SHORT COMMUNICATION

The Determination of Two Morphologically Distinct Types of Pilus by Plasmids of Incompatibility Group I₂

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Electron microscopy revealed that IncI₂ plasmids determined thick pili as well as the thin flexible ones previously detected. Phage PR4, which typifies the broad host-range, plasmid-specific, lipid-containing bacteriophages, formed plaques on bacterial strains carrying derepressed IncI₂ plasmids, and adsorbed to the tips of the thick pili rather than the thin ones.

INTRODUCTION

Plasmids of the I complex of incompatibility groups have been tentatively rearranged into two groups: IncI₁ and IncI₂ (Coetzee *et al.*, 1982). Like IncB and IncK plasmids, they determine thin flexible pili (Bradley, 1980*a*), which show distinct serological relationships (Bradley, 1980*b*). The I₁ serotype contains I₁, B and K pili, and the unrelated I₂ pili form the I₂ serotype. Pili of all I complex plasmids act as receptors for several different bacteriophages (Meynell & Lawn, 1968; Coetzee *et al.*, 1980; Coetzee *et al.*, 1982), none of which infect bacteria carrying IncB or IncK plasmids. While attempting to isolate an I₂-specific phage from sewage, we obtained a virus which apparently multiplied specifically on I₂⁺ strains. However, we subsequently found that it was morphologically identical with, and had a similar plasmid host-range to the broad host-range, lipid-containing phage PR4 (Stanisich, 1974; Bradley & Rutherford, 1975), which also formed clear plaques on strains harbouring derepressed IncI₂ plasmids.

In this communication, we describe the determination by IncI₂ plasmids of thick pili in addition to the thin flexible I₂ pili described previously (Bradley, 1980*b*). We also demonstrate the adsorption of phage PR4 to the tips of the thick pili.

METHODS

Bacteria, plasmids, and bacteriophage. *Escherichia coli* bacterial strains used were JE2571 (*leu thr str fla pil*), J53 (*Lac*⁺ *pro met*), its nalidixic acid-resistant derivative J53-1, and S38R475 (prototroph supplied by H. Smith). *Salmonella typhimurium* LT2 strains were SQ1139 (*purC proA ilv str fla pil*), its nalidixic acid-resistant derivative SQ1139-1, and M827 (Spratt *et al.*, 1973). Plasmids were from the Hammersmith collection, being listed with their drug resistance markers in Bradley (1980*b*) save for pIN2b, which was supplied by S. B. Levy, who obtained it in Indonesia. R721*drd* is a mutant of R721 that determines conjugative pili constitutively (constructed by R. W. Hedges). Bacteriophage PR4 (authors' collections) was described by Stanisich (1974) and Bradley & Rutherford (1975).

Media and growth methods. Brain Heart Infusion Agar (BBL) was used throughout. The standard spot test for the lytic activity of bacteriophages was done by placing a drop of PR4 suspension (about 10¹⁰ p.f.u. ml⁻¹) on a soft agar overlay containing the organism under test. The soft layer contained 0.5% (w/v) agar. Clearing by the phage after overnight incubation at 37 °C indicated sensitivity.

Electron microscopy. Pili were mounted for electron microscopy as described by Bradley (1980*a*), bacteria being

grown by the temporary derepression method where indicated. Phage plus pilus adsorption mixtures were prepared for electron microscopy by thoroughly suspending a loopful of bacteria in a drop of phage PR4 suspension (about 10^{10} p.f.u. ml⁻¹), incubating the mixture for 1 h at 37 °C, then touching a carbon-coated electron microscope specimen support grid on to the surface. After bacteria had been removed by dabbing the grid on a bath of 0.1 M-ammonium acetate solution, negative staining was carried out with a mixture of equal volumes of 2% (w/v) sodium phosphotungstate and 0.1 M-ammonium acetate solutions (also used for all other electron microscope preparations).

RESULTS

Occurrence and morphology of thick I₂ pili. For the purposes of this work we will refer to the two kinds of pili determined by IncI₂ plasmids as 'thick I₂ pili' and 'thin I₂ pili', the latter being those described previously (Bradley, 1980*b*).

Electron microscopy revealed both the well-known thin (6 nm) I₂ pili and, in addition, thick 10 nm diameter pili associated with three IncI₂ plasmids: pIN2b (repressed), R721*drd* (derepressed), and TP114*drp1* (derepressed). Under normal growth conditions on nutrient agar no pili of any sort could be found for strain JE2571(pIN2b), but using the temporary derepression method of growth (Bradley, 1980*a*), numerous thin flexible I₂ pili and a few thick ones were visible in the electron microscope (Fig. 1*a*). Some of the thick ones had a structure at one end (Fig. 1*b*) similar to that found on other types of thick pilus (Bradley, 1980*a, b*). Figure 1(*b*) also shows a dark axial line which was not often seen. Strain SQ1139-1(R721*drd*) synthesized many pili, both thick and thin, under normal growth conditions. The one in Fig. 1(*c*) shows a typical pointed end. We were unable to ascertain with certainty whether or not the thick I₂ pili were of the rigid or the thick flexible kind (Bradley, 1980*b*), but on the whole they more closely resembled the former. Strain JE2571(TP114*drp1*) only synthesized a few thick pili compared with SQ1139-1(R721*drd*); they were similar in appearance to those illustrated. Thin I₁ and I₂ pili aggregated strongly (Bradley, 1980*b*), but thick I₂ pili did not.

It is emphasized that the thin I₂ pili determined by the three plasmids are all serologically related, belonging to the I₂ serotype (Bradley, 1980*b*, and unpublished for pIN2b), but the thick I₂ pili have yet to be tested. Thick I₂ pili did not react with anti-TP114*drp1* serum (not illustrated), presumably because too few thick I₂ pili were determined by the plasmid to produce an immune reaction in the rabbit. This negative result also indicated that there was no serological relationship between the thick and thin I₂ pili determined by the same plasmid.

Host-range of bacteriophage PR4. A number of new incompatibility groups of plasmids have been identified since the original isolation of PR4 and other similar bacterial viruses. In view of this, and the isolation of a PR4-like phage on bacteria carrying IncI₂ plasmids (see Introduction), we checked the PR4 plasmid host-range by the spot test (see Methods) for lytic activity on strains carrying representative plasmids of most currently recognized incompatibility groups. Each plasmid-bearing strain was screened for the constitutive synthesis of conjugative pili, a prerequisite for PR4 lysis, either using electron microscopy (Bradley, 1980*b*) or by lysis with an appropriate conjugative pilus-specific phage. Phage PR4 gave well-defined lysis with strains carrying plasmids of *E. coli* incompatibility groups I₂ [J53(R721*drd*), JE2571(R721*drd*), SQ1139(R721*drd*), M827(R721*drd*), S38R475(TP114*drp1*)], N (N3 in several strains), P (RP4 in several strains), W [SQ1139(Sa)], and the single plasmid R775 [J53-1(R775)]. No lysis was obtained with strains harbouring plasmids of incompatibility groups B, C, D, FI, FII, HII, I₁, J, M, T, U, X and the single plasmid F₀*lacdrd*. Tests were not done for groups HI, K, V and com9, no plasmids synthesizing conjugative pili constitutively being available.

Adsorption of phage PR4 to thick I₂ pili. The fact that PR4 lysed strains carrying derepressed IncI₂ plasmids suggested that the phage adsorbed to either thick or thin I₂ pili, or both. The electron microscopy of an adsorption mixture of bacteriophage and pili revealed no virions attached to the easily distinguishable thin I₂ pili (not illustrated), but many examples where PR4 was attached to thick I₂ pili (Fig. 1*d, e*). The tail of phage PR4 was not always visible (Bradley & Rutherford, 1975), but with the few phage plus pilus complexes where it was, the point of attachment was clearly not the tail, but an apex of the head (Fig. 1*d*). Occasionally, virions were found with two pili attached (Fig. 1*e*); this also suggested that the site of pilus attachment was

Fig. 1. (a) Thick and thin I_2 pili determined by *E. coli* strain JE2571(pIN2b) using the temporary derepression method of growth (Bradley, 1980a). (b) A thick I_2 pilus showing a probable basal structure and dark axial line, synthesized by strain JE2571(pIN2b). (c) Thick and thin I_2 pili synthesized by *S. typhimurium* strain SQ1139(R721drd), the thick pilus showing a pointed end. (d) An empty PR4 phage virion, the tail pointing downwards, with a thick I_2 pilus from strain SQ1139(R721drd) attached to an apex of the head. (e) Another PR4 virion with two thick I_2 pili from the same strain adsorbed to the head; no tail is visible. The bar markers represent 100 nm for all micrographs.

not the tail since there is only one tail on the virion. These observations are similar to those described by Bradley (1978, 1979) where PR4 was found to adsorb by an apex of the head to P, N and W pili. Thus far, the conjugative pili to which PR4 adsorbs have all been of the rigid type, suggesting that thick I_2 pili might also be rigid rather than flexible.

DISCUSSION

The discovery that IncI₂ plasmids determine two morphologically different types of pili raises a number of questions perhaps the most important being whether or not similar thick pili are also determined by IncI₁, IncB or IncK plasmids in addition to their I_1 -serotype thin flexible pili. Thus far, we have not found thick I_1 pili. If they do exist, it seems unlikely that they will act as receptors for phage PR4 like thick I_2 pili since we have shown that strains carrying de-repressed IncI₁ or IncB plasmids do not specify PR4 lysis. We have found that thick I_2 pili are determined by R721*drd*, TP114*drp1*, and pIN2b, but obviously all other IncI₂ plasmids should be checked if possible. The present classification of the I complex plasmids is based on incompatibility relationships and thin I pilus serotype, but the new pili could be a useful additional character. They would easily be detectable by sensitivity to phage PR4, using multiplication in liquid medium for repressed plasmids.

There is considerable experimental evidence in favour of the involvement of thin I_1 pili in the conjugation process (see Meynell, 1978). By analogy, the structurally similar thin I_2 pili are likely to perform the same function. Thick I_2 pili resemble other conjugative pili in appearance (Bradley, 1980*a, b*) so that there is a definite possibility that two transfer operons may be carried by IncI₂ plasmids. Some of the many questions raised by the discovery of this two-pilus system are currently being investigated.

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