SHORT COMMUNICATION

Phage C-1: an IncC Group Plasmid-specific Phage

By F. A. SIRGEL,¹ J. N. COETZEE,^{1*} R.W. HEDGES² AND G. LECATSAS¹

¹ Department of Microbiology, University of Pretoria and Bacterial Genetics Research Unit of the South African Medical Research Council, P.O. Box 2034, Pretoria 0001, South Africa

² Department of Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS

(Received 22 July 1980)

A phage was isolated from sewage and shown to form plaques on *Salmonella typhimurium* strains carrying C plasmids. It failed to multiply on strains lacking plasmids of this group. It also plated on *Proteus mirabilis* and *Serratia marcescens* strains carrying various IncC plasmids but failed to form plaques on *Escherichia coli* strains harbouring most of these plasmids, although in all cases, phage multiplication on the strains was demonstrated. No phage increase occurred in any of the strains which lacked an IncC plasmid or contained plasmids of other incompatibility groups. The phage is small, hexagonal in outline, contains RNA, is resistant to chloroform and adsorbs to the shafts of pili coded for by IncC plasmids.

INTRODUCTION

With the use of a segregant of a recombinant between the IncC plasmid R57b and plasmid RA1, Datta & Hedges (1973) were able to distinguish phenotypically between the *Aeromonas liquefaciens* plasmids RA1 and RA2 (Aoki *et al.*, 1971) and proved the latter two plasmids to be incompatible. The IncA group was then established with RA1 and RA2 as sole members. Even before the formation of this group it was known (Datta & Hedges, 1972) that, although RA1 is compatible with the IncC plasmids R40a and R57b, the latter two plasmids interact with the former at levels of exclusion, dislodgement or recombination reminiscent of plasmids belonging to the same incompatibility group. Doubt was again cast on the incompatibility grouping of RA1 by the finding (Datta & Olarte, 1974) that the IncC plasmid R806a is incompatible with RA1 although it is not excluded by this plasmid. The IncA and IncC groups are probably closely related and Hedges (1974) arranged RA1 and R57b in an 'A-C complex'. Recently this practice has been discontinued (Bradley, 1980) and IncA plasmids are now incorporated in the IncC group. Some IncC plasmids are readily transferable to *Pseudomonas aeruginosa* in which they are members of incompatibility group P-3 (Jacoby, 1977).

The IncC group is large, composed of diverse plasmids and has been compared to the IncF or IncI plasmid complexes (Datta & Olarte, 1974; Hedges, 1974). The latter complex is defined (Grindley *et al.*, 1972; Falkow *et al.*, 1974) as consisting of plasmids which code for pili the tips of which adsorb the λ phages (Meynell & Lawn, 1968; and see Coetzee *et al.*, 1980). This paper describes some properties of a phage which adsorbs specifically to bacteria harbouring IncC plasmids.

METHODS

Bacteria and plasmids. Bacteria were *Proteus mirabilis* PM5006, *Salmonella typhimurium* LT2trpA8, *Escherichia coli* K12 strains J53, J62-1 (Coetzee *et al.*, 1979), RC24Pil⁻ (Datta *et al.*, 1966) as well as *Serratia*

Table 1. *Host range of phage C-1*

Drops of the phage suspension (titre 1×10^9 p.f.u. ml⁻¹) were spotted on lawns of organisms contained in top-layer agar. Phage multiplication was assessed as outlined in Methods.

Host	Plasmid Inc group	Phage action*		Reference
		Plaque formation	Multiplication	
LT2trpA8(RA1)	A†	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(RA1-1)	A†	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(RA2)	A†	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(R714b)	A-C†	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(P-lac)	A-C†	+	+	Jacob <i>et al.</i> (1977)
PM5006(P-lac)	A-C†	+	+	Jacob <i>et al.</i> (1977)
J62-1(P-lacR1drd19)‡	A-C†	+	+	Jacob <i>et al.</i> (1977)
SM89(P-lacR1drd19)	A-C†	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(R724)	B	—	—	Jacob <i>et al.</i> (1977)
LT2trpA8(R16a)	C	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(R40a)	C	+	+	Jacob <i>et al.</i> (1977)
J53(R40a)	C	—	+	Jacob <i>et al.</i> (1977)
LT2trpA8(R57b)	C	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(R746)	C	+	+	Jacob <i>et al.</i> (1977)
LT2trpA8(R1184)	C	+	+	Datta <i>et al.</i> (1980)
RC24(R1184)	C	—	+	Datta <i>et al.</i> (1980)
LT2trpA8(R840)	D	—	—	R. W. Hedges (unpublished)
LT2trpA8(R1drd19)	FII	—	—	Jacob <i>et al.</i> (1977)
LT2trpA8(EDP208)	FV	—	—	Bradley & Meynell (1978)
LT2trpA8(R478)	H2	—	—	Bradley (1980)
LT2trpA8(R144drd3)	I	—	—	Jacob <i>et al.</i> (1977)
LT2trpA8(R391)	J	—	—	Jacob <i>et al.</i> (1977)
LT2trpA8(R992)	J	—	—	Bradley (1980)
LT2trpA8(RIP69)	M	—	—	Jacob <i>et al.</i> (1977)
LT2trpA8(R447b)	N	—	—	Jacob <i>et al.</i> (1977)
LT2trpA8(RP4)	P-1	—	—	Jacob <i>et al.</i> (1977)
LT2trpA8(R402)	T	—	—	Coetzee <i>et al.</i> (1972)
LT2trpA8(RA3)	U	—	—	R. W. Hedges (unpublished)
LT2trpA8(R769)	V	—	—	Hedges (1975)
LT2trpA8(RSa)	W	—	—	Coetzee <i>et al.</i> (1979)
LT2trpA8(R6Kdrd)	X	—	—	Bradley (1980)

* +, Plaque formation or increase in titre; —, no visible action or titre increase.

† Recently grouped as IncC (Bradley, 1980).

‡ Also named plasmid D (Coetzee, 1978).

marcescens SM89 (supplied by R. E. Ambrosio). The plasmids (Table 1) were from the Hammersmith collection and were transferred to the appropriate organisms.

Bacteriophages. Phages f2 (Loeb & Zinder, 1961), PR64FS (Coetzee *et al.*, 1980) and PR772 (Coetzee *et al.*, 1979) were used to identify sex pili on host cells harbouring plasmids of the F, I, and P-1, N and W incompatibility groups, respectively.

Media, antibiotics and conjugal transfer of plasmids. These were as described (Coetzee *et al.*, 1979). The incubation temperature was 37 °C for all plasmids apart from the IncT group where it was 20 °C.

Phage isolation. Attempts were made to isolate an IncC plasmid-specific phage from sewage obtained from the main Pretoria sewage works using the method of Meynell & Lawn (1968). Briefly, this involved the addition of 1 ml of exponentially growing culture of *E. coli* J62-1(P-lacR1drd19) or *P. mirabilis* PM5006(P-lac) to an equal volume of sewage. After 20 min adsorption, each mixture was diluted with 10 ml warm broth and incubated overnight. The mixtures were then treated as described previously (Coetzee *et al.*, 1979) and finally plated on the above organisms and isogenic strains lacking the plasmids.

General phage techniques, production of phage lysates and organic solvent sensitivity of phages. These were done as described and referred to by Coetzee *et al.* (1979).

Concentration of phage. Phage lysates were concentrated using polyethylene glycol (mol. wt 6000) and NaCl at final concentrations of 11% (w/v) and 0.5 M, respectively (Yamamoto *et al.*, 1970). Pellets were resuspended in small amounts of distilled water.

Bacteriophage increase. This was assessed by the double agar layer technique (Adams, 1956). Top layer (4 ml) contained 1 ml of an exponentially growing culture of the test organism (Table 1) as well as 0.1 ml phage suspension [1×10^4 plaque-forming units (p.f.u.) ml⁻¹]. One control consisted of 1 ml of an isogenic strain lacking the plasmid to which the same amount of phage and top layer was added. In the other control the same volume of phage was added to the top layer agar which contained 1 ml broth instead of the host organism. The mixtures were then poured over the surface of nutrient agar plates and allowed to set. After overnight incubation, 8 ml saline was added to each plate and the soft agar layer was scraped off completely. The suspensions were then centrifuged at 8000 g for 10 min and phage titres of the supernatants were determined.

Ribonuclease sensitivity. Drops of the phage suspension (1×10^7 p.f.u. ml⁻¹) were spotted on plates containing *S. typhimurium* LT2trpA8(P-lac) with pancreatic RNAase (BDH) at a final concentration of 25 µg ml⁻¹ in top-layer agar (Watanabe & August, 1967). Similar plates seeded with strain LT2trpA8(RP4) were spotted with a double-stranded DNA phage, PR772, (Coetzee *et al.*, 1979) as a control.

Electron microscopy. *Escherichia coli* strain RC24Pil⁻ carrying plasmid R1184 was used to determine the adsorption site of phage C-1. The method was that of Bradley (1980). Bacteria from plates grown overnight were suspended at high concentration in 0.2 ml of a neutral 0.1 M-ammonium acetate solution. An equal volume of phage suspension (5×10^{11} p.f.u. ml⁻¹) was added and the mixture was incubated for 30 min. Subsequently, a drop of the suspension was deposited on a Formvar-carbon coated grid, repeatedly rinsed with 0.1 M-ammonium acetate and negatively stained. Specimens were examined in a Philips EM300 electron microscope at an instrumental magnification of 42000.

RESULTS AND DISCUSSION

Isolation of a P-lac-dependent bacteriophage

A phage was isolated which plated on both the strains used for isolation but not on isogenic strains lacking the plasmids. The phage was named C-1 and was routinely propagated on *S. typhimurium* LT2trpA8(P-lac).

Properties of phage C-1

Plaque morphology. The phage formed slightly turbid plaques 1.0 to 2.0 mm in diameter on *S. typhimurium* LT2trpA8(P-lac) and *E. coli* J62-1(P-lacR1drd19). When plated on *P. mirabilis* PM5006(P-lac) or *Ser. marcescens* SM89(P-lacR1drd19) the plaques were more turbid but of the same size.

Morphology. The phage had an hexagonal outline (Fig. 1a) indicating possible icosahedral symmetry. The average diameter of 60 phage particles was 27 ± 2 nm between opposite apices.

Adsorption site. Phage particles were seen attached to the shaft of pili (Fig. 1b) produced by *E. coli* RC24 carrying the IncC plasmid R1184.

Ribonuclease sensitivity. Plaque formation by phage C-1 was totally inhibited by RNAase. Phage PR772 plated to the same titre on RNAase-containing plates as on control plates which lacked RNAase.

Organic solvent sensitivity. Phage C-1 was stable in the presence of chloroform and diethyl ether. Suspensions of the phage were routinely maintained above a layer of chloroform.

Host range. The phage plated on *S. typhimurium* LT2trpA8 carrying plasmids of incompatibility groups A, A-C and C (Table 1). It also plated on *P. mirabilis* PM5006, *E. coli* J62-1 and *Ser. marcescens* SM89 carrying various plasmids of the above mentioned groups. Phage C-1 did not plate on isogenic strains lacking the plasmids or on strains carrying plasmids of other incompatibility groups tested, and no multiplication of the phage, as demonstrated by an increase in titre, could be demonstrated on these strains. *Escherichia coli* strains carrying some A, A-C or C incompatibility group plasmids failed to plate the phage although a titre increase of at least 100-fold was demonstrated in all these cases (Table 1, lines 12, 16 and not shown) and it is fortunate that *E. coli* strain J62-1(P-lacR1drd19), which was used for sewage enrichment, plated the phage. *Escherichia coli* strains lacking the plasmids failed to produce phage in a titre above that of controls. Bradley (1980) could not demonstrate pilus formation electron microscopically by *E. coli* strains harbouring the IncC

Fig. 1. (a) Morphology of phage C-1. (b) Adsorption of phage C-1 to the shaft of a detached pilus produced by *E. coli* RC24 carrying plasmid R1184. Bar markers represent 100 nm.

plasmid R40a with the use of a temporary derepression method. Failure of *E. coli* strains bearing the particular plasmids to produce pili is an unlikely explanation for our results as intraspecific transfer frequencies of these plasmids in *E. coli* and *S. typhimurium* were similar and greater than 1×10^{-3} per donor (not shown). Bradley (1977) encountered a reverse situation with an *E. coli* and a *S. typhimurium* strain carrying the IncX (now IncD; R. W. Hedges, unpublished) plasmid R711b. When phage fd was spotted on lawns of the two plasmid-bearing organisms, plaque formation was only detected with the former strains, although a small phage titre increase on *S. typhimurium* indicated the susceptibility which the plasmid conferred on the latter organism.

Plasmid P-lac was originally thought (Coetzee *et al.*, 1973; Coetzee, 1974) to belong to the P incompatibility group, but Datta & Hedges (Hedges, 1974) finally reassigned it to the A-C

complex. The fact that strains harbouring plasmid (P-lacR1drd19) plate phage C-1 and not phage f2 confirms the compatibility studies of Hedges (1974) who found that the fused plasmid belongs to the IncA-C complex and not to IncFII (Coetzee, 1974).

The fact that pilus formation (not shown) by plasmids of incompatibility groups I, FII, and N, P-1 and W was demonstrated by the susceptibility they conferred on host bacteria to phages PR64FS, f2 and PR772, respectively, but not to phage C-1, is an indication of the specificity of the latter phage. Pilus shaft-adsorbing phages are more specific than those which adsorb to pilus tips (Coetzee *et al.*, 1979) and bacteria harbouring all plasmids of incompatibility groups A, A-C and C tested have supported growth of phage C-1. Hedges (1974) concluded, on the basis of compatibility studies, that groups A and C should be merged. In view of the implications of the present study we propose that all these plasmids are best regarded as members of a single group. Although affinity studies have not been done, such an investigation could possibly subdivide the IncC plasmids in a manner similar to that achieved by the shaft-adsorbing phages MS2, μ 2 or f2 for F-like plasmids (Meynell, 1978).

REFERENCES

- ADAMS, M. H. (1956). Methods of study of bacterial viruses. *Methods in Medical Research* **2**, 1-73.
- AOKI, T., EGUSA, S., OGATA, Y. & WATANABE, T. (1971). Detection of resistance factors in fish pathogen *Aeromonas liquefaciens*. *Journal of General Microbiology* **65**, 343-349.
- BRADLEY, D. E. (1977). Characterization of pili determined by drug resistance plasmids R711b and R778b. *Journal of General Microbiology* **102**, 349-363.
- BRADLEY, D. E. (1980). Determination of pili by conjugative bacterial drug resistance plasmids of incompatibility groups B, C, H, J, K, M, V and X. *Journal of Bacteriology* **141**, 828-837.
- BRADLEY, D. E. & MEYNELL, E. (1978). Serological characteristics of pili determined by the plasmids R711b and F_{lac}. *Journal of General Microbiology* **108**, 141-149.
- COETZEE, J. N. (1974). Properties of *Proteus* and *Providencia* strains harbouring recombinant plasmids between P-lac R1drd19 or R447b. *Journal of General Microbiology* **80**, 119-130.
- COETZEE, J. N. (1978). Mobilization of the *Proteus mirabilis* chromosome by R plasmid R772. *Journal of General Microbiology* **108**, 103-109.
- COETZEE, J. N., DATTA, N. & HEDGES, R. W. (1972). R factors from *Proteus rettgeri*. *Journal of General Microbiology* **72**, 543-552.
- COETZEE, J. N., DATTA, N., HEDGES, R. W. & APPELBAUM, P. C. (1973). Transduction of R factors in *Proteus mirabilis* and *P. rettgeri*. *Journal of General Microbiology* **76**, 355-368.
- COETZEE, J. N., LECATSAS, G. & COETZEE, W. F. (1979). Properties of R plasmid R772 and the corresponding pilus-specific phage PR772. *Journal of General Microbiology* **110**, 263-273.
- COETZEE, J. N., SIRGEL, F. A. & LECATSAS, G. (1980). Properties of a filamentous phage which adsorbs to pili coded by plasmids of the IncI complex. *Journal of General Microbiology* **117**, 547-551.
- DATTA, N. & HEDGES, R. W. (1972). R factors identified in Paris, some conferring gentamicin resistance, constitute a new compatibility group. *Annales de l'Institut Pasteur* **123**, 849-852.
- DATTA, N. & HEDGES, R. W. (1973). R factors of compatibility group A. *Journal of General Microbiology* **74**, 335-336.
- DATTA, N. & OLARTE, J. (1974). R-factors in strains of *Salmonella typhi* and a *Shigella dysenteriae* 1 isolated during epidemics in Mexico: classification by compatibility. *Antimicrobial Agents and Chemotherapy* **5**, 310-317.
- DATTA, N., LAWN, A. M. & MEYNELL, E. (1966). The relationship of F type piliation and F phage sensitivity to drug resistance transfer in R⁺F⁻ *Escherichia coli* K12. *Journal of General Microbiology* **45**, 365-376.
- DATTA, N., DACEY, S., HUGHES, V., KNIGHT, S., RICHARDS, H., WILLIAMS, G., CASEWELL, M. & SHANNON, K. P. (1980). Distribution of genes for trimethoprim and gentamicin resistance in bacteria and their plasmids in a general hospital. *Journal of General Microbiology* **118**, 495-508.
- FALKOW, S., GUERRY, P., HEDGES, R. W. & DATTA, N. (1974). Polynucleotide sequence relationships among plasmids of the I compatibility complex. *Journal of General Microbiology* **85**, 65-76.
- GRINDLEY, N. D. F., GRINDLEY, J. N. & ANDERSON, E. S. (1972). R factor compatibility groups. *Molecular and General Genetics* **119**, 287-297.
- HEDGES, R. W. (1974). R factors from *Providencia*. *Journal of General Microbiology* **81**, 171-181.
- HEDGES, R. W. (1975). R factors from *Proteus mirabilis* and *P. vulgaris*. *Journal of General Microbiology* **87**, 301-311.
- JACOB, A. E., SHAPIRO, J. A., YAMAMOTO, L., SMITH, D. I., COHEN, S. N. & BERG, D. (1977). Plasmids studied in *Escherichia coli* and other enteric bacteria. In *DNA Insertion Elements, Plasmids and Episomes*, pp. 607-638. Edited by A. I. Bukhari, J. A. Shapiro & S. L. Adhya. New York: Cold Spring Harbor Laboratory.
- JACOBY, G. A. (1977). Classification of plasmids in *Pseudomonas aeruginosa*. In *Microbiology - 1977*, pp. 119-126. Edited by D. Schlessinger. Washington, D.C.: American Society for Microbiology.
- LOEB, T. & ZINDER, N. D. (1961). A bacteriophage containing RNA. *Proceedings of the National*

- Academy of Sciences of the United States of America* **47**, 282–289.
- MEYNELL, E. (1978). Experiments with sex pili: an investigation of the characters and function of F-like and I-like sex pili based on their reactions with antibody and phage. In *Pili*, pp. 207–233. Edited by D. E. Bradley, E. Raizen, P. Fives-Taylor & J. Ou. Washington, D.C.: International Conferences on Pili.
- MEYNELL, G. G. & LAWN, A. M. (1968). Filamentous phages specific for the I sex factor. *Nature, London* **217**, 1184–1186.
- WATANABE, M. & AUGUST, J. T. (1967). Methods for selecting RNA bacteriophage. *Methods in Virology* **3**, 337–350.
- YAMAMOTO, K. R., ALBERTS, B. M., BENZINGER, R., LAWHORNE, L. & TREIBER, G. (1970). Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. *Virology* **40**, 734–744.