J5.3 (R1.19). The efficiency of plating was less than 10^{-6} , but the plaques formed were clear, suggesting that the phages adsorb efficiently. As might be predicted, they resulted from host range mutants with new specificity patterns (Table 3).

Very little is known about the details of the interaction between RNA phages and pili. It is not even possible to predict whether phage mutations altering the host range should specify alterations in the major coat protein (Weber & Konigsberg, 1967) or a minor component (Roberts & Steitz, 1967). This question can be settled using these mutants. Since it is possible to obtain both pili (Brinton, 1965) and phages (Gesteland & Boedtke, 1964) in large quantities such mutants and mutant sex pili unable to adsorb particular phages may be useful in investigations of the specificity of interaction between the protein molecules of phage and receptor.

The peculiar 'satellite-ringed' plaques of phage GA may be produced by a mechanism similar to that responsible for star mutant plaques of phage T2 (Symonds, 1958), and comparable plaque mutants of several other phages (reviewed by Symonds, 1958). If phage GA is inhibited from lysing infected cells near the edge of the plaque (possibly because of superinfection) the fuzzy edges of the plaques would be explained. Mutants (not susceptible to the inhibition) arising in the peripheral regions of the plaque would be expected to form satellite plaques of the type observed.

The pseudomonas phage Pp7 failed to form plaques on any of the strains of *E. coli* tested. It seems that it must adsorb to pili of a specificity different from either F or I.

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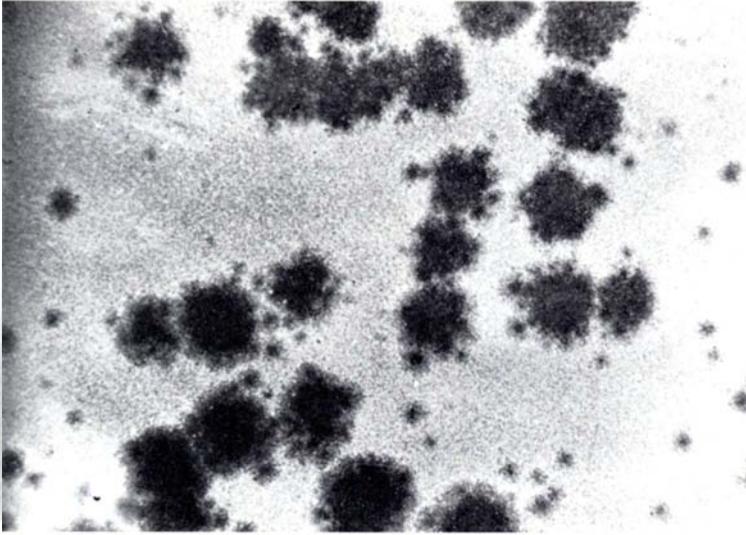


Fig. 1.

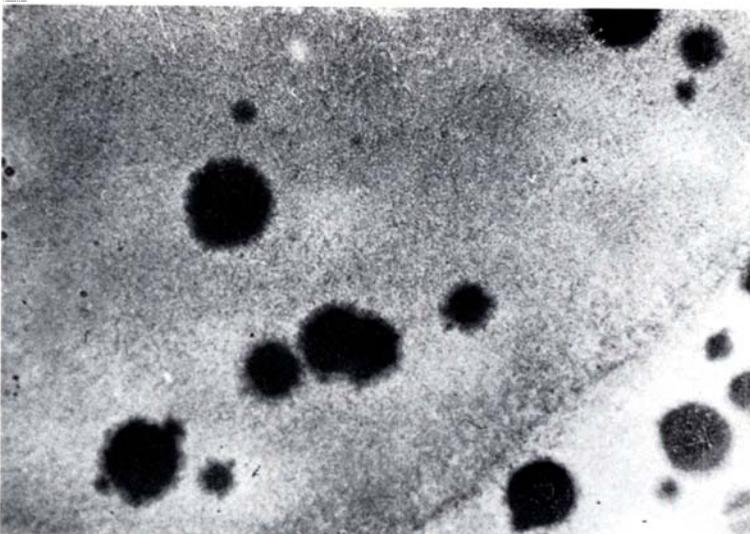


Fig. 2.

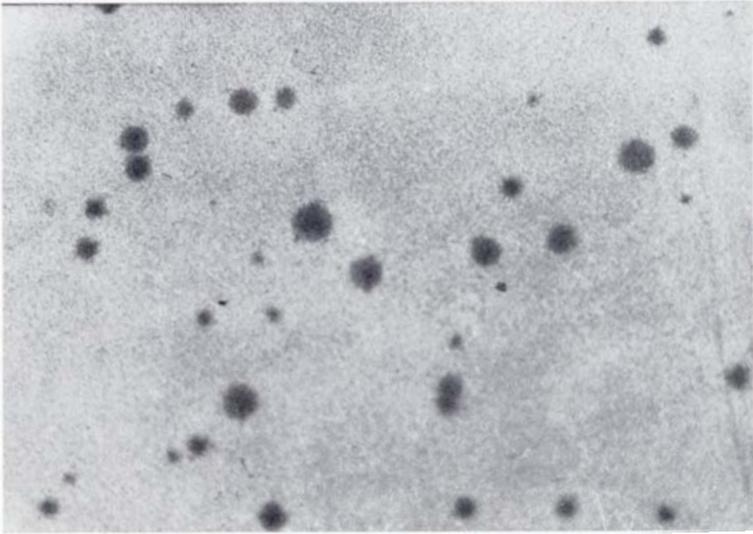


Fig. 3.

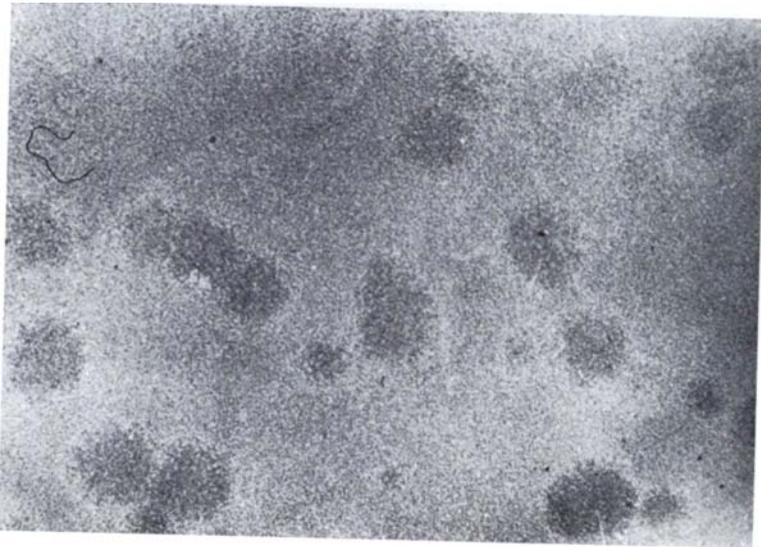


Fig. 4.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Plaques formed by phage GA on J 5. 3 (F).

Fig. 2. Plaques formed by phage GA on J 5. 3 (R 538. 1 drd).

PLATE 2

Fig. 3. Plaques formed by phage SP on J 5. 3 (R 538. 1 drd).

Fig. 4. Plaques formed by phage GA on J 5. 3 (R 386 drd).