Properties of *Proteus* and *Providencia* Strains Harbouring Recombinant Plasmids between P-*lac* R1drd19 or R447b

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SUMMARY

Recombinant plasmids between R1drd19 and P-*lac* and between R447b and P-*lac* were produced by phage PL25 transductions of the respective R factors to *Providencia* p29 strains harbouring P-*lac*. The P-*lac*R1drd19 (the superior line indicating the transduced R factor) recombinant plasmid transfers *lac*⁺ and all determinants of the R factor to *Proteus mirabilis* strains and *Escherichia coli* at high frequencies which approach inter-*Proteus* transfer of the parental plasmids. The P-*lac*R447b recombinant transfers *lac*⁺ and only marker K of the parent R factor, at frequencies of P-*lac* inter-*Proteus* transfer. Both recombinants possess the compatibility property and possibly most other features of P-*lac*. Transductions of the P-*lac*R1drd19 recombinant with phages PL25 and 34 differ. Those with the former vector to p29 yield transductants which have markers ACKSu but not *lac*⁺ or S and do not transmit markers conjugally. Conjugal transferability is restored with transductions of the recombinant to p29 carrying a resident P-*lac*; these transductants then transmit *lac*⁺ACKSu as a unit. Transductions of the recombinant P-*lac*R1drd19 with phage 34 to strains of *P. mirabilis* produce transductants which possess all the resistance determinants and which transfer these conjugally, but in which *lac*⁺ does not appear. A spontaneous *lac* segregant of the P-*lac*R1drd19 recombinant retained the high transfer rate and other properties of the parent. Transduction of this segregant to strains of p29 and *P. mirabilis* resulted in transductants which carried markers ACKSu and ACKSSu respectively but could not transfer them by conjugation. Transductions of the segregant to strains harbouring P-*lac* again produced recombinant plasmids at low rates which transmitted the above groups of resistance determinants and *lac*⁺ at frequencies similar to those of the original recombinant. Transductions of the P-*lac*R447b recombinant with either phage yielded transductants carrying K (and able to transmit it conjugally) but never any carrying *lac*⁺.

INTRODUCTION

Recombination between R factors and plasmids not associated with resistance determinants has not been frequently reported. Harada, Kameda, Suzuki & Mitsuhashi (1964) described a recombinant between F-*lac* and a non-transmissible R factor. In addition to possessing the drug-resistance marker of the R factor the recombinant had all the properties of F-*lac*. Watanabe & Ogata (1966) investigated properties of a recombinant between an R factor and F. The hybrid had features distinct from either parent. Kondo & Mitsuhashi (1966) described a recombinant between a non-transferable R factor and F. The recombinant had the properties of the F component and the chloramphenicol resistance of the R factor. Gosden, Irving & Bishop (1971) described a sex factor FR5 which is a

recombinant of F'8 with R factor R1drd19. This R factor is a mutant of the *fi*⁺ R factor R1 (Meynell & Datta, 1967) which lacks a functional repressor of genes affecting transfer (Meynell & Cooke, 1969). FR5 carries the marker for galactose fermentation and all resistance determinants of R1drd19 apart from kanamycin. Cooper (1971) described properties of a recombinant between a non-transmissible ColV and R538drd1. The fused plasmid comprised the col determinant and transmissibility genes of the R factor. It appeared to have compatibility properties of both parents. Meynell (1973) mentions a recombinant of R1drd19 with F which produces sex pili of F not R1 serotype.

Plasmids which carry *lac*⁺ determinants may exist stably side by side with R factors, as demonstrated by Reeve (1970), Reeve & Braithwaite (1970) and Brenchley & Magasanik (1972), who isolated wild strains of *Klebsiella aerogenes* which carried both a *lac*⁺ plasmid and drug resistance plasmids as separate entities.

Falkow, Wohlhieter, Citarella & Baron (1964) demonstrated that a *lac*⁺ determinant present in a strain of *Proteus mirabilis* isolated from clinical material (see Sutter & Foecking, 1962) is associated with an infectious element and is readily transmissible to many other enterobacteria. The plasmid was named P-*lac*. The DNA of the plasmid has an average guanine plus cytosine molar content of 50 % and a molecular weight of 10×10^6 (Wohlhieter, Falkow, Citarella & Baron, 1964).

Proteus morganii strain 16 (Hedges, Datta, Coetzee & Dennison, 1973) yielded a factor R447b of compatibility group N which is unique among wild N plasmids in that it confers kanamycin resistance. It does not confer host specificity type II on its hosts.

This paper describes the isolation and some properties of recombinants between P-*lac* and R1drd19 or R447b.

METHODS

Bacteria, plasmids and phages. These are listed in Table 1. Streptomycin-resistant variants were selected by the method of Coetzee & Sacks (1960). Mutants resistant to nalidixic acid were selected by the same method on agar containing 50 µg nalidixic acid/ml.

Media. These were as previously used (Coetzee, Datta, Hedges & Appelbaum, 1973) with the exception that the minimal medium contained 0.3 % (w/v) lactose instead of glucose and 0.01 % (w/v) tetrazolium chloride (Grabow, 1972). This medium was supplemented with amino acids (20 µg/ml) to suit auxotrophic strains when necessary.

Antibacterial drugs, conjugal transfer of plasmids, preparation of phage lysates and transduction techniques. These were as described by Coetzee *et al.* (1973), with the exception that streptomycin was used at 15 µg/ml and also at 1 mg/ml for selection of chromosomally resistant organisms.

Propagation of F-specific phages. This was done according to the method of Haapala & Falkow (1971) with titration on *Escherichia coli* strain K12 Hfrc.

Coexistence of plasmids and selection of bacteria in which segregation of plasmids had occurred. Plasmid-containing bacteria were grown overnight in broth and dilutions were plated on MacConkey agar and on this agar with antibiotics. Colonies on MacConkey agar were also replicated to this agar containing antibiotics. About 12 000 colonies of each plasmid-containing strain were examined.

Effect of F on transfer of P-lac. This was examined according to methods of Falkow *et al.* (1964).

Table 1. *Bacteria, plasmids and phages*

Bacteria	Properties*		Reference or source
PM13	Recipient for transducing phage 34. T resistant		Coetzee & Sacks (1960)
PM13 <i>str-r</i>	—		Coetzee & Sacks (1960)
PM5006	Recipient for transducing phage 34. T resistant		Coetzee & Smit (1970)
P29	Providence strain NCTC 9295. Recipient for transducing phage PL25. A,T resistant		Coetzee, Smit & Prozesky (1966)
E27 <i>nal-r</i>	<i>Escherichia coli</i> recipient for R factors		Coetzee <i>et al.</i> (1973)
<i>E. coli</i> K12 strains:			
HFIC	<i>met</i>		Clowes & Hayes (1968)
J62	F ⁻ <i>lac, pro, his, trp</i>		Clowes & Hayes (1968)
J62-1	J62 <i>nal-r</i> mutant		Coetzee, Datta & Hedges (1972)
Plasmids and hosts	Markers and compatibility group*		
PM13(P- <i>lac</i>)	<i>lac</i> ⁺	Undetermined	Coetzee <i>et al.</i> (1973), Dr N. Datta
PM13(R1 <i>drd</i> 19)	ACKSSu	FII	Coetzee <i>et al.</i> (1973)
PM13(R1-1)	ACSSu	FII	Coetzee, Datta & Hedges (1972)
PM13(R395)	ACSSpSut <i>hsp</i> II	N	Coetzee <i>et al.</i> (1973)
P29(R1 <i>drd</i> 19)	ACKSSu	FII	Datta & Hedges (1972)
P29 <i>str-r</i> (R447b)	AK	N	Hedges <i>et al.</i> (1973)
<i>E. coli</i> K12 58-161	F ⁺ <i>lac met</i>	FI	Clowes & Hayes (1968), Falkow & Baron (1962). <i>lac</i> obtained by u.v. irradiation
Phages			
34	—	—	Coetzee & Sacks (1960)
PL25	—	—	Coetzee <i>et al.</i> (1966)
MS2	—	—	Davis, Straus & Sinsheimer (1961)

* A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamide; Sp, spectinomycin, T, tetracycline.

Table 2. *Intra- and inter-strain transfer of plasmids*

Donor	Recipient	Selected marker	Transfer frequency/donor cell
PM13(P- <i>lac</i>)	PM13 <i>str-r</i>	<i>lac</i> ⁺ *	1 × 10 ⁻¹
	P29 <i>str-r</i>	<i>lac</i> ⁺ *	8 × 10 ⁻²
	5006 <i>str-r</i>	<i>lac</i> ⁺ *	1 × 10 ⁻¹
PM13(R1 <i>drd</i> 19)	PM13 <i>nal-r</i>	K†	1 × 10 ⁻¹
	P29 <i>nal-r</i>	K†	8 × 10 ⁻²
	PM5006 <i>nal-r</i>	K†	2 × 10 ⁻¹
P29 <i>str-r</i> (R447b)	PM5006 <i>nal-r</i>	A†	9 × 10 ⁻⁴
	P29 <i>nal-r</i>	K†	9 × 10 ⁻⁴

* Conjugal mixtures washed and plated on lactose minimal medium with streptomycin 1 mg/ml.

† Selection on MacConkey agar containing nalidixic acid and appropriate antibiotic.

RESULTS

Transfer of plasmids from strains of PM13

The rates at which P-*lac* and R1*drd*19 are transferred to strains of *Proteus mirabilis* and Providence are high and practically identical (Table 2). A strain of *Salmonella typhosa* carrying P-*lac* transfers it to *lac* strains of *Escherichia coli* at frequencies greater than 10⁰/donor cell while the transfer frequency between strains of *E. coli* is about 10⁻² (Falkow

Table 3. *Transduction of R1drd19, R447b by phage PL25*

Donor		Recipient	Recipient markers	Selected marker	Resistance of transductants*	Transduction rate/adsorbed p.f.u.	Conjugal transfer of selected marker†
Lysate	Resistance						
P29(R1drd19)	ACKSSuT	P29	AT	S	ACKSSuT	5×10^{-8}	0/19
				K	ACKSSuT	9×10^{-8}	0/20
				C	ACKSSuT	5×10^{-8}	0/20
		P29str-r(P-lac)	ASTlac ⁺	K	ACKSSuT‡	8×10^{-8}	19/21
				C	ACKSSuT‡	8×10^{-8}	15/15
P29nal-r(R447b)	AKT	P29	AT	K	AKT	8×10^{-7}	20/20
		P29str-r(P-lac)	ASTlac ⁺	K	AKST‡	8×10^{-7}	20/20

* Suspensions of purified transductants spotted on selective media.

† Recipient *Escherichia coli* E27nal-r. Denominator refers to number of clones tested.‡ All clones lac⁺.Table 4. *Conjugal transfer of transductant markers*

Transductant	Recipient	Transfer frequency selection for:†		Markers of both plasmids present in exconjugant clones derived from columns:‡	
		Resident plasmid	Transduced marker	3	4
P29str-r(P-lacR1drd19)*	P29nal-r	1×10^{-1} (lac ⁺)	1×10^{-1} (ACKSSu)	20/20 both present	20/20 both present
	PM13nal-r	9×10^{-2} (lac ⁺)	9×10^{-2} (ACKSSu)		
	PM13nal-r	1×10^{-2} (lac ⁺)	1×10^{-2} (ACKSSu)		
	PM13nal-r	6×10^{-3} (lac ⁺)	6×10^{-3} (ACKSSu)		
	PM13nal-r	1×10^{-4} (lac ⁺)	1×10^{-4} (ACKSSu)		
	PM5006nal-r	1×10^{-2} (lac ⁺)	1×10^{-2} (ACKSSu)		
P29(R447b)	E27nal-r	§	8×10^{-2} (ACKSSu)	—§	—§
	P29nal-r	1×10^{-4} (K)	—	—	—
	PM5006nal-r	1×10^{-4} (K)	—	—	—
		1×10^{-4} (A)	—	—	—
P29str-r(P-lacR447b)	P29nal-r	1×10^{-1} (lac ⁺)	1×10^{-1} (K)	30/30 0/30	30/30 0/30
		1×10^{-1} (lac ⁺)	0 (A)		
	PM5006nal-r	1×10^{-1} (lac ⁺)	1×10^{-1} (K)	30/30 0/30	30/30 0/30
		1×10^{-1} (lac ⁺)	0 (A)		

The italicized symbol in columns 3 and 4 is the marker used for selection. The remaining symbols in column 4 represent unselected markers detected by replication to appropriate media. lac⁺ was selected on minimal medium. Drug resistance was selected on MacConkey agar containing individual antibiotics. Both media contained nalidixic acid.

* Superior line indicates transduced R factor.

† Transfer frequency/donor cell.

‡ Denominator equals no. clones tested.

§ E27nal-r is lac⁺.

et al. 1964). R1drd19 transfers at rates even higher than those presented here between strains of *E. coli* (Harden & Meynell, 1972). R447b transmits at very much lower frequencies which approximate to those of its inter-*E. coli* transfer (Hedges *et al.* 1973).

Transduction of R1drd19 and R447b to Providence strain P29

Preparations of phage PL25 on P29(R1drd19) and P29nal-r(R447b) had titres of 1 to 5×10^{10} p.f.u./ml. Table 3 shows results of transductions with these lysates. While all markers of R1drd19 which can be selected are transduced to P29 at low rates, transductants

Table 5. Transduction of recombinant plasmids *P-lacR1drd19*, *P-lacR447b*

Expt no.	Phage	Donor		Recipient	Re-cipient markers	Selected markers	Markers of transductants	Transduction rate (per adsorbed p.f.u.)	Con-jugal transfer of selected marker
		Lysate	Markers						
1	PL25	P29(<i>P-lacR1drd19</i>)	ACKSSuT <i>lac</i> ⁺	P29	AT	K	ACKSuT	5 × 10 ⁻⁸	0/20*
2						C	ACKSuT	5 × 10 ⁻⁸	0/20*
3						<i>lac</i> ⁺	—	0	—
4				P29(<i>P-lac</i>)	AT <i>lac</i> ⁺	K	ACKSuT†	5 × 10 ⁻⁹	10/10*
5						C	ACKSuT‡	5 × 10 ⁻⁹	10/10*
6	34	PM5006(<i>P-lacR1drd19</i>)	ACKSSuT <i>lac</i> ⁺	PM13	T	K	ACKSSuT	8 × 10 ⁻⁸	15/20†
7						<i>lac</i> ⁺	—	0	—
8	PL25	P29(<i>P-lacR447b</i>)	AKT <i>lac</i> ⁺	P29	AT	K	AKT	1 × 10 ⁻⁸	20/20*
9						<i>lac</i> ⁺	—	0	—
10	34	PM5006(<i>P-lacR447b</i>)	KT <i>lac</i> ⁺	PM5006	T	K	KT	5 × 10 ⁻⁸	20/20†
11						A	—	0	—
12						<i>lac</i> ⁻	—	0	—

Methods were as for the experiments in Table 3. Selection for *lac*⁺ was on minimal medium.

* Transfer to PM13*nal-r*.

† Transfer to P29*nal-r*.

‡ All clones *lac*⁻.

cannot transmit the markers (Coetzee, 1972). These results differ from transductions of *R1drd19* by phage 34 to strains of PM13 (Coetzee *et al.* 1973). The latter transductants only registered markers C and S. Transduction of *R1drd19* into P29*str-r*(*P-lac*) yields transductants which transfer markers A, C, K, S and Su to *Escherichia coli* E27*nal-r* (Table 3). Table 4 reveals that *lac*⁺ is also transferred to *Proteus* strains at the same high frequency as marker K and that all exconjugants have markers ACKSSu. The resident *P-lac* thus confers conjugal transmissibility to transductant markers of *R1drd19*, and *lac*⁺ invariably accompanies them. With selection for A, C or S the frequency of transfer is decreased in that order, but all exconjugant clones carry *lac*⁺ACKSSu whatever the frequency of recovery of the drug marker. The apparent decrease in frequency of conjugal transfer of the complex with selection for markers K, A, C and S (in that order – Table 4) merely reflects differences in efficiency of expression of newly acquired genes by exconjugants. It is concluded that *P-lac* and portions of transduced *R1drd19* are closely associated in transductants. Both A and K markers of *R447b* are transmissible in P29 transductants (Table 4) but with a resident *P-lac* (Tables 3, 4) markers *lac*⁺ and K (but not A) register and are jointly transferred at the high frequency characteristic of *P-lac*. The complexes of *lac*⁺ with *R1drd19* and *R447b* respectively are readily transferred from *nal-r* exconjugant clones (including E27*nal-r*) to P29, PM13 and PM5006 as well as *str-r* variants of these strains and also strains of *E. coli* K12 without contraselection for the donor. The 34 transductant clones of *P-lacR1drd19* (Table 3) had the same phenotype. This also applies to the 20 *P-lacR447b* clones investigated (Table 3).

Transduction of *P-lacR1drd19* and *P-lacR447b* complexes

The *lac*⁻ marker has not registered in transduction experiments with phage 34 lysates of PM13*str-r*(*P-lac*) or 5006*str-r*(*P-lac*) to PM13 and phage PL25 lysates of P29*str-r*(*P-lac*) to P29 with selection for *lac*⁺ on minimal agar. These lysates transferred the *str-r* chromosomal marker at high frequency (unpublished). Phage lysates were prepared of exconjugants

PM5006(P-*lac* $\overline{R1drd19}$), P29(P-*lac* $\overline{R1drd19}$), P29(P-*lac* $\overline{R447b}$) and PM5006(P-*lac* $\overline{R447b}$). Lysates had titres of at least 1×10^{10} p.f.u./ml and were used in the transduction experiments listed in Table 5. The *lac*⁺ marker never registered either as a selected or an unselected marker. Markers ACKSu appear in P29 transductants at low frequencies but the latter do not transmit them in mating experiments (Table 5, Expts 1, 2). These results differ from those of $\overline{R1drd19}$ transductions to P29 listed in Table 3 in that the S marker is never expressed in the present transductants. By employing a resident P-*lac*, P29 transductants appeared at a low rate but all were infective for *lac*⁺ACKSu (Table 5, Expts 4, 5). Again the S marker does not register. These results may mean that PL25 only conveys ACKSu of the fused plasmid and that these are rendered transmissible by recombination with a resident P-*lac*. Why the S marker is not found is not known as it is conveyed in transductions of the R factor. Phage 34-mediated transductants of the fused plasmid register all resistance determinants and are infective for them (Table 5, Expt 6). Again, marker *lac*⁺ is not expressed in transductants. Transduction of the P-*lac* $\overline{R447b}$ complex by both vectors is the same: neither markers *lac*⁺ nor A register (Table 5, Expts 9, 11) but transductants are conjugally infectious for K (Table 5, Expts 8, 10).

Segregation of markers

Overnight broth cultures of P29(P-*lac* $\overline{R447b}$) and PM5006(P-*lac* $\overline{R447b}$) plated on MacConkey agar containing kanamycin showed no pale colonies among about 12000 clones scanned. The same cultures plated on MacConkey agar had 10 and 16 pale colonies respectively per 12000 clones studied. All the *lac*⁺ colonies on these plates replicated to kanamycin MacConkey agar but the pale clones did not transfer. Cultures of PM5006*nal-r*(P-*lac* $\overline{R1drd19}$), PM13*nal-r*(P-*lac* $\overline{R1drd19}$), P29*nal-r*(P-*lac* $\overline{R1drd19}$) and PM5006*str-r*(P-*lac* $\overline{R1drd19}$) plated on MacConkey agar containing kanamycin or streptomycin did not produce pale colonies. The same cultures plated on MacConkey agar had 14, 0, 11 and 3 pale colonies respectively per 12000 clones. *lac*⁺ colonies on these plates grew when replicated to MacConkey agar containing kanamycin, ampicillin, chloramphenicol or streptomycin. The 11 pale clones from P29*nal-r*(P-*lac* $\overline{R1drd19}$) were sensitive to C, K, S and Su. All 14 pale clones derived from PM5006*nal-r*(P-*lac* $\overline{R1drd19}$) had lost markers ACKSSu. Two of the three pale clones derived from PM5006*str-r*(P-*lac* $\overline{R1drd19}$) had also lost ACKSu resistance. The third pale clone retained resistance to these markers. These results demonstrate that the complexes, like components P-*lac* (Wohlhieter *et al.* 1964), $\overline{R1drd19}$ and $\overline{R447b}$ (unpublished), are stable in *Proteus* and that almost invariably all markers are lost simultaneously. The clone which had lost only the *lac*⁺ marker is designated PM5006*str-r*(P- $\overline{R1drd19}$) and differs from P29($\overline{R1drd19}$) and PM13*nal-r*($\overline{R1drd19}$) (Coetzee *et al.* 1973) in that it is conjugally infective for markers ACKSSu at a frequency equal to that of the parent (data not shown). Phage lysates prepared on exconjugates of the segregant with PM13*nal-r* and P29*nal-r* were used in transduction experiments to strains PM5006 and P29 respectively. Providence strain P29 transductants express markers ACKSu but are not infective for them (Table 6, Expts 1, 2). Again the S marker is not expressed and results are similar to those of phage PL25 transductions of the parent recombinant. In experiments where the recipient carried P-*lac*, transduction occurred at a low rate but all transductants were conjugally fertile for *lac*⁺ACKSu (Table 6, Expts 3, 4). Transduced markers of the segregant appear to have recombined with the resident P-*lac*. Results of phage 34 transductions of the segregant differ from those of the parent recombinant in that although all markers are transduced, transductants are not conjugally infective (Table 6, Expts 5 to 7). Conjugal fertility can be restored by employing P-*lac* as a resident (Table 6, Expts 8 to 10). Again the

Table 6. Transduction of *lac* segregant of *P-lacR1drd19*

Expt no.	Phage	Donor		Recipient	Re-cipient markers	Selected marker	Resistance of transductants	Transduction rate (per adsorbed p.f.u.)	Con-jugal transfer of selected marker
		Lysate	Resistance						
1	PL25	<i>P29nal-r(P-R1drd19)</i>	ACKSSuT	P29	AT	K	ACKSuT	9×10^{-8}	0/20*
2						C	ACKSuT	9×10^{-8}	0/18*
3				P29(P- <i>lac</i>)	AT <i>lac</i> ⁺	K	ACKSuT [†]	7×10^{-9}	18/18*
4						C	ACKSuT [†]	7×10^{-9}	15/15*
5	34	<i>PM13nal-r(P-R1drd19)</i>	ACKSSuT	PM5006	T	A	ACKSSuT	1×10^{-7}	0/20 [†]
6						K	ACKSSuT	1×10^{-7}	0/20 [†]
7						C	ACKSSuT	$\times 10^{-7}$	0/20 [†]
8				PM5006(P- <i>lac</i>)	T <i>lac</i> ⁺	A	ACKSSuT [†]	6×10^{-9}	14/14 [†]
9						K	ACKSSuT [†]	6×10^{-9}	15/15 [†]
10						C	ACKSSuT [†]	6×10^{-9}	13/13 [†]

Methods were as for the experiments in Table 3.

* Transfer to PM5006*nal-r*. † Transfer to P29*nal-r*. ‡ All clones were *lac*⁺.

transduced segregant plasmid fragment appears to have recombined with *P-lac*. The fused plasmids have the high transmissibility rates of the original recombinant, the resistance markers and *lac*⁺ being invariably transferred together; the only difference is that the plasmids derived from phage PL25 transductions do not possess the S marker (data not shown)

Effect of F on conjugal transfer of the fusion plasmid

The presence of F in a *lac* strain 58-161 of *Escherichia coli* K12 also harbouring the fused plasmids *P-lacR1drd19* or *P-lacR447b* reduces their transfer by about 1000-fold. Similar results have been reported in experiments where *P-lac* is present in *E. coli* F⁺ strains (Falkow *et al.* 1964).

Superinfection immunity of P-lacR1drd19, its lac segregant and P-lacR447b

Table 7 shows that the frequency of transfer of the *P-lacR1drd19* complex and its *lac* segregant to recipients which carry a resident *P-lac* is reduced 1000-fold or more in comparison with transfer to strains not carrying the latter plasmid or strains harbouring R1-1. The fused plasmid *P-lacR447b* is also excluded by *P-lac*. Exclusion by *P-lac* is not complete, as happens between I-like factors, but resembles N plasmids where the frequency of transfer to a cell carrying a resident of the same compatibility group is greatly reduced (Datta & Hedges, 1971). It is concluded that both complexes and the *lac* segregant have the surface exclusion properties of *P-lac*. Stable coexistence of the plasmids was tested in Expts 5 to 8, 10 and 11 of Table 7. In Expt 5 no pale chloramphenicol resistant segregants were observed, while many pale kanamycin resistant clones were obtained from exconjugants of Expt 6. These experiments suggest that *P-lacR1drd19* coexists stably with R1-1 but not with its *lac* segregant and it may be inferred (Meynell, 1969) that the recombinant and its segregant belong to the same compatibility group. Expt 7 never yielded kanamycin-sensitive clones while Expt 8 produced many *lac* colonies resistant to kanamycin. Results of the latter conjugation experiment differ from those of transduction experiments reported in Table 6 (Expts 8, 9): although transduction rates were more than tenfold lower than those to non-*P-lac*-carrying recipients, pale clones were not observed. Many *lac*⁺ kanamycin sensitive clones were segregated by exconjugants derived from Expt 10. Cultures derived from Expt 11

Table 7. *Frequency of transfer*

Donor	Expt no.	Recipient	Com- patibility group of resident plasmid	Transfer frequency	
				<i>lac</i> ⁺ *	K [†]
PM13 <i>nal-r</i> (P- <i>lacR1drd19</i>)	1	PM13 <i>str-r</i> (P- <i>lac</i>)	?	—	5 × 10 ⁻⁷
	2	PM13 <i>str-r</i>	—	8 × 10 ⁻²	8 × 10 ⁻²
	3	P29 <i>str-r</i> (P- <i>lac</i>)	?	—	5 × 10 ⁻⁷
	4	P29 <i>str-r</i>	—	8 × 10 ⁻²	8 × 10 ⁻²
	5	PM13 <i>str-r</i> (R1-1)	F II	5 × 10 ⁻²	5 × 10 ⁻²
	6	PM5006 <i>str-r</i> (P- <i>R1drd19</i>)	?	5 × 10 ⁻⁷	—
PM5006 <i>nal-r</i> (P- <i>R1drd19</i>)	7	PM13 <i>str-r</i> (R1-1)	F II	—	5 × 10 ⁻²
	8	PM13 <i>str-r</i> (P- <i>lac</i>)	?	—	1 × 10 ⁻⁷
	9	PM13 <i>str-r</i>	—	—	9 × 10 ⁻²
PM5006(P- <i>lacR447b</i>)	10	PM13 <i>str-r</i> (P- <i>lac</i>)	?	—	1 × 10 ⁻⁶
	11	PM13 <i>str-r</i> (R395)	N	8 × 10 ⁻²	8 × 10 ⁻²

Due to marker overlap transfer frequency of both markers was not always determined.

* Selection for *lac*⁺ was also on minimal medium with S (1 mg/ml).

† Selection on MacConkey agar with K and S (1 mg/ml).

yielded colonies which were almost all *lac*⁺ on MacConkey agar, which all replicated to this agar containing kanamycin and chloramphenicol. Expts 10 and 11 thus suggest that P-*lacR447b* is compatible with an N plasmid but not with P-*lac*.

Bacteriophage propagation

Phage MS2 did not increase in p.f.u. when incubated with strains of PM13(P-*lac*), P29*nal-r*(P-*lac*), PM13(R1*drd19*), P29(R1*drd19*) P29*str-r*(P-*lacR1drd19*), PM13*nal-r*(P-*lacR1drd19*), PM5006*str-r*(P-*R1drd19*) P29*nal-r*(P-*R1drd19*) or fertile exconjugants from Expts 6, 8 and 10 of Table 5. Control mixtures of the phage and PM13(R1*drd19*) showed at least a 10 000-fold increase in titre. These results confirm those of Falkow *et al.* (1964) for P-*lac* and demonstrate that neither transductants of R1*drd19* nor the plasmid complex or its segregant or organisms harbouring the P-*lacR447b* complex are susceptible to the F-specific phage.

DISCUSSION

The P-*lac* plasmid is stable in *Proteus mirabilis* (Wohlhieter *et al.* 1964) and in Providence strain P29 (unpublished). R1*drd19* is unstable in P29 (unpublished) but segregation studies show that the complex of the two factors is remarkably stable in P29 and in *P. mirabilis* exconjugants. R447b is stable in Providence and *P. mirabilis*. The complex P-*lacR447b* retains this characteristic. Stability of the associations was a feature from the onset. The complexes were discovered during an investigation of transduction of R factors by phage PL25 (unpublished) and differ from the association between R1*drd19* and *Fgal* which was unstable initially (Gosden *et al.* 1971). A feature which accords well with recombination between the plasmids is that apart from one segregant of P-*lacR1drd19* all markers of the complexes are lost simultaneously.

Cells harbouring the recombinant plasmids, like *Proteus* carrying only P-*lac* (Falkow *et al.* 1964) or strain 29 transductants of R1*drd19* or R447b, do not permit MS2 multiplication. The nature of sex pili, if any, formed by the complexes is not known and attempts to isolate phages specific for organisms carrying P-*lac* or the complexes have not succeeded to

date. It is not known whether P-*lac* causes specific surface changes in cells carrying it. The fact that cells harbouring recombinants do not support phage MS2 multiplication is no argument for inferring that recombinant plasmids have the surface modifying properties of P-*lac*. Alfaro & Willetts (1972) found that transfer systems of F-like plasmids did not necessarily correspond to their compatibility properties. The fused plasmids have the compatibility properties of P-*lac*, not R1*drd*19 or N (Table 7), and transfer at frequencies approaching that of intra-strain passage of the former plasmid. The *lac* segregant of the PlacR1*drd*19 fused plasmid differs from parent R1*drd*19 in that it does not confer phage MS2 sensitivity on its host and – like the complex – does not exclude R1*drd*19. Although the presence of P-*lac* does not interfere with the functions of F, transfer of P-*lac* from strains carrying F is markedly inhibited (Falkow *et al.* 1964). The nature of the interaction is not known but may not necessarily implicate a specific repressor (see also Meynell, 1973). The inhibition of transfer of the P plasmid RP4 by the I-like factor R64 in established cultures may be a similar phenomenon (Datta *et al.* 1971) and probably means that the regulation of fertility is more complex than a simple repressor-operator interaction (Clowes, 1972).

The fact that the complexes described here differ in some genetic properties from the ancestors, and that the *lac* segregant of P-*lac*R1*drd*19 also differs from its parents, favours genetic recombination between the plasmids rather than a mere physical association. The recombinant between an R factor and F described by Watanabe & Ogata (1966) had the high transmissibility and compatibility properties of F, was susceptible to F-specific phages but differed from F in that it was not eliminated by acridines and did not restrict a particular phage. It differed from the parent R factor by not suppressing F-mediated fertility. Watanabe and Ogata explained features of the recombinant on the grounds that transfer of the complex is still mediated by F, but that the acridine sensitivity and phage restriction regions of the latter DNA were simply replaced by the R factor.

Apart from physical studies (Nisioka, Mitani & Clowes, 1970) one of the surest ways of demonstrating recombination between plasmids is joint transduction of markers (Clowes, 1972). With the use of phage P1, Mitsuhashi *et al.* (1962), Watanabe & Lyang (1962), Harada, Kameda, Suzuki & Mitsuhashi (1964), Watanabe *et al.* (1964), Hashimoto & Hirota (1966), Kondo & Mitsuhashi (1966) and Kameda, Harada, Suzuki & Mitsuhashi (1969) demonstrated co-transduction of markers from different plasmids and suggested that they recombined. Watanabe & Ogata (1966) could not transduce a complex of an R factor and F with phage P1 although it had features of a recombinant. The failure of phages 34 and PL25 to transduce all markers of the fusion plasmids reported here may possibly be explained by the magnitudes of the molecular weights of the plasmids concerned. The mol. wt of phage PL25 DNA is 24.7×10^6 daltons and that of phage 34 is 29.5×10^6 daltons (van Rensburg, 1970). The mol. wt of P-*lac* is only 10×10^6 (Wohlhieter *et al.* 1964) but the composite molecule of R1*drd*19 has a mol. wt of 65×10^6 daltons (Cohen & Miller, 1969; Silver & Falkow, 1970) of which the transfer element contributes 52×10^6 daltons and the resistance determinant replicon the remainder (Cohen & Miller, 1970; Haapala & Falkow, 1971). The mol. wt of R447b is not known. The contributions of the two parents to the two fusion plasmids is not known, but complexes may have weights too large for these vectors to accommodate. The PL25 vector can contain all resistance determinants of R1*drd*19 (Table 3) but only markers ACKSu of the complex are expressed in transductants of the phage (Table 5, Expts 1, 2). It may mean that due to the structure of the complex only these markers can be taken up by the phage or, if markers S or *lac*⁺ are also conveyed, only genes of the former cluster are assembled in a non-transferable replicon or are incorporated in the bacterial chromosome (Coetzee *et al.* 1973). The fact that only the

ACKSu cluster recombines with a resident *P-lac* to form a highly transferable plasmid (Expts 3, 4, Table 6) may favour the view that only the latter cluster of the complex is conveyed by vector PL25. Phage 34 transduction of the complex results in transductants which register all resistance determinants (but not *lac*⁺) and are conjugally infectious (Table 5, Expt 6). The vector may thus be able to select (and convey) a transferable replicon from the genes of the complex. Results differ from those of Coetzee *et al.* (1973) where, although *P-lac* was not employed as a resident, only C and S markers registered in transductants by phage 34. *P-lac* was, however, used with phage 7. R49 transductions of R1drd19 to *Proteus rettgeri* strain R49 but resistance determinants did not register. Transduction of R447b by phage PL25 differs from those of R1drd19 by the same vector in that transductants are conjugally infectious for the resistance determinants (Tables 3, 4). R447b is quite stable in P29 and it is surprising that total recombination between it and *P-lac* was obtained on first attempt, as there is no pressure to mobilize the genes as in the case of R1drd19 determinants in strain P29 or in *E. coli* with *Fgal* (Gosden *et al.* 1971). Transduction of R447b by phage 34 to PM13 (Coetzee *et al.* 1973) yielded transductants which were only infectious for marker A while transduction to PM13 harbouring *P-lac* did not yield recombinant plasmids at first attempt and the experiments were not pursued. Transduction of the recombinant *P-lac*R447b by both phages yielded results which resemble transductions of recombinant *P-lac*R1drd19 by phage 34, in the sense that although transductants do not register *lac*⁺ they do claim enough of the transduced material to constitute independent transmissible plasmids for the resistance determinants carried by the two recombinants respectively.

The *lac* segregant of the *P-lac*R1drd19 complex behaves in an identical fashion to the parent recombinant as far as the PL25 vector is concerned (Table 5, Expts 1 to 5; Table 6, Expts 1 to 4), and the conclusion may be that its genes retain the steric relationship of the complex and the *lac* phenotype is not caused by a deletion. However, the phage 34 vector produces transductants of the segregant which are non-infectious for the resistance determinants (Table 6, Expts 5 to 7), unlike transductants of the complex (Table 5, Expt 6), and the segregant may thus have a disturbed structure. It is as if the vectors employed have a preference for the resistance determinant region no matter whether these are carried by the R factors, recombinant or segregant. This region has a ready partner in the resident *P-lac* with which it may recombine to form a transmissible plasmid.

An explanation to accommodate features of the association described may be that vector PL25 conveys only the resistance determinant regions of the R factors to P29. This material then integrates into the resident *P-lac* and transfer of the complex is mediated under control of *P-lac* transfer genes. This is an adequate explanation for the *P-lac*R447b recombinant because it transfers at the same high rate as the *P-lac* parent. The *P-lac*R1drd19 recombinant transfers at a rate similar to both parents, and the possibility that transfer genes of R1drd19 may participate in transmission of the complex cannot be excluded.

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