Phage Fₙlac: An Fₙlac Plasmid-dependent Bacteriophage

By D. E. BRADLEY,¹ J. N. COETZEE,²* TINA BOTHMA² and
R. W. HEDGES³

¹ Faculty of Medicine, Memorial University of Newfoundland, St John’s, Newfoundland, Canada A1B 3V6
² 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
³ Department of Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS, U.K.

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By enriching sewage with Escherichia coli or Salmonella typhimurium strains harbouring the plasmid EDP208, a constitutive pilus-producing derivative of plasmid Fₙlac, a phage was isolated which plated on these two organisms but not on isogenic strains without the plasmid. The phage was named Fₙlac; it had a hexagonal outline with a diameter of 28 nm, contained RNA, was resistant to chloroform, and probably adsorbed preferentially to the sides of EDP208 pili very near the tip. Phage multiplication could be demonstrated on E. coli or S. typhimurium strains carrying the plasmid Fₙlac, but an increase in titre did not occur on E. coli strains carrying plasmids of the F complex. Results of phage multiplication experiments on strains carrying the depressed pilus-producing plasmids R71 or TP224-Tc, which determine pili serologically related to those of EDP208, were inconclusive. Phage Fₙlac was found to be serologically related to phage UA-6, another isolate specific for EDP208.

INTRODUCTION

The plasmid Fₙlac, present in a strain of Salmonella typhosa, was described as readily transmissible to some other members of the family Enterobacteriaceae (Falkow & Baron, 1962). A relationship between Fₙlac and F was suspected and Lawn et al. (1967) allocated Fₙlac to an F-like group with other plasmids like F, ColV and R1 on the grounds of morphological similarity and apparent antigenic relatedness of sex pili. However, Fₙlac pili differ from F pili because, while they adsorb the tip-specific, broad host range, filamentous phage M13 and related phages, they do not adsorb the shaft-specific RNA phage MS2. Datta (1975) arranged F-like plasmids into groups FI to FV, Fₙlac being the sole member of the last group.

Bradley & Meynell (1978) and Bradley (1978) found that Fₙlac pili were unrelated to all F pilus serotypes and Fₙlac has now been removed from the F incompatibility complex and is classified as a unique plasmid (Datta, 1979). Bradley (1980b, and unpublished) found that the Inc9 (=Com9) plasmid R71 (Scavizzi, 1973) and the plasmid TP224-Tc (strain E7476 in Scotland et al., 1979) determine pili which are serologically related to those of plasmid EDP208, a constitutive pilus-producing derivative of plasmid Fₙlac constructed by N. Willetts (see Armstrong et al., 1980).
A further distinction between F pili and those synthesized by EDP208 was made by the discovery of an EDP208-specific RNA phage (Armstrong et al., 1980) which did not adsorb to F pili.

In this paper we describe the properties of another EDP208-specific phage which adsorbs to F,lac pili.

METHODS

**Bacteria and plasmids.** Bacteria were Proteus mirabilis strain PM5006, Salmonella typhimurium strain LT2irpA8, and Escherichia coli strains J62-2 (Coetzee et al., 1979) and JE257I (Bradley, 1980a). Plasmids were from the collection of Naomi Datta (Datta, 1977) unless otherwise indicated and were transferred to appropriate organisms. Plasmid-carrying strains are listed in Table 1.

**Bacteriophages.** Phage UA-6 (Armstrong et al., 1980) was kindly provided by W. Paranchych. Phage PR772, a DNA-containing P-1 plasmid-specific phage (Coetzee et al., 1979), was used as a negative control in ribonuclease sensitivity tests. The F-specific RNA-containing phage MS2 was used for serological comparison.

**Media, antibiotics, transfer of plasmids by conjugation, general phage techniques, production of phage lysates and organic solvent sensitivity tests for phages.** The materials and methods used were as described and referred to by Coetzee et al. (1979). Organisms were incubated at 37 °C apart from those harbouring T group plasmids when incubation was at 25 °C.

**Isolation of a bacteriophage specific for bacteria harbouring plasmid EDP208.** The general method of Coetzee et al. (1979) was used to isolate the phage from sewage derived from a Pretoria hospital. One modification was introduced. This consisted of first thoroughly mixing the sewage with 0-25 vol. chloroform to inactivate (Marvin & Hohn, 1969) F complex pilus-tip adsorbing filamentous bacteriophages (see above). The chloroform was then evaporated and the sewage was enriched with partner strains J62-2(EDP208) or LT2irpA8(EDP208). After the incubation period a Millipore membrane filtrate of the enriched sewage was titrated on the other bacterial partner. The F-specific RNA-containing phage MS2 was used for serological comparison.

**Electron microscopy.** For phage adsorption studies, strain JE2571(EDP208) cells were suspended in a few drops of phage F,lac suspension at about 1 × 10¹⁰ plaque-forming units ml⁻¹ in a small Petri dish, which was then covered and incubated for 45 min at 37 °C. A support grid was then touched on to the surface of the mixture and negatively stained with a mixture of equal volumes of neutral 2% (w/v) sodium phosphotungstate solution and 0-1 m-ammonium acetate solution. Alternatively, to reduce the number of free phage virions on the grid by dilution, a droplet of the adsorbed phage-suspension mixture was introduced into the top of a conical centrifuge tube filled with 0-1 m-ammonium acetate solution and with an electron microscope support grid (carbon side up) at the bottom (see Bradley, 1977). Bacteria with phages adsorbed to their pili were centrifuged on to the grid at about 1000 g for 15 min. The grid was then removed, washed free of excess bacteria and negatively stained.

**Preparation of antisera and immune electron microscopy.** Phage F,lac antiserum was prepared according to the general method of Coetzee et al. (1979) and the serum obtained was absorbed with J62-2(EDP208). For immune electron microscopy, a carbon-coated support grid was touched on to the surface of a phage suspension and then placed gently on the surface of a mixture of equal volumes of the phage F,lac antiserum (inactivation constant: 6-17 min⁻¹) and 0-1 m-ammonium acetate solution. The surface layer of phage suspension on the grid did not mix with the antiserum mixture but it appeared that antibodies diffused into the surface layer causing aggregates of serologically related phage to form and stick to the support film. After 5 min the grid was dabbed several times on the surface of the antiserum mixture and allowed to float for a further 5 min before washing and negative staining.

**RESULTS AND DISCUSSION**

At the first attempt, a phage was isolated which plated on both the strains carrying plasmid EDP208 used for sewage enrichment but not on isogenic strains lacking the plasmid. It was named phage F,lac and was routinely propagated on strain J62-2(EDP208).
Fig. 1. (a) A group of three $F_{lac}$ phage virions adsorbed near the tip of a detached EDP208 pilus with a fourth apparently attached further down the shaft. (b) A pair of adsorbed $F_{lac}$ phage virions on a detached EDP208 pilus. (c) A single $F_{lac}$ phage virion adsorbed near the tip of an EDP208 pilus on a cell. (d) An aggregate of phage UA-6 virions after treatment with antiserum to phage $F_{lac}$. (e) MS2 phage virions attached to an F pilus after treatment with antiserum to phage $F_{lac}$, showing no antibody adsorption. The bar marker represents 100 nm for all micrographs.

Properties of phage $F_{lac}$

*Plaque morphology.* The phage formed clear plaques with a diameter of about 2 mm on both strains used for phage isolation. The plaque morphology did not vary like that of some other phages that adsorb to pili (Coetzee et al., 1979, 1980; Sirgel et al., 1981) and could imply that phage adsorption sites were plentiful (see Meynell, 1978). Armstrong et al.
(1980) found that the level of pilation of *E. coli* K12 cells carrying plasmid EDP208 was at least an order of magnitude greater than that normally seen with F or F-like plasmids.

**Phage morphology.** Phage F₀lac appeared similar to other RNA-containing bacteriophages in the electron microscope (Fig. 1a, b, c) and may be compared with the F-specific RNA phage MS2 in Fig. 1(e). Phage F₀lac had a diameter of 28 nm (using the catalase crystal lattice for calibration).

**Adsorption site.** Phage F₀lac adsorbed very inefficiently to EDP208 pili as was the case with phage UA-6 (Armstrong et al., 1980). Where an excess of phage virions was present in an adsorption mixture, they were found attached in small numbers (usually one to three) near the tips of a small proportion of detached EDP208 pili (Fig. 1a, b). In addition, some virions appeared to adsorb further down the shafts (Fig. 1a) but, because of the numerous particles scattered over the support film, it was not possible to be certain whether they were adsorbed to the pili or simply lying beside them. When the number of excess virions were decreased by dilution (see Methods), most virions were found attached near the tips of pili attached to cells (Fig. 1c), the proportion adsorbed further down the sides being reduced. However, too few attached phages were found to permit a quantitative estimate of this reduction. Since it is unlikely that virions would adsorb non-specifically to a localized region of the pilus in preference to further down the shaft, it is probable that the adsorption site is near the pilus tip. Similar results have been obtained with the group T-specific phage t (Bradley et al., 1981b).

**Organic solvent sensitivity.** It was confirmed that the phage was chloroform-resistant. It was also stable in the presence of diethyl ether.

**Ribonuclease sensitivity.** Plaque formation by phage F₀lac was totally inhibited in the presence of 25 μg RNAase ml⁻¹ whereas a phage titre of 1 × 10⁹ plaque-forming units ml⁻¹ was obtained on control plates which lacked RNAase. The titre of phage PR772 (Coetzee et al., 1979) was the same (5 × 10⁸ plaque-forming units ml⁻¹) on RNAase-containing plates as on plates without the enzyme.

**Host range.** The phage plated on the plasmid EDP208-bearing organisms used for isolation but on no other strains (Table 1). Phage titre increases also occurred on strains J62-2(F₀lac) and LT2trpA8(F₀lac), but no increase in the titre above that of the negative control could be demonstrated on strains carrying plasmids of the F complex (Table 1). This confirms the uniqueness of pili specified by plasmid F₀lac. The phage did not plate on PM5006(EDP208) although a phage titre increase was demonstrated (Table 1). Plasmid EDP208 was transferable from this *Proteus mirabilis* strain to strain J62-2 at high frequency (not shown). The phenomenon of plasmid-specific phage only plating on particular strains carrying the same plasmid has been frequently reported (see Bradley, 1977; Coetzee et al., 1979; Sirgel et al., 1981). Baumberg & Dennison (1975) and Morgan & Kaplan (1977) found that F pili are poorly expressed in *P. mirabilis*. Also, *Proteus morganii* strain 2815 carrying N group plasmids does not propagate phage IKe although N-linked IKe sensitivity is transferable from the strains (Dennison & Baumberg, 1975), and Hua et al. (1981) demonstrated that a strain of *Caulobacter vibrioides* modified plasmid RP1-specified pili to the extent that the latter did not adsorb phage PRR1 while still retaining their conjugal function.

The host range of sex pilus-specific bacteriophages usually extends to include serologically related sex pili (see Meynell, 1978) but results of phage multiplication tests on strains of *E. coli* and *S. typhimurium* carrying plasmids R71 or TP224-Tc were inconsistent. On four occasions titre increases at least 10-fold above that of the negative control were obtained for phage added to each strain, but in five other experiments no increase in phage titre could be demonstrated for phage added to either organism. The reason for these results is not understood but an explanation may be that pilus production by these organisms under the experimental conditions used is variable. Bradley (1980b) found that R71-bearing strains did not produce many pili with the temporary derepression method used. Attempts to select for mutants of these two plasmids constitutive for pilus synthesis have thus far failed.

**Serological comparison with phage UA-6.** Using immune electron microscopy, it was
Table 1. Host range of phage \(F_{\text{lac}}\)

Drops of phage suspension (titre \(1 \times 10^9\) plaque-forming units ml\(^{-1}\)) were spotted on lawns of organisms contained in top-layer agar. Phage multiplication was determined as indicated in Methods.

<table>
<thead>
<tr>
<th>Host</th>
<th>Plasmid incompatibility group</th>
<th>Plaque formation*</th>
<th>Multiplication†</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT2-trpA48 (R724)</td>
<td>B</td>
<td>-</td>
<td>ND</td>
<td>Sirgel et al. (1981)</td>
</tr>
<tr>
<td>LT2-trpA48 (F-lac)</td>
<td>C</td>
<td>-</td>
<td>ND</td>
<td>Sirgel et al. (1981)</td>
</tr>
<tr>
<td>J62-2 (R711b)</td>
<td>D</td>
<td>-</td>
<td>ND</td>
<td>Bradley (1977); R. W. Hedges (unpublished)</td>
</tr>
<tr>
<td>J62-2 (R386)</td>
<td>F1</td>
<td>-</td>
<td>-</td>
<td>Wollman et al. (1956)</td>
</tr>
<tr>
<td>HfrH</td>
<td>F1</td>
<td>-</td>
<td>-</td>
<td>Jacob et al. (1977)</td>
</tr>
<tr>
<td>J62-2 (R1drd19)</td>
<td>F1II</td>
<td>-</td>
<td>-</td>
<td>Sirgel et al. (1981)</td>
</tr>
<tr>
<td>J62-2 (R124)</td>
<td>F1IV</td>
<td>-</td>
<td>-</td>
<td>Sirgel et al. (1981)</td>
</tr>
<tr>
<td>LT2-trpA48 (EDP208)</td>
<td>(F_{\text{lac}})</td>
<td>+</td>
<td>ND</td>
<td>Bradley &amp; Meynell (1978)</td>
</tr>
<tr>
<td>LT2-trpA48 (FZac)</td>
<td>(F_{\text{lac}})</td>
<td>-</td>
<td>+</td>
<td>Falkow &amp; Baron (1962)</td>
</tr>
<tr>
<td>J62-2 (R64drd11)</td>
<td>H2</td>
<td>-</td>
<td>ND</td>
<td>Bradley (1980a)</td>
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<td>I2</td>
<td>-</td>
<td>ND</td>
<td>Datta et al. (1971)</td>
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<tr>
<td>LT2-trpA48 (R997)</td>
<td>J</td>
<td>-</td>
<td>ND</td>
<td>Grindley et al. (1973)</td>
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<tr>
<td>LT2-trpA48 (Rip69)</td>
<td>M</td>
<td>-</td>
<td>ND</td>
<td>Jacob et al. (1977)</td>
</tr>
<tr>
<td>J62-2 (N3)</td>
<td>N</td>
<td>-</td>
<td>ND</td>
<td>Hedges (1972)</td>
</tr>
<tr>
<td>J62-2 (R1)</td>
<td>T</td>
<td>-</td>
<td>ND</td>
<td>Coetzee et al. (1972)</td>
</tr>
<tr>
<td>LT2-trpA48 (RA3)‡</td>
<td>U</td>
<td>-</td>
<td>ND</td>
<td>R. W. Hedges (unpublished)</td>
</tr>
<tr>
<td>LT2-trpA48 (R769)</td>
<td>V</td>
<td>-</td>
<td>ND</td>
<td>Hedges (1975)</td>
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<tr>
<td>LT2-trpA48 (R5a)</td>
<td>W</td>
<td>-</td>
<td>ND</td>
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<tr>
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<td>X</td>
<td>-</td>
<td>ND</td>
<td>Bradley (1980b)</td>
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<tr>
<td>J62-2 (R71)§</td>
<td>Com9</td>
<td>-</td>
<td>?</td>
<td>Scavizzi (1973)</td>
</tr>
<tr>
<td>LT2-trpA48 (R71)i†</td>
<td>Com9</td>
<td>-</td>
<td>?</td>
<td>Scavizzi (1973)</td>
</tr>
<tr>
<td>LT2-trpA48 (TP224-Tc)§</td>
<td>?</td>
<td>-</td>
<td>?</td>
<td>Scotland et al. (1979)</td>
</tr>
</tbody>
</table>

* †, +, Plaques observed; −, no plaques observed.
† +, At least a 10-fold titre increase above that of the negative control obtained on three separate occasions; −, no increase in phage titre above that of the negative control; ?, inconsistent results obtained (see text); ND, not done.
‡ Originally classified as belonging to incompatibility group W (Hedges & Datta, 1971).
§ Pili serologically related to EDP208 pili (Bradley, 1980b, and unpublished). TP224 determines a heat-stable enterotoxin and is marked with tetracycline resistance using a transposon, TP224-Tc (M. McConnell, personal communication).

found that phage UA-6 was strongly labelled with antibodies to phage \(F_{\text{lac}}\) (Fig. 1d), whereas the F-specific RNA phage MS2 was not (Fig. 1e). It was concluded that phages UA-6 and \(F_{\text{lac}}\) were serologically related.

The ready isolation of two phages with \(F_{\text{lac}}\) specificity from widely separated geographical locations could imply the abundant presence of \(F_{\text{lac}}\)-type pili in the environment. Bradley (1978) and Bradley & Meynell (1978) have commented on the widespread occurrence of the F pilus morphological form determined by unrelated plasmids and exhibiting different properties. \(F_{\text{lac}}\) pili are of this morphology and the serologically related pili specified by the compatible plasmids R71 and TP224-Tc (Bradley 1980b, and unpublished) could (see above) be a case in point.

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