Plasmids Determining I Pili Constitute a Compatibility Complex

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SUMMARY

Plasmids which determine I pili were subdivided by their compatibility properties. The original examples, R64, R144 and Col Ib-P9, together with others more recently isolated, constituted one group, termed I α . Another group, I β , was compatible with I α plasmids. The I β group was represented by some newly observed trimethoprim-resistance factors. A group incompatible with both of these (possibly the ancestral form) was designated I ω . Yet another group, designated I γ was compatible with all the above plasmids, and with members of all other known compatibility groups. Its exemplar repressed piliation by some, but not all, mutant I α plasmids derepressed for pilus synthesis. Exclusion was not necessarily correlated with incompatibility.

INTRODUCTION

Colicin factor Col Ib-P9 and certain *fi*⁻ R factors (e.g. R64, R144) are self-transmissible and determine the production of sex pili (I pili) which are related by serological and phage-adsorption criteria (Meynell & Datta, 1967; Meynell & Lawn, 1967; Meynell & Lawn, 1968). R factors R64 and R144 are incompatible with one another and with Col Ib-P9. They thus belong to a single compatibility group, I (Datta & Hedges, 1971).

In early work on classification of R factors, it was shown that in a high proportion of cases, transfer to *Escherichia coli* K12 of antibiotic resistance from wild-type strains of *Salmonella* and *Escherichia coli* was associated with acquisition of I pili (Lawn, Meynell, Meynell & Datta, 1967; Meynell & Datta, 1969).

Anderson (1968) showed that strains of *Salmonella* carried resistance plasmids which were non-self-transmissible and also separate transfer (or sex) factors which brought about their transfer. Some of these sex factors (e.g. that termed Δ) determined I pili (Anderson, 1968). Many of the R factors found by Lawn *et al.* (1967) to be associated with I pilus production seem to be aggregates of the sort described by Anderson (1968), since their resistances can co-exist with the prototypic I group plasmids. I group R factors, previously reported (Meynell & Datta, 1969) as the most abundant of the *fi*⁻ class, are thus rather rare.

This paper describes the relationships between plasmids which determine I pili using exclusion and compatibility as our criteria.

METHODS

Bacteria. All experiments were carried out using nutritionally distinct strains of *Escherichia coli* K12.

Plasmids. Plasmids determining I pili are listed in Table 1.

Phages. If1 (Meynell & Lawn, 1968); P1 (Lennox, 1955).

Table 1. *Plasmids determining I pili*

Plasmid	Resistance* and colicine determinants	Pilus production	Reference
R64	ST	Repressed	Meynell & Datta (1967)
R144	TK Col Ib	Repressed	Meynell & Datta (1967)
R144 <i>drd</i> 3a†	Col Ib	Derepressed	
R648**	ASK	Repressed	
Col Ia-CA53	Col Ia	Repressed	Lawn <i>et al.</i> (1967)
Sex factor I-16 <i>drd</i>		Derepressed	Meynell (1968)
R483	STp	Repressed	Hedges, Datta & Fleming (1972)
JR66a	SK	Repressed	Datta & Hedges (1973).
R621a‡	T	Repressed	

* Drug resistance markers abbreviated as follows: S, streptomycin; T, tetracycline; A, ampicillin; K, kanamycin; Tp, trimethoprim.

† R144*drd*3a a spontaneous segregant of R144*drd*3 (Meynell & Datta, 1967) from which antibiotic resistance was lost.

** R factor derived from a strain of *Salmonella typhimurium* supplied by Mr W. J. Sojka.

‡ R factors derived from strains of *Salmonella typhimurium* supplied by Professor J. N. Coetzee.

Media. These were as described by Datta, Hedges, Shaw, Sykes & Richmond (1971).

Transfer of R factors. Methods were as described by Datta *et al.* (1971).

Compatibility of R factors and transfer from doubles. Methods were as described by Coetzee, Datta & Hedges (1972).

Recognition of I pili. (i) Constitutive production of I pili was recognized if lysis was visible when an undiluted preparation of If1 (approx. 10^{11} plaque-forming units/ml) was spotted on cultures spread on nutrient agar plates. (ii) Repressed production of I pili was recognized by an increase in titre of phage If1 in the culture under test. The method was as described by Datta *et al.* (1971).

PI transduction was as described by Jacob (1955), selection being for antibiotic resistance.

RESULTS

Identification of R factors determining I pili

In strains of bacteria which possess transferable drug-resistance determinants and produce I pili, the genes for resistance and pili may be carried on a single plasmid, or the pilus genes may be carried by a sex factor separate from the drug-resistance plasmid (Anderson, 1968). The plasmids which we investigated were of the former kind. Evidence for this is as follows.

R144 and R64 have been shown by physical methods to be single circular molecules (Falkow, Tompkins, Silver, Guerry & Le Blanc, 1971; Falkow, personal communication; Vapnek, Lipman & Rupp, 1971). R621a was transduced by phage P1 between strains of *Escherichia coli* K12. Transduced R621a was transmissible and determined I pili. After co-existence with I plasmids R648 or JR66a, R621a still determined tetracycline resistance and I pili.

The evidence that JR66a and R483 were plasmids in which determinants for I pili were carried on the same molecule as drug resistance rests on their compatibility properties (Datta & Hedges, 1973, and Table 2). In addition, R483, after co-existence with R144, retained streptomycin and trimethoprim resistances and still determined I pili. Col Ia-CA53 produces I pili (Lawn *et al.* 1967) and its incompatibility with R64[†] and R144 indicates that its colicin gene is carried on the same molecule as the I pilus genes.

Table 2. *Exclusion and compatibility relationships of plasmids determining I pili*

R factor	Transfer to host carrying	Frequency/donor cell	Characters of purified transipients
R64	—	1×10^{-3}	—
	R144	2×10^{-7}	2/2 R64 only
	R648	1×10^{-3}	20/20 R64 only
	Col Ia	1×10^{-5}	20/20 R64 only
	R483	1×10^{-3}	20/20 both plasmids*
	JR66a	5×10^{-5}	19/20 R64 only: 1/20 recombinant†
	R621a	Not testable‡	—
R144	—	1×10^{-3}	—
	R64	$< 10^{-7}$	No transfer observed
	R648	$< 10^{-7}$	No transfer observed
	Col Ia	$< 10^{-7}$	No transfer observed
	R483	1×10^{-3}	20/20 both plasmids*
	JR66a	$< 10^{-7}$	No transfer observed
	R621a	Not testable	—
R648	—	1×10^{-3}	—
	R64	1×10^{-4}	20/20 R648 only
	R144	$< 10^{-7}$	No transfer observed
	Col Ia	5×10^{-5}	20/20 R648 only
	R483	6×10^{-4}	10/20 both plasmids*: 2/20 R648 only
	JR66a	Not testable	—
	R621a	5×10^{-5}	10/20 both plasmids*: 10/20 R648 only
R483	—	2×10^{-3}	—
	R64	1×10^{-3}	20/20 both plasmids, but unstable§
	R144	1×10^{-3}	20/20 both plasmids, but unstable§
	R648	1×10^{-3}	20/20 both plasmids, but unstable§
	Col Ia	2×10^{-5}	20/20 both plasmids*
	JR66a	1×10^{-3}	5/20 R483 only: 15/20 both present but unstable§
	R621a	1×10^{-3}	20/20 both plasmids*
JR66a	—	3×10^{-3}	—
	R64	2×10^{-4}	20/20 JR66a only
	R144	2×10^{-7}	10/10 JR66a only
	R648	Not testable	—
	R483	2×10^{-3}	8/20 JR66a only: 12/20 Tp ^r retained but non-transmissible
	Col Ia	$< 10^{-7}$	No transfer observed
	R621a	2×10^{-3}	20/20 both plasmids*
R621a	—	2×10^{-3}	—
	R64	Not testable	—
	R144	Not testable	—
	R648	2×10^{-5}	18/20 both plasmids*: 2/20 R621a only
	Col Ia	2×10^{-4}	20/20 both plasmids*
	R483	2×10^{-3}	20/20 both plasmids*
	JR66a	4×10^{-4}	20/20 both plasmids*

* Both plasmids: indicates that resistances of each were stably inherited and separately transmissible.

† Recombinant: indicates that resistances of each were always transmissible together, whichever drug was used for selection.

‡ Not testable: overlap of markers prevents recognition of double.

§ Unstable: symmetrical loss of plasmids observed in drug-free medium.

|| See text. Tp^r indicates trimethoprim-resistance.

Fig. 1. Relationships between plasmids determining I pili as shown in Table 2. —□— between plasmids indicates compatibility, —■— between plasmids indicates incompatibility, —●— indicates exclusion, —○— indicates lack of exclusion, by the plasmid beside which the symbol appears.

Exclusion

When R64 was mated with strains carrying R144 (with which it is incompatible; see below), the rate of transfer was reduced so drastically that only rarely were any transciipients observed. On the other hand, transfer of R64 to a recipient carrying the similarly incompatible R648 was as efficient as to an R⁻ recipient. Thus in crosses between incompatible plasmids, exclusion was sometimes very efficient and sometimes absent.

In matings between strains carrying compatible pairs of R factors, exclusion was sometimes but not always observed, e.g. R621a excluded R648 (and vice versa) but R621a did not exclude (and was not excluded by) R483. Results are presented in Table 2 and Fig. 1.

Compatibility

Among R factors determining I pili, four patterns of compatibility were distinguished. R64, R144, R648 and Col Ia were incompatible with one another. These we call typical I group plasmids or I α . R483 was compatible with the I α plasmids and is designated I β . In crosses in which an I α plasmid was introduced into an R483⁺ strain, the doubles were stable. But when R483 was transferred into a strain carrying R64, R144 or R648 the resulting doubles were unstable, segregating clones carrying one or other plasmid (Table 2). The stability or instability of doubles depended upon which was the resident and which the newly introduced plasmid, and was independent of the host strain of *Escherichia coli* K12. Col Ia differed from the other I α plasmids in co-existing stably with introduced R483. JR66a was incompatible with plasmids of both I α and I β groups and we propose the designation I ω . When one I α plasmid was transferred into a strain already carrying an I α plasmid, incom-

patibility was expressed so rapidly that doubles, even unstable, were never observed. The same was true in matings between $I\alpha^+$ and $I\omega^+$ strains. But in matings between R483 ($I\beta$) and JR66a ($I\omega$) the results were more complicated. When R483 was transferred to a JR66a $^+$ strain, a proportion of the trimethoprim-resistant transipients retained JR66a; all (15/15) of these strains were unstable. From overnight broth cultures, a majority of clones carried one or other plasmid but not both. When JR66a was transferred to an R483 $^+$ strain, a proportion of the kanamycin-resistant transipients retained trimethoprim resistance, but in this case trimethoprim resistance was stable but non-transmissible (Datta & Hedges, 1973).

R621a was compatible with $I\alpha$, $I\beta$ and $I\omega$ plasmids. This R factor was also compatible with plasmids of all other defined compatibility groups. (The only untestable group was Fiv whose only known member, R124, confers tetracycline resistance only, as does R621a itself.)

Repression of I pilus synthesis

Since R621a is compatible with all other I-pilus determining plasmids, we tested its ability to repress pilus production by mutant I plasmids which produce pili constitutively. R621a cannot be distinguished in the presence of R factors which determine tetracycline resistance, but was tested with R144*drd*3a and the derepressed sex factor of Col Ib, I-16 *drd* (Meynell, 1968). Pilus production remained constitutive in 10 of 10 independently isolated clones of the double J53 (R144*drd*3a) (R621a) but was repressed in 10 of 10 clones of J62 (I-16 *drd*) (R621a).

DISCUSSION

R factors and other transmissible plasmids of Gram-negative bacilli can be classified by compatibility in *Escherichia coli* K12 (Datta & Hedges, 1971) or by the specificity of their sex pili (Lawn *et al.* 1967). These criteria are correlated, in that any known plasmid determining F-like pili is compatible with any determining I pili (Meynell, Meynell & Datta, 1968). However, numerous groups of plasmids have been identified which do not appear to determine either F-like or I pili and which are compatible with plasmids of either pilus type (e.g. the N and P compatibility groups [Datta & Hedges, 1971; Datta *et al.* 1971]).

Plasmids determining F-like pili constitute several compatibility groups (Hedges & Datta, 1972). In this paper we show that plasmids determining I pili can also be subdivided by their compatibility properties.

To distinguish between I pili determined by R factors and I pili determined by a separate sex factor we used two techniques, P1 transduction and the construction of doubles with known I group plasmids. For example, R621a, transduced by P1 with selection for tetracycline resistance, still determined I pili. Secondly, when JR66a was transferred to an R621a $^+$ strain, each R factor was separately transferable from the double. R621a transipients retained I piliation. Had the I pili been determined by a separate I group sex factor, it would have been eliminated by JR66a.

The classic I group factors, R144 and R64, together with R648 and Col Ia-CA53 constitute the group we term $I\alpha$. These exclude one another strongly and are incompatible. The incompatibility is so immediately expressed that even unstable doubles were never observed.

R483, which determines resistance to streptomycin and trimethoprim, is the prototype of the group which we designate $I\beta$. When this R factor was first described, it was allocated to a new compatibility group, B (Hedges, Datta & Fleming, 1972). We have subsequently shown that R483 determines I pili and is incompatible with JR66a (Datta & Hedges, 1973).

and we withdraw therefore the designation B and substitute $I\beta$. R483 does not exclude and is not excluded by any of the other I-pilus producing plasmids. It co-exists stably with plasmids of group $I\alpha$ (Hedges *et al.* 1972) if an $I\alpha$ plasmid is introduced into a strain carrying resident R483. But when the cross was made in the other direction, R483 being transferred into a strain carrying R64, R144 or R648, the transciipients were unstable doubles from which one or other plasmid was lost during growth in non-selective medium (Table 2). We are investigating the molecular basis of this phenomenon. Col Ia differed from the other $I\alpha$ plasmids in forming stable doubles with R483, even when the latter was introduced into a col Ia⁺ strain (Table 2).

JR66a is incompatible with plasmids of groups $I\alpha$ and $I\beta$. The incompatibility with $I\alpha$ plasmids was immediately expressed, but unstable doubles were observed with the $I\beta$ plasmid R483. We have termed the compatibility pattern of JR66a, $I\omega$, and postulated that this is the ancestral form from which $I\alpha$ and $I\beta$ have diverged (Datta & Hedges, 1973). JR66a excluded and was excluded by plasmids of group $I\alpha$, but markedly less efficiently than the intragroup exclusion by $I\alpha$ plasmids.

R621a is an *fi*⁻ plasmid determining tetracycline resistance and I pilus production. It is compatible with all plasmids tested, including members of all compatibility groups so far defined. In compatibility studies, R621a showed no interaction (with one exception) with plasmids of groups $I\alpha$, $I\beta$ or $I\omega$ and we have designated the group to which it belongs $I\gamma$. The exception was dislodgement (Coetzee *et al.* 1972) of R621a by R648 and vice versa, i.e. introduction of one of these factors eliminated the other from some recipient clones, even though the two were compatible (Table 2). It was excluded by $I\alpha$ and $I\omega$ plasmids, but less efficiently than the intragroup $I\alpha$ exclusion.

Thus, among I pilus-determining plasmids there is not complete correlation between exclusion and incompatibility, for example, R621a and R648 are compatible but exclude one another, whereas R483 and JR66a are incompatible yet do not exclude one another. It is on compatibility that we base our classification.

Since R621a is compatible with the $I\alpha$ group, we can test whether it represses constitutive pilus synthesis by mutant plasmids of this group. R621a repressed pilus synthesis by the derepressed sex factor I-16 *drd* but not that of R144 *drd3a*. We conclude that R621a produces a *transactive* repressor capable of acting on the pilus synthesizing genes of I-16 *drd* but not on those of R144 *drd3a*.

Note: after this paper was submitted for publication, Grindley, Grindley & Anderson (1972) reported that two R factors, TP114 and T- Δ *drp1*, a laboratory construct, were mutually compatible although both determined I pili. The relationship of these two plasmids to the plasmids described in this paper has not been tested.

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