pHH502, a Plasmid with IncP and IncI α Characters, Loses the Latter by a Specific *recA*-independent Deletion Event

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Plasmid pHH502, of molecular weight 70×10^6 , determined resistance to tetracycline, chloramphenicol, trimethoprim, sulphonamides and mercuric chloride and was incompatible with members of IncP and IncI α . It resembled other plasmids of IncI α in the following properties: it determined pili that were morphologically and serologically I α pili, whose production was repressed in established plasmid-carrying (R⁺) cultures; its transfer was equally efficient in liquid or on solid medium; it exerted surface exclusion against other IncI α plasmids; it was non-transferable to *Proteus*. In a reproducible, *recA*-independent event, pHH502 gave rise to pHH502-1, a plasmid of molecular weight 40×10^6 , lacking determinants for resistance to tetracycline and chloramphenicol and all detectable IncI α characteristics. pHH502-1 was incompatible only with IncP plasmids and resembled other IncP plasmids in determining constitutive production of rigid pili, in its surface exclusion, in transferring at greater frequency on solid than in liquid medium and in being transmissible to *Proteus mirabilis*. It differed from other IncP plasmids in the morphology and serological type of its pili and in failing to transfer to *Pseudomonas aeruginosa* or *Acinetobacter calcoaceticus*. Small numbers of pHH502-1 rigid pili were present on bacteria carrying pHH502. Possible mechanisms for the generation of pHH502 and pHH502-1 are discussed.

INTRODUCTION

pHH502, a plasmid of molecular weight 70×10^6 conferring resistance to tetracycline, chloramphenicol and trimethoprim, was previously shown to be incompatible with RP4 and therefore classified as belonging to incompatibility group P (IncP) (Datta *et al.*, 1979*b*). Classification of plasmids into incompatibility (Inc) groups is based on the inability of closely related plasmids to co-exist stably in a growing bacterial cell line, i.e. they are incompatible. Compatible plasmids can and do thus co-exist. Many plasmids can be grouped unequivocally by compatibility testing and it is generally found that conjugative plasmids within an Inc group determine pili of the same morphological and serological type, while plasmids of different groups determine different pili (Bradley, 1980*a, b*). Plasmids of IncP are recognized to have a broad host range and may be carried by aerobic bacteria of most, perhaps all, Gram-negative genera (Datta & Hedges, 1972; Olsen & Shipley, 1973).

Plasmids incompatible with members of IncP and IncI α have been described by Grant & Pittard (1974) and Grant *et al.* (1980). In re-examining pHH502 we confirmed its incompatibility with RP4 and other IncP plasmids and found that it was also incompatible with R144, a prototype of IncI α (Jacob *et al.*, 1977). We also found that pHH502 regularly loses the genetic

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determinants for tetracycline and chloramphenicol resistance and all detectable IncI α characters, undergoing loss of about 30×10^6 of DNA to give plasmid pHH502-1. pHH502 was previously recorded as being compatible with plasmids of all Inc groups other than IncP (Datta *et al.*, 1979*b*). We think this error resulted from using trimethoprim-resistance as the marker for pHH502, so that the compatibility reactions of pHH502-1 were sometimes recorded rather than those of pHH502.

We describe here the deletion event and the incompatibility, surface exclusion and conjugative properties of pHH502 and its variant, pHH502-1. The observations cannot be explained on the basis of known examples of illegitimate recombination.

METHODS

Bacteria and plasmids. Bacterial strains, plasmids and phages used are given in Table 1.

Culture media. These were as described by Datta *et al.* (1980).

Transfer of plasmids by conjugation. (a) Transfer between lines of *E. coli* K12. Plasmids were tested for transfer in broth or on plates (Bradley *et al.*, 1980) or by a cross-streaking method (Barth, 1979). In comparing frequencies of transfer in liquid and on solid media, the method of Bradley *et al.* (1980) was used slightly modified as follows. Donor and recipient strains were J62-2 and J53-1, respectively; after incubation, mixtures from broth or nutrient agar were diluted 100-fold and treated with a vortex mixer to prevent further conjugation. Dilutions were plated on minimal salts agar with appropriate nutrients and antibiotics to provide viable counts of donors, recipients and transconjugants.

(b) Transfer from *E. coli* K12 to other genera. Methods were as above. If no transfer was detected, an additional surface method was used: 0.25 ml of donor and recipient cultures, each at approximately 2×10^9 cells ml⁻¹, were mixed on a filter paper (Schleicher and Schüll, type 595) on nutrient agar. After overnight incubation at 37 °C, the cells were resuspended from the filter and plated on selective medium for the isolation of transconjugants.

Surface exclusion. The frequency of plasmid transfer by conjugation to *E. coli* K12 R⁻ and, in parallel, to the same strain carrying a plasmid was measured. The donor culture was the same for both matings, the recipients were cultured in parallel and their densities adjusted to equality if necessary. If the transfer frequency was reduced by the presence of a plasmid in the recipient, it was taken as evidence for surface exclusion by that plasmid.

Compatibility tests. Plasmids were tested for their ability to co-exist with members of all known incompatibility (Inc) groups. The method was to introduce a standard plasmid into the strain carrying the plasmid under test, selecting only for a resistance marker of the incoming plasmid. In the same experiment, selection was made for transfer of the unclassified plasmid into the strain carrying the standard plasmid. Incompatibility was recognized by elimination of the resident plasmid from all clones tested. If 'doubles' carrying both plasmids were obtained, the stability of the plasmid combination was tested when the clone was grown in drug-free broth through at least 20 generations, cell density being kept low to prevent reinfection. Instability indicated incompatibility. Compatibility was scored when the two plasmids co-existed stably during growth in drug-free broth, their separate identities being shown by separate transmissibility to another K12 strain, or sometimes by their separate identification in agarose gels after electrophoresis of lysates.

Isolation of plasmid DNA and restriction enzyme analysis. Two small-scale methods were used to prepare DNA for determining plasmid molecular weights (mol. wt) by agarose gel electrophoresis, the single colony lysis method of Eckhardt (1978) and that described by Broome-Smith (1980). Plasmid DNA for restriction enzyme digestion was isolated by the method of Clewell & Helinski (1969). Restriction enzymes were obtained from BRL and digestion conditions were as described by them. Gel electrophoresis of restriction endonuclease generated fragments was as described by Datta *et al.* (1980).

Nick translations and Southern hybridization. Four DNA preparations of phage λ , each carrying an insertion sequence, IS1, IS2, IS3 or IS4 were provided by Dr N. J. Grinter who obtained the λ -carrying strains from P. L. Bergquist. ³²P-labelled 'probes' of λ IS1, λ IS2, λ IS3 and λ IS4 were prepared by the method of Rigby *et al.* (1977). Hybridization of ³²P-labelled 'probes' with DNA fragments immobilized on cellulose nitrate filters was carried out as described by Southern (1975).

Identification of plasmid-determined pili. Pili were examined by electron microscopy, with and without the addition of pilus-specific antiserum, by the methods described by Bradley (1980*a*). Pilus-specific phages used were If1, which adsorbs to the tips of I α pili (Meynell & Lawn, 1968), I α , which adsorbs to their sides (J. N. Coetzee, unpublished), PR4 and PRD1, which adsorb to the tips of P, N or W pili (Bradley, 1976, 1979; Bradley & Cohen, 1977), and PRR1, which adsorbs to the sides of P pili (Bradley, 1974).

Propagation of phage If1 was demonstrated by growing a test culture, and positive and negative control cultures, overnight in nutrient broth in the presence of phage at low titre (approximately 10^4 p.f.u. ml⁻¹), then centrifuging and titrating the phage in the supernatants. 'Spot' tests for phage sensitivity were made by placing a drop of high-titre phage preparation on a soft-agar overlay containing the test culture.

Table 1. *Bacteria and plasmids*

Bacterial strain	Genotype or phenotype	Reference or source
<i>Salmonella typhimurium</i> LT2 M329	<i>trp</i>	G. Meynell
<i>Enterobacter cloacae</i> D1786	prototrophic	V. M. Hughes
<i>Klebsiella aerogenes</i> K21 D1739B	prototrophic	V. M. Hughes
<i>Proteus mirabilis</i> PM13	<i>nad</i>	Coetzee (1972)
<i>Providencia stuartii</i> P29	<i>nad</i>	Coetzee (1972)
<i>Serratia marcescens</i>	prototrophic	Falkow <i>et al.</i> (1966)
<i>Pseudomonas aeruginosa</i> 280	<i>met rif</i> ⁺	Bryan <i>et al.</i> (1972)
<i>Acinetobacter calcoaceticus</i> EBF 65/65 C4161	<i>phe thi</i>	Hinchliffe & Vivian (1980)
<i>Escherichia coli</i> K12 strains:		
J53-1	<i>pro met nal</i>	Coetzee <i>et al.</i> (1972)
J53-2	<i>pro met rif</i> ⁺	Coetzee <i>et al.</i> (1972)
J62-2	<i>pro his trp</i>	Coetzee <i>et al.</i> (1972)
PB1150	<i>his recA rif</i> ⁺	Bergquist <i>et al.</i> (1982)
JE2571	<i>leu thr fla pil</i>	Bradley (1980 <i>a</i>)

Plasmid*	Resistance characters†	Inc group	Reference
RP4	ApTcKm	P	Jacob <i>et al.</i> (1977)
R702	SmTcKmSu	P	Jacob <i>et al.</i> (1977)
R144	TcKmCollb	Ia	Jacob <i>et al.</i> (1977)
R64	SmTc	Ia	Jacob <i>et al.</i> (1977)
R300B	SmSu	Q	Jacob <i>et al.</i> (1977)
pHH502‡	TcCmTpHg[Su]	Ia and P	Datta <i>et al.</i> (1979 <i>b</i>)
pHH502-1	TpHg[Su]	P	This paper

* In addition to those listed here, examples were used of plasmids in each Inc group listed by Jacob *et al.* (1977) and Bradley (1980*a*). Standard reference plasmids for gel analysis were: RA1, mol. wt 86×10^6 ; R1, mol. wt 62×10^6 ; R702, mol. wt 46×10^6 and RP4, mol. wt 36×10^6 (Jacob *et al.*, 1977).

† Resistance characters: Ap, ampicillin; Sm, streptomycin; Tc, tetracycline; Cm, chloramphenicol; Km, kanamycin; Su, sulphonamides; Tp, trimethoprim; Hg, mercuric chloride.

‡ pHH502 was reported (Datta *et al.*, 1979*b*) as conferring resistance only to tetracycline, chloramphenicol and trimethoprim. It confers resistance also to mercuric chloride and sulphonamides. Expression of the latter is easily overlooked in disc tests: a disc containing 200 µg or 500 µg sulphafurazole, on a plate spread with a diluted culture of a pHH502⁺ strain, produced, after 18 h incubation, a zone of inhibition equal in size to that of an R⁻ control. Within the zone there appeared a variable number of colonies that increased in both size and number with further incubation. This was not the case with the R⁻ control, nor with plasmids, such as R751, that determine trimethoprim but not sulphonamide resistance. This kind of sulphonamide resistance, indicated as [Su] above, was described by Datta *et al.* (1979*a*) but has not been investigated.

RESULTS

Break-down of pHH502

Strains J53-1(pHH502) and PB1150(pHH502) *recA*, were plated to give single colonies on nutrient agar containing 10 mg tetracycline l⁻¹. Five colonies of each strain were shown, by the single colony lysis method, to contain a single plasmid species of mol. wt 70×10^6 . Single colonies of each strain were then grown overnight without selection in 10 ml nutrient broth at 37 °C. The cultures were diluted and plated on nutrient agar and the resulting colonies were streaked across plates with three ditches containing, respectively, chloramphenicol, tetracycline and trimethoprim, to look for any loss of resistance. Of 460 colonies of J53-1(pHH502), 30 were resistant to trimethoprim only and 430 to trimethoprim, tetracycline and chloramphenicol. Similar results were obtained for PB1150(pHH502): 31 colonies were resistant to trimethoprim only and 425 to all three antibiotics.

Eight colonies resistant to trimethoprim, and sensitive to tetracycline and chloramphenicol, were investigated further. A single plasmid of mol. wt 40×10^6 was demonstrated in each. Plasmid DNA from these eight cultures was digested with *EcoRI* and the resulting fragments compared with *EcoRI* digests of pHH502. All eight gave the same sized fragments and four fragment sizes of 11.5×10^6 , 3.2×10^6 , 2.8×10^6 and 0.85×10^6 , were the same as four of

Table 2. *Digests of pHH502 and pHH502-1 with EcoRI*

Purified pHH502 and pHH502-1 DNA was digested with *EcoRI* and electrophoresed on a 0.7% (w/v) agarose slab gel for 3 h at 150V constant voltage. An *EcoRI* digest of plasmid R1, run in parallel, was used for calibration; (for the mol. wts of R1 fragments see Blohm & Goebel, 1978).

10 ⁻⁶ × Fragment mol. wt*	
pHH502	pHH502-1
11.5	11.5 (× 2)†
7.8	
7.2	
6.0	
5.1	
4.7	
	4.5
	4.4
3.9	
3.5	
3.2 (× 2)†	3.2
2.8	2.8
2.05	2.1
1.85	
1.75 (× 2)†	
1.45	
1.35	
1.20	
0.85	0.85
Total 71.35	40.85

* Using the present electrophoresis conditions, molecular weights can be determined to within a value of 0.05 × 10⁶ for fragments up to a size of approximately 2.1 × 10⁶.

† There are two fragments of this mol. wt as shown by the intensity of staining with ethidium bromide.

pHH502. Since all eight variants appeared identical, the 40 × 10⁶ mol. wt plasmid was designated pHH502-1. It determined mercuric chloride resistance and the same type of sulphonamide resistance as pHH502 (Table 1). The restriction enzyme patterns of pHH502 and pHH502-1 are shown in Table 2. The second fragment of 11.5 × 10⁶ in pHH502-1 must have resulted from deletion of one of the *EcoRI* cut sites of pHH502. The additional appearance of fragments with mol. wts 4.5 × 10⁶ and 4.4 × 10⁶ in pHH502-1, not present in pHH502, cannot then be explained on the basis of there having been deletion of a single arc of the pHH502 circumference; a second deletion or other rearrangement of DNA must have occurred.

Detection of known insertion sequences in pHH502-1

Agarose gel electrophoresis of *EcoRI* digestions of pHH502-1 and λ IS1, λ IS2, λ IS3 and λ IS4 DNA was carried out and the fragments were transferred to cellulose nitrate filters. The filters were tested with ³²P-labelled 'probes' of λ IS1, λ IS2, λ IS3 and λ IS4 and the resulting autoradiograms were examined for evidence of homology between the probe and the digest fragments. Although there was strong binding of probe to λ IS DNA, no homology was seen with pHH502-1 DNA.

Properties of pHH502 and pHH502-1

Incompatibility. Incompatibility of pHH502 with RP4 (Datta *et al.*, 1979*b*) was confirmed and pHH502-1 was also incompatible with RP4. The transfer of pHH502 or pHH502-1 led to elimination of resident RP4 from a high proportion of clones. Transfer of RP4, however, did not eliminate resident pHH502 or pHH502-1. Many clones were isolated in which both plasmids were present and their carriage was always unstable (Table 3). pHH502 and pHH502-1 showed the same relationships to R702 as to RP4 (results not shown).

pHH502 was also incompatible with the IncIα plasmid R144. The resident plasmid was lost with approximately the same frequency regardless of the direction of transfer but when 'doubles'

Table 3. *Incompatibility properties of pHH502 and pHH502-1 with RP4 and R144*

Chloramphenicol was used for selecting pHH502 and trimethoprim for pHH502-1.

Donor strain	Recipient strain	Markers lost*	
		Initially	During stability tests on doubles
J53-1(RP4)	J62-2(pHH502)	0	CmTp (7) ApKm (30)
J62-2(pHH502)	J53-1(RP4)	ApKm (25)	ApKm (100)
J53-1(RP4)	J62-2(pHH502-1)	0	Tp (60) ApKm (40)
J62-2(pHH502-1)	J53-1(RP4)	ApKm (75)	ApKm (93) Km† (7)
J53-1(R144)	J62-2(pHH502)	CmTp (69)	CmTp (100)
J62-2(pHH502)	J53-1(R144)	Km (66)	CmTp (100)
J53-1(R144)	J62-2(pHH502-1)	0	0
J62-2(pHH502-1)	J53-1(R144)	0	0

* Values are the percentage of colonies tested.

† Ampicillin resistance was not transferable from these clones and we attribute this result to transposition of Tn1 into the chromosome.

Table 4. *Transfer frequencies of pHH502 and pHH502-1 between strains of E. coli K12*

Donor host strains were J62-2, recipient was J53-1. Frequencies were calculated from viable counts at the end of the 1 h incubation period. Donor and recipient counts were approximately equal after incubation in broth or on plates.

	Transfer frequency per donor cell in 1 h		Ratio agar/broth
	In broth	On nutrient agar	
pHH502	4.5×10^{-4}	4.9×10^{-4}	1.1
pHH502-1	5.9×10^{-4}	1.4×10^{-1}	237

were tested for stability it was always the markers of pHH502 that were lost (Table 3). No incompatibility was seen between R144 and pHH502-1.

Surface exclusion. Surface exclusion of RP4 (*IncP*) and R144 (*IncI α*) by pHH502 and pHH502-1 was measured. The presence of pHH502 in the recipient strain reduced R144 transfer frequency 100-fold, and had no effect on RP4 transfer. A 100-fold reduction in RP4 transfer frequency was observed when the recipient strain carried pHH502-1, while the transfer of R144 was not significantly altered.

Transfer by conjugation between K12 strains. pHH502 transferred at approximately equal frequencies in broth and on nutrient agar; frequency of transfer of pHH502-1 was at least 200 times higher on agar than in broth (Table 4).

Host ranges. In transmissibility to the strains of various genera listed in Table 1, pHH502 and pHH502-1 differed from one another and from plasmids RP4 and R751, of *IncP*, used as donor controls.

Both pHH502 and pHH502-1 transferred to the strains of *Salmonella typhimurium*, *Enterobacter cloacae* and *Klebsiella aerogenes*. pHH502-1, but not pHH502, transferred also to *Proteus mirabilis*, *Providencia stuartii* and *Serratia marcescens* as shown by the acquisition of resistance to trimethoprim and mercuric chloride and the appearance of the 40×10^6 plasmid band in lysates.

The *IncI α* plasmids R144 and R64, unlike members of many plasmid groups, appear to be non-transmissible by conjugation from *E. coli* K12 to *Proteus* or *Providencia* species. They are, however, able to mediate conjugative transfer of plasmid R300B to *Proteus mirabilis* (Datta &

Fig. 1. (a) RNA bacteriophage Ix adsorbed to an aggregate of thin flexible pili synthesized by bacteria newly infected with pHH502, natural repression of pilus synthesis being temporarily lifted; (b) short rigid pili synthesized by JE2571(pHH502-1); (c) part of an aggregate of pili synthesized by JE2571(pHH502) reacted with antiserum to R64*drd*-11 pili (IncI α) by the method of Lawn (1967), showing heavy antibody adsorption. The bar markers represent 100 nm.

Table 5. *Phage-sensitivity determined by pHH502 and pHH502-1*

Plasmid in <i>E. coli</i> JE2571	Phage sensitivity indicated by:					Propagation If1
	Spot lysis					
	PR4	PRD1	PRR1	I α *	If1*	
pHH502	—	—	—	+	+	+
pHH502-1	+	+	—	—	—	—

* Spot lysis of JE2571(pHH502) was only visible with newly-infected cells showing temporary depression of pilus synthesis (Bradley, unpublished). Propagation of If1, i.e. phage titres increased $\times 10^3$, was observed with established R⁺ cultures.

Hedges, 1972). pHH502 behaved like R144 and R64 in these respects. J53(pHH502) was grown in nutrient broth with tetracycline (10 mg l⁻¹) to prevent breakdown to pHH502-1, and mixed in broth with *Proteus mirabilis* PM13. No plasmid transfer was demonstrated using any selective agent. When the donor culture was J53(R300B)(pHH502), transfer of R300B occurred; the transferred plasmid expressed its resistance genes and was visible in lysates of the recipient *Proteus mirabilis* in gel electrophoresis.

Many attempts were made to demonstrate transfer of pHH502 or pHH502-1 to *Pseudomonas aeruginosa*, but none succeeded, though from the numbers of bacteria in the test mixtures, a frequency of 10⁻⁸ should have been detectable. R751, tested in parallel, was transferred at frequencies between 10⁻⁴ and 10⁻⁵ per donor cell. RP4 and R751, but not pHH502 or pHH502-1, were transferable to *Acinetobacter calcoaceticus*.

Pili determined by pHH502 and pHH502-1. In the electron microscope, JE2571(pHH502) showed two types of pilus, one thin, about 6 nm across, and flexible (Fig. 1*a*), and the other thick, about 10 nm across, and rigid (Fig. 1*b*), both in small numbers. The numbers of flexible pili were greatly increased (Fig. 1*a*) in newly infected cells obtained by the method of Bradley (1980*b*), indicating that the normal repression of pilus synthesis was temporarily lifted after transfer, as occurred with plasmids R64 or R144 of *IncI α* (Meynell & Datta, 1967). No parallel increase in the numbers of rigid pili was observed. The thin flexible pili reacted with antiserum raised against the I α pili of R64*drd-11* (Fig. 1*c*) and acted as receptors for phage I α (Fig. 1*a*), which contains RNA and attaches specifically to I α pili (J. N. Coetzee, personal communication).

JE2571(pHH502-1) synthesized the rigid pili alone, not the flexible ones. Unlike cultures carrying pHH502, the pili were synthesized constitutively (in large numbers). They were morphologically different from the P pili determined by RP4 in that they were thicker and they did not aggregate like P pili. Pili of pHH502-1 showed no reaction with antisera raised against P pili (not illustrated), or rigid pili of any other type (*Inc* groups M, N, U, W, or the single plasmid R775), nor did they react with antiserum to I α pili.

The sensitivity of cultures carrying pHH502 or pHH502-1 to pilus-specific phages is shown in Table 5. The reactions determined by pHH502 were those of a typical I α plasmid, the sensitivity to phages I α and If1 being much greater when repression of pilus synthesis was temporarily lifted after transfer of the plasmid to a new host (D. E. Bradley, unpublished). No lysis by PR4, PRD1, or PRR1 of pHH502-carrying cultures was seen. pHH502-1 gave no sensitivity to the I-pilus-specific phages. It determined sensitivity to PR4 and PRD1 but not to PRR1. All these phages lyse bacteria carrying *IncP* plasmids, but PR4 and PRD1 are less specific than PRR1, being able to lyse, in addition, strains carrying plasmids of *IncN* or *IncW* (Bradley & Rutherford, 1975).

DISCUSSION

When plasmids are classified into incompatibility (*Inc*) groups, it is generally true that within each group, they have conjugative pili of the same serotype (Bradley, 1980*a*) and, to a large extent, homologous polynucleotide sequences (Grindley *et al.*, 1973; Falkow *et al.*, 1974; Roussel

& Chabbert, 1978) indicating that each group has evolved separately. Plasmid pHH502 has dual incompatibility, being incompatible with plasmids of IncI α and IncP. Several examples of naturally-occurring plasmids with dual incompatibility have been reported (Grant & Pittard, 1974; Grant *et al.*, 1980; Monti-Bragadin & Samer, 1975; Kontomichalou *et al.*, 1976; Nugent & Hedges, 1979; Taylor *et al.*, 1981). In molecular weight, host range and incompatibility pHH502 resembled plasmids pMU700–pMU707 described by Grant *et al.* (1980). However, the antibiotic resistance markers were different and there were differences in detailed results of incompatibility testing, for example, RP4 was preferentially lost from 'doubles' with pHH502, the reverse was true with the pMU plasmids. More significant, perhaps, variants of the pMU plasmids, e.g. pMU700-1, were still IncI and no longer IncP, whereas the pHH502 variant, pHH502-1, was incompatible only with IncP, not with IncI α plasmids nor with those of any other known group.

The breakdown of pHH502 to give pHH502-1 was a specific event that occurred with the same frequency and yielded the same end product in Rec⁺ or *recA* strains. The dissociation of the IncFII plasmids, R100, R1 and R6, in Rec⁺ strains into separate molecules, termed RTF and R-determinants, results from recombination between the two IS1 sequences on those plasmids; the separated molecules each contain IS1 (Rownd & Mickel, 1971; Hu *et al.*, 1975; Ptashne & Cohen, 1975). The mechanism of breakdown of pHH502 is unknown, but our results indicate that there are no copies of IS1, IS2, IS3 or IS4 in pHH502-1. Another, as yet uncharacterized transposable sequence may be involved. It has recently been found that pHH502 and pHH502-1 both carry a trimethoprim-resistance transposon, Tn735, of mol. wt 9.85×10^6 and containing three internal *Eco*RI fragments of mol. wts 3.2×10^6 , 2.8×10^6 and 0.85×10^6 (M. E. Nugent, unpublished). 'Illegitimate' recombination, i.e. not resulting from reciprocal exchange, between unrelated plasmids can be mediated by insertion sequences or transposons which, in bringing about their own transposition, link two unrelated replicons into a cointegrate structure. In the transposition process, the cointegrate is then resolved into its two components, differing from the parent replicons in that the transposing sequence is now present in both instead of in one. It may be that pHH502 resulted from cointegration of an IncI α and an IncP plasmid in an abortive transposition event. The breakdown of pHH502 to pHH502-1 perhaps represents what would normally be the resolution of the cointegrate into its two components, but in this case it is only the IncP moiety that survives. We have never obtained from pHH502 an IncI α plasmid without the IncP character. *RecA*-independent deletions or duplications of DNA can occur from the margins of insertion sequences or transposons, extending variable distances into vector replicons (Kleckner, 1981); such effects could not explain the regularity of the event that generates pHH502-1.

pHH502 behaved like a typical IncI α plasmid not only in its incompatibility with members of that group, but also in its conjugative functions. It differed from other IncI α plasmids in its IncP incompatibility property and in determining another class of pilus in addition to the I type, rigid pili present in small numbers.

The variant of pHH502, pHH502-1 had no detectable IncI characteristics but was in many respects a typical IncP plasmid. Most of the IncP-like features of pHH502-1, other than incompatibility, were unexpressed in pHH502. We suppose that the full expression of its pilus, conjugation, surface exclusion and phage-sensitivity determinants had suffered mutation as a result of insertion of DNA with the IncI α determinants of pHH502, the effect being reversed when the IncI α characters were lost.

pHH502-1 differed from other IncP plasmids in the morphology and serotype of its pili. The different structure of its pili presumably accounted for the insensitivity of its host bacteria to phage PRR1, whose attachment site is the sides of P pili. Phages PR4 and PRD1, which lysed bacteria carrying pHH502-1, attach to the tips of several different kinds of conjugative pili (Bradley, 1976, 1979; Bradley & Cohen, 1977) and are thus less specific. The host range of pHH502-1 differed from that of other IncP plasmids. It was not transmissible to strains of *Pseudomonas aeruginosa* or *Acinetobacter calcoaceticus*.

A broad host range, shown by transmissibility to, and maintenance in bacteria of all genera within and many outside the family *Enterobacteriaceae*, is considered characteristic of IncP

plasmids. It has been most thoroughly demonstrated with the interrelated plasmids, RP1, RP4 and RK2 (Datta & Hedges, 1972; Olsen & Shipley, 1973; Meyer, 1979) and observations on other members of the incompatibility group support the generalization (Beringer, 1974; Stanisich & Ortiz, 1976; K. Ellis, unpublished). However, the *IncP* plasmid, pAV1, behaved differently, being transmissible only between strains of *Acinetobacter calcoaceticus* (Hinchliffe & Vivian, 1980); pHH502-1 is another exception, with a host range resembling that of plasmids of *IncFII* (Harada *et al.*, 1960; Datta & Hedges, 1972). It is possible that pHH502-1 was derived from a plasmid with broader host range, determinants required for that property being lost as the result of the changes in plasmid DNA that generated first pHH502 and subsequently pHH502-1.

Incompatibility grouping of plasmids is an attempt at taxonomic classification and is useful in ecological and epidemiological studies. Assignment of a new plasmid to an incompatibility group, though providing a pointer to its likely pilus type and host range properties, is not, as illustrated by pHH502-1, sufficient evidence for them. The appearance of apparently recombinant plasmids, such as pHH502, is perhaps to be expected in view of the ubiquity in bacteria of elements that can bring about recombination between unrelated DNA sequences. When a plasmid is classified by its incompatibility properties, it can evidently not be assigned to any one group until it has been tested with members of all groups.

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