**Escherichia coli tolQ Mutants Are Resistant to Filamentous Bacteriophages That Adsorb to the Tips, not the Shafts, of Conjugative Pili**

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The *tolQ* (previously *fii*) mutation in *Escherichia coli* K12 inhibits infection by filamentous bacteriophages *f1* and *IKe* but not by RNA-containing phage *f2*. This work extends these observations to other plasmid-specific bacteriophages including various filamentous, RNA-containing, and lipid-containing isolates. Only tip-adsorbing filamentous phages were affected by *tolQ* and not shaft-adsorbing ones. Electron microscopy showed that RP4-specific filamentous phage *Pf3* was one of the latter kind. Several tip-adsorbing filamentous phages inhibited conjugation between *tolQ* strains carrying their specific plasmids, implicating the phage receptors (conjugative pili) as mating organelles. *tolQ* mutant strains were as proficient as their parents in conjugation mediated by a wide range of plasmids.

**INTRODUCTION**

Plasmid-specific filamentous and RNA-containing bacteriophages of *Escherichia coli* adsorb to conjugative pili (Bradley, 1967; Marvin & Hohn, 1969). Most filamentous phages attach to the pilus tips, but a few isolates, together with all RNA phages, adsorb to the pilus shafts (sides). Sun & Webster (1986) described a mutation, *fii*, which caused the inhibition of infection of *F*⁺ bacteria by filamentous bacteriophage *f1*, but not by RNA-containing phage *f2*. Similarly, the mutation inhibited the infection of bacteria harbouring plasmids of incompatibility group N (IncN) by filamentous phage *IKe*. The *fii* mutation also provided tolerance against *E* coli cains, which are classified as low-*M*, bacteriocins, as opposed to high-*M*, bacteriocins of bacteriophage origin (Bradley, 1967). Sun & Webster (1987) sequenced the *fii* region, and renamed the mutation for filamentous phage inhibition *tolQ*. The entire gene cluster, *tolQRAB*, encodes proteins which form a transport system across the bacterial envelope for large molecules. The main purpose of this work has been to extend the observations of Sun & Webster (1986) to the additional plasmid-specific bacteriophages in our collection. Furthermore, the hypothesis that plasmid DNA transfer in conjugation (see Willetts & Wilkins, 1984) might be affected by *tolQ* has been tested.

The inhibition of *F* plasmid transfer by the presence in mating mixtures of inactivated pilus-specific filamentous bacteriophages demonstrated unequivocally that *F* pili were involved in conjugation (Ippen & Valentine, 1967; Novotny et al., 1968). Similar experiments have been done here with filamentous phages specific for plasmids other than *F*. The presence of the *tolQ* mutation rather than phage inactivation was used to prevent infection of donor strains.

**METHODS**

*Strains, bacteriophages and plasmids.* The parent strain used by Sun & Webster (1986) was *E. coli* K12 P90C [araΔ (lac pro) thi]. Their strain GM1 was P90C/F lac pro. The *tolQ* (= *fii*-1) derivative of GM1 was TPS13. For this work we cured GM1 and TPS13 of the F lac pro plasmid by overnight growth in broth containing novobiocin (150 μg ml⁻¹); Lac⁻ colonies were then sought on MacConkey agar; plasmid loss was checked by agarose gel electrophoresis. Cured GM1 (= P90C) was designated P90C; cured TPS13 (= P90CtolQ) was designated TPS.
Nalidixic acid- and rifampicin-resistant derivatives of both strains (NalR, RifR; suffix -1 and -2 respectively) were isolated. In addition to phage fd, tolerance to colicin E1 was used to check the toIQ mutation. A loopful of E. coli K12 strain E8705(ColE1::Tn7) was suspended in 2 ml broth and about 10 μl of this suspension was spotted on the toIQ strain (see spot test for phages below); after incubation, lack of clearing around the bacterial overgrowth indicated tolerance. Strains JE2571 (lac leu thr str fla pil) and 553 (Lac+ pro met) or their NalR or RifR derivatives were used as plasmid hosts.

Bacteriophages and references are listed in Table 1. Most known filamentous phages were used with the exception of SF (Coetzee et al., 1986), which was an fd-like phage with an extended host range, but is no longer available. The two filamentous phages If1 (Meynell & Lawn, 1968) and PR64 (abbreviated from PR64FS; Coetzee et al., 1980) adsorb to the thin flexible pili as opposed to the thick rigid pili of I complex plasmids (Bradley, 1984). Since they are serologically related (Coetzee et al., 1982) we used only PR64. We could not test the only other filamentous phage known to adsorb to the pilus shaft, C-2 (Bradley et al., 1982a), since it only infected Salmonella typhimurium strains carrying transfer-derepressed IncC plasmids, not similar E. coli K12 strains.

Plasmids were selected on the basis of availability and for good responses to plasmid-specific phages with host strain P90C; most are cited by Jacob et al. (1977) with the exceptions of EDP208 (transfer-derepressed F0lac mutant constructed by N. Willetts; see Worobec et al., 1983), which is IncSI (Coetzee et al., 1986), pMG110 (IncHI1; Wolfson et al., 1982), pIN25 (isolated by S. B. Levy; see Bradley & Whelan, 1985), pAr-32 (Bradley et al., 1982b) and R687 (IncD; see Datta, 1979). Strain TPS-2(R687) gave poor clearing in spot tests with phage fd, so the plasmid could not be used in some experiments.

**Media, bacteriophage techniques and mating methods.** Brain Heart Infusion broth or agar (BBL) was used for most cultures. The minimal salts medium used was M9, and drug concentrations were as listed by Bradley (1984).

Methods for phage growth, and the spot test for phage activity, were as described or cited in Bradley (1967). Filamentous phages were grown using strain JE2571 harbouring the appropriate plasmid. Brain Heart Infusion broth was inoculated with bacteria and phage, then grown statically overnight at 37 °C (30 °C for phages tf-1, tf, and pilHa). After removing bacteria by several cycles of centrifugation (filtration removes filamentous phage virions and is unsuitable), the suspension was titrated by spotting 10 μl samples of serial dilutions on a soft agar layer plate of the host strain, and counting plaques after incubation. Mating methods were as described previously (Bradley et al., 1980; Bradley, 1984).

*Electron microscopy.* Techniques were those previously employed (Bradley et al., 1981a, b, c).

**RESULTS**

**Effect of toIQ mutation on infection by plasmid-specific bacteriophages**

As many plasmid-specific bacteriophages as possible, including filamentous, RNA-containing, and lipid-containing types were tested for inhibition of infection by the toIQ mutation. Results obtained with spot tests for lytic activity, together with the locations of the phage adsorption sites on their conjugative pilus receptors, are given in Table 1. In agreement with the results of Sun & Webster (1986), it was found that filamentous phage fd infected the ToIQ+ strain P90C-2(F::Tn5) but not its toIQ derivative TPS-2(F::Tn5), and that RNA-containing phage of QΦ infected both strains. Similar results were obtained with R1drl9 (IncFII) and EDP208 (IncS); the RNA phage F0lac (Bradley et al., 1981c) replaced QΦ for the latter. The toIQ mutation in strain TPS-2(N3) also inhibited infection by the IncN-specific filamentous phage IK (in agreement with Sun & Webster, 1986). Filamentous phages PR64 (specific for I complex plasmids) and X-2 (IncX-specific) were also inhibited. Three other filamentous phages, Pf3, tf-1, and X, together with all non-filamentous plasmids, were not.

**Effect of toIQ mutation on plasmid transfer**

A series of plasmids selected by incompatibility grouping and pilus type was tested for inhibition of transfer from JE2571 or 553 to toIQ strains TPS-1 or -2. The following transfer-derepressed plasmids with surface-obligatory conjugation systems (exceptions R1drl9 and pIN25) were tested by cross-streak mating (Bradley et al., 1980): R1drl9 (IncFII), N3 (IncN), RP4 (IncP), pIN25 (IncT), pAr-32 (IncU), R905 (IncV), Sa (IncW). Quantitative broth matings were used for R453 (IncFII), R478 (IncHI2), MIP233 (H13), R144 (IncI1, +B), R391 (IncJ), F0lac (IncSI) and quantitative plate matings for RIP64 (IncC), R687 (IncD), pMG110 (IncHI1), RIP69 (IncM) and TP228 (IncX). A significantly lower transfer frequency with recipients TPS-1 or -2 compared with recipients P90C-1 or -2 was not detected with any plasmid. The possibility
that $tolQ$ prevented plasmid entry was therefore eliminated. In view of these results, and because of the nature of the conjugation mechanism (Willetts & Wilkins, 1984), it seemed highly unlikely that plasmid exit from $tolQ$ donor strains would be prevented by the mutation. Sun & Webster (1986) found that TPS13 functioned normally as a donor for plasmid F, and their equivalent of TPS-2(N3) for N3. While a complete series of transfer tests from TPS strains was not carried out, a few examples are given in Table 2, where matings were done for another purpose.

### Inhibition of plasmid transfer between $tolQ$ strains by filamentous phages

The effect of various filamentous phages on plasmid transfer frequencies (see Introduction) was assessed by determining the percentage reduction obtained (inhibition) using the plasmid-specific phage with reference to a non-specific filamentous phage control (Table 2). Repressed plasmids (determining few pili) were employed to avoid having too many receptors which might have used up all the phage virions, exceptions being F : : Tn5 (only determined about one pilus per cell) and N3 (pili detach in a liquid; Bradley et al., 1980). In all cases with over 90% inhibition, the pilus receptors were clearly implicated as mating organelles. The 45% inhibition obtained with phage IKe and thick I2 pili was considered inconclusive and probably reflected a poor adsorption efficiency. Frequencies of $<1.0 \times 10^{-4}$ transconjugants per donor h$^{-1}$ were approximate, an example being the negative inhibition obtained with phage X-2 and IncX plasmid TP231. These frequencies were a mean of three matings (two with Tc, one with Cm selection) and indicate no effect on plasmid transfer by phage X-2. The value of $-19\%$ reflects the inaccuracy of mating techniques at low frequencies since it is clear that X-2 could not enhance mating.

**Table 1. Inhibition of plasmid-specific bacteriophage infection by $tolQ$ mutation**

<table>
<thead>
<tr>
<th>Phage</th>
<th>Type</th>
<th>Adsorption site on pili</th>
<th>Plasmid</th>
<th>Inc</th>
<th>Phage sensitivity of plasmid host strain*</th>
<th>Reference for phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>fd</td>
<td>Filamentous</td>
<td>Tip</td>
<td>F: :Tn5</td>
<td>FI</td>
<td>+</td>
<td>Marvin &amp; Hohn (1969)</td>
</tr>
<tr>
<td>Qβ</td>
<td>RNA</td>
<td>Shaft</td>
<td>F: :Tn5</td>
<td>FI</td>
<td>+</td>
<td>Watanabe (1964)</td>
</tr>
<tr>
<td>fd</td>
<td>Filamentous</td>
<td>Tip</td>
<td>R1dr19</td>
<td>FII</td>
<td>+</td>
<td>Marvin &amp; Hohn (1969)</td>
</tr>
<tr>
<td>Qβ</td>
<td>RNA</td>
<td>Shaft</td>
<td>R1dr19</td>
<td>FII</td>
<td>+</td>
<td>Watanabe (1964)</td>
</tr>
<tr>
<td>fd</td>
<td>Filamentous</td>
<td>Tip</td>
<td>EDP208</td>
<td>S</td>
<td>+</td>
<td>Marvin &amp; Hohn (1969)</td>
</tr>
<tr>
<td>Fλ,lac</td>
<td>RNA</td>
<td>Shaft†</td>
<td>EDP208</td>
<td>S</td>
<td>(+)</td>
<td>Bradley et al. (1981c)</td>
</tr>
<tr>
<td>PR64</td>
<td>Filamentous</td>
<td>Tip‡</td>
<td>R163dr7</td>
<td>I1, +B</td>
<td>-</td>
<td>Coetzee et al. (1980)</td>
</tr>
<tr>
<td>Iα</td>
<td>RNA</td>
<td>Shaft‡</td>
<td>R163dr7</td>
<td>I1, +B</td>
<td>+</td>
<td>Coetzee et al. (1982)</td>
</tr>
<tr>
<td>IKe</td>
<td>Filamentous</td>
<td>Tip</td>
<td>N3</td>
<td>N</td>
<td>-</td>
<td>Khatoon et al. (1972)</td>
</tr>
<tr>
<td>PR4</td>
<td>Lipid</td>
<td>Tip</td>
<td>N3</td>
<td>N</td>
<td>+</td>
<td>Stanisch (1974)</td>
</tr>
<tr>
<td>Pf3</td>
<td>Filamentous</td>
<td>Shaft§</td>
<td>RP4</td>
<td>P</td>
<td>+</td>
<td>Stanisch (1974)</td>
</tr>
<tr>
<td>PR4</td>
<td>Lipid</td>
<td>Tip</td>
<td>RP4</td>
<td>P</td>
<td>+</td>
<td>Stanisch (1974)</td>
</tr>
<tr>
<td>tf-1</td>
<td>Filamentous</td>
<td>Shaft†</td>
<td>pIN25</td>
<td>T</td>
<td>+</td>
<td>Coetzee et al. (1987)</td>
</tr>
<tr>
<td>t</td>
<td>RNA</td>
<td>Shaft†</td>
<td>pIN25</td>
<td>T</td>
<td>(+)</td>
<td>Bradley et al. (1981b)</td>
</tr>
<tr>
<td>PR4</td>
<td>Lipid</td>
<td>Tip</td>
<td>Sa</td>
<td>W</td>
<td>+</td>
<td>Stanisch (1974)</td>
</tr>
<tr>
<td>X</td>
<td>Filamentous</td>
<td>Tip</td>
<td>R6K</td>
<td>X</td>
<td>(+)</td>
<td>Bradley et al. (1981a)</td>
</tr>
<tr>
<td>X-2</td>
<td>Filamentous</td>
<td>Unknown§</td>
<td>R6K</td>
<td>X</td>
<td>-</td>
<td>Coetzee et al. (1988)</td>
</tr>
<tr>
<td>pilHz</td>
<td>RNA</td>
<td>Shaft</td>
<td>pMG110</td>
<td>HII</td>
<td>(+)</td>
<td>Coetzee et al. (1985)</td>
</tr>
</tbody>
</table>

* Using the spot test for sensitivity to bacteriophages (see Methods): +, complete or slightly hazy clearing; (+), hazy but definite clearing; -, no visible clearing.
† Adsorption limited to the tapered region of the pilus shaft near the pointed tip; virions do not attach to the point itself.
‡ IncI1, + B plasmids encode thin flexible and thick rigid pili (Bradley, 1984). Phages PR64 (= PR64FS) and Iα adsorb to the thin pili, which stabilize mating aggregates, thick pili mediating DNA transfer.
§ The adsorption site of Pf3 on the P pilus was not known so was determined for this study as being on the shaft (sides) of the pilus (see Results). Attempts to visualize phage X-2 virions attached to X pili were unsuccessful (Coetzee et al., 1988).
Table 2. Plasmid transfer from TPS-2 in the presence of tip-adsorbing filamentous phages

| Test phage | Control phage | Pilus type* | Donor plasmid | Selection† | Transfer frequency‡ (titre p.f.u. ml⁻¹) | Inhibition§ (%)
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>fd (10⁻²)</td>
<td>IKe (10⁶)</td>
<td>F</td>
<td>F::Tn5</td>
<td>Km</td>
<td>1.0 x 10⁻³, 6.1 x 10⁻²</td>
<td>98</td>
</tr>
<tr>
<td>fd (10⁻²)</td>
<td>IKe (10⁹)</td>
<td>D</td>
<td>R6871</td>
<td>Km</td>
<td>2.2 x 10⁻⁶, 2.6 x 10⁻⁵</td>
<td>92</td>
</tr>
<tr>
<td>fd (10⁻²)</td>
<td>IKe (10⁹)</td>
<td>S</td>
<td>Fₙlac</td>
<td>Lac</td>
<td>4.9 x 10⁻⁸, 1.4 x 10⁻⁶</td>
<td>97</td>
</tr>
<tr>
<td>PR64 (10⁹)</td>
<td>fd (10⁻²)</td>
<td>Iₕf</td>
<td>R144</td>
<td>Km</td>
<td>&lt;6.9 x 10⁻⁸, 1.3 x 10⁻⁵</td>
<td>&gt;100</td>
</tr>
<tr>
<td>PR64 (10⁹)</td>
<td>fd (10⁻²)</td>
<td>Iₕf</td>
<td>R721</td>
<td>Tp</td>
<td>&lt;4.3 x 10⁻⁸, 8.3 x 10⁻⁵</td>
<td>&gt;100</td>
</tr>
<tr>
<td>IKe (10⁹)</td>
<td>fd (10⁻²)</td>
<td>Iₐr</td>
<td>R721</td>
<td>Tp</td>
<td>6.1 x 10⁻⁵, 1.1 x 10⁻⁴</td>
<td>45</td>
</tr>
<tr>
<td>IKe (10⁹)</td>
<td>fd (10⁻²)</td>
<td>N</td>
<td>N3</td>
<td>Tc</td>
<td>1.1 x 10⁻⁵, 3.6 x 10⁻⁴</td>
<td>97</td>
</tr>
<tr>
<td>X-2 (10⁻¹)</td>
<td>fd (10⁻²)</td>
<td>X</td>
<td>TP231</td>
<td>Tc, Cm</td>
<td>3.1 x 10⁻⁴, 2.6 x 10⁻⁵</td>
<td>-19</td>
</tr>
</tbody>
</table>

* Correspond to incompatibility groups F, D, S, N, X and I complex. The latter plasmids determine both thin flexible (f) and rigid (r) pili (Bradley, 1984).
† Plasmid selection: Cm, chloramphenicol; Km, kanamycin; Lac, lactose (M9 medium); Tc, tetracycline; Tp, trimethoprim. For concentrations see Bradley (1984).
‡ Transconjugants per donor h⁻¹. The test phage was specific for the pilus type indicated, whereas the control phage was not. Both were made similarly from strain JE2571 carrying the appropriate plasmids. Where no transfer could be detected, a ‘<‘ frequency was calculated assuming one colony on plates at the lowest dilution used (10⁻¹).
§ Frequency with the test phage expressed as a percentage of that with the control phage and subtracted from 100%. For an explanation of the single negative inhibition found, see text.

TPS-2 (R687) was resistant to phage fd although R687 normally confers sensitivity to K12 strains (D. E. Bradley, unpublished). Transfer frequencies were approximate due to the instability of IncD plasmids in E. coli K12. TPS-2 (R687) was resistant to phage fd although R687 normally confers sensitivity to K12 strains (D. E. Bradley, unpublished). Transfer frequencies were approximate due to the instability of IncD plasmids in E. coli K12.

N3 is a derepressed surface-mating plasmid (Bradley et al., 1980) not inhibited for transfer by nalidixic acid. Surface mating occurred here on transconjugant-selecting plates at the low dilutions required to detect transfer frequencies in a liquid environment. Transconjugant colonies from a liquid mating are much larger than those from subsequent plate mating; the latter originate from bacteria having a shorter time to express drug resistance, and then to grow into colonies (D. E. Bradley, unpublished). Frequencies were obtained by counting the distinctive larger colonies.

Adsorption site of filamentous phage Pf3

The only filamentous phage whose adsorption site on conjugative pili had not been previously studied was Pf3 (specific for IncP plasmids; Stanisich, 1974). Its infection was not inhibited by the mutation in tolQ. Electron microscopy showed that it attached along the pilus shaft (Fig. 1) and was not specific for the tip or any area close to it, as was the case for tf-1 (Coetzee et al., 1987). This explained why the tolQ mutation did not affect it.

DISCUSSION

It is clear that the tolQ mutation only inhibits infection by tip-adsorbing, not shaft-adsorbing filamentous phages, an exception being phage X. This virus appears to adsorb precisely at the pilus tip (Bradley et al., 1981a) and yet is not inhibited; only single virions are attached at this locus, multiple adsorption being expected if attachment is proximal from the tip. Phage X has a very wide plasmid specificity range, but it is not known whether this is connected with its insensitivity to the tolQ effect. Filamentous phage X-2 (IncX-specific), whose receptor was not identifiable due to poor adsorption (Coetzee et al., 1988), was also inhibited by tolQ; because of this, it seems very likely that it attaches to the tips of X pili. The inhibition of phage PR64 infection by tolQ is noteworthy. PR64 adsorbs to the tips of thin I₁ or thin I₂ pili, which act as ‘grappling hooks’ in conjugation, forming stable mating aggregates in a liquid. Cell-to-cell contact between outer membranes together with plasmid DNA transfer is believed to be mediated by rigid pili, the I₂ type being phage IKe receptors (see Bradley, 1984). Inhibition of
phage PR64 infection suggests that these thin flexible pili may retract like thick flexible pili (see below).

The morphological type of pilus receptor, whether thick flexible (F, S, X), thin flexible (I complex), or rigid (N), does not affect filamentous phage inhibition. Possibly, after a filamentous phage has adsorbed precisely to the conjugative pilus tip and the pilus has retracted, the virion is pulled through a molecular assembly in the outer membrane as if it was an additional part of the pilus. After the capsid is removed, probably in the periplasm, the DNA is further processed through the inner membrane by the tolQRAB gene products. We suggest that, unless the phage attaches exactly at the pilus tip, it cannot be pulled through the outer membrane, so that a different transport mechanism would be required for the DNA of shaft-adsorbing filamentous phages. Clearly phages with a larger profile like those containing RNA or lipid could not be pulled through the outer membrane either; indeed their modes of infection are quite different (Bradley, 1972, 1978; Silverman & Valentine, 1969). The function of the tolQRAB gene products seems to be limited at present to the transport of tip-adsorbing filamentous phage DNA and E. coli colicins across the bacterial envelope. The transport mechanism and natural function of the gene cluster might be easier to elucidate if other affected macromolecules could be identified.

We have used tolQ TPS strains as plasmid hosts to demonstrate mating inhibition by filamentous phages that adsorb to conjugative pilus tips. The pilus types that we have implicated as mating organelles in this way are D, S, thin I₁, thin I₂, and N. The F pilus acted as a control since it has been tested in similar experiments (Ippen & Valentine, 1967; Novotny et al., 1968). Other pili have been shown to be involved in conjugation using several different agents for specifically inhibiting mating. They are thin I₁ (anti-pilus serum; Harden & Meynell, 1972), W (inactivated lipid-phage; Bradley, 1978), the H13 pili of plasmid MIP233 (anti-pilus serum; Bradley, 1986) and N and P pili (concentrated pilus preparations blocking receptors on recipient cells; Bradley & Chaudhuri, 1980). We have thus confirmed the roles of thin I₁ and N pili, and added D, S, and thin I₂ pili.

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