Intra-species Transduction with *Proteus mirabilis* High Frequency Transducing Phages

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SUMMARY

The properties of three additional *Proteus mirabilis* hosts for the high frequency transducing (HFT) phages 5006MHFT k and 5006MHFT a_k are described. The phages transduce resistance to kanamycin and to ampicillin plus kanamycin, respectively, and were produced by ultraviolet induction of derivatives of *P. mirabilis* strain PM5006. Strain PM804 could not be shown to adsorb the phages although it yielded a few transductants. All transductants, even those produced at low multiplicities of phage input, were lysogenic and segregated markers at high frequency. Ultraviolet induced phage lysates of these transductants transduced PM804 at higher frequencies and PM5006 at lower frequencies than the original phages. Strain PM804 or its derivatives did not adsorb phage from these lysates. Transmission experiments through PM5006 of phage in the transductant lysates confirmed that PM804 had a host-controlled modification system which modified HFT phage from PM5006. That PM804 also possessed a restriction system was inferred from the greater numbers of transductants obtained with phage which bore a compatible modification pattern. Strain N and a restrictionless mutant of it named NH1, adsorbed the HFT phages avidly and failed to modify their host range. Transduction frequencies of the phage markers were about 10^{-4} /p.f.u. adsorbed to strain NH1 and only about tenfold lower to strain N which did not plate the phages. Transductants also had the features of heterogenotes and those obtained at low multiplicities of infection were non-lysogenic. The latter transductants adsorbed homologous phage while NH1 transductants also plated the HFT lysates. Strain NH1, lysogenized by the parent phage 5006M, did not adsorb the HFT phages. These findings suggest that the HFT phages were most likely defective for lysogenic conversion to homologous phage non-adsorption. The postulated restriction enzymes of PM804 and strain N could not be shown to be thermolabile.

INTRODUCTION

High frequency transducing (HFT) phages for *Proteus mirabilis* strain PM5006 have been described (Coetzee, 1974, 1975*a*). The first was derived by u.v. irradiation of a kanamycin-resistant transductant of PM5006 produced by phage 34 (Coetzee & Sacks, 1960) transduction of plasmid R394 (Coetzee, Datta & Hedges, 1972). It is now named phage 5006MHFT k (Coetzee, 1975*b*). The other lysate was produced by irradiation of a particular ampicillin- and kanamycin-resistant clone of PM5006 produced by conjugal transfer of R394 to the above-mentioned kanamycin-resistant transductant. This phage is named phage 5006MHFT a_k (Coetzee, 1975*b*).

Proteus mirabilis strains PM5006 and PM13 harbour a cryptic prophage 5006M (Krizsanovich, 1973) which is similar to phage 34 and serologically identical to both the HFT phages. The presence of this prophage complicated analysis of PM5006 transductants by the HFT phages (Coetzee, 1974, 1975*a*) and thus a prophage-free susceptible host was required.

High frequency transduction of antibiotic resistance provides a means of selection for transductants from bacterial strains not normally sensitive to the phage (Goldberg, Bender & Streicher, 1974). Coetzee (1975*b*) reported that a *P. vulgaris* strain PV127 could be transduced to kanamycin resistance by the *P. mirabilis* HFT phages. Strain PV127 was not an entirely satisfactory host as it adsorbed the phages poorly, did not plate them and was ampicillin-resistant. Nevertheless, heterogenote-like transductants (Morse, Lederberg & Lederberg, 1956; Luria, Adams & Ting, 1960) formed at low multiplicities of phage input, adsorbed homologous phage. This was a unique situation because strains PM13 and PM5006 lysogenized by phage 5006M did not adsorb this phage. The latter phenomenon was due to lysogenic conversion to homologous phage non-adsorption (Coetzee, 1961), possibly produced by the interaction of resident cryptic and superinfecting prophage (see Krizsanovich, 1973). This raised the possibility that the HFT phages were defective in the converting function but, as PV127 did not plate phage 5006M, an essential control was missing.

Because the ampicillin resistance marker of phage 5006MHFTak would provide the necessary selection for retransduction of phage 5006MHFTk transductants if the latter adsorbed the phage, a large number of ampicillin- and kanamycin-sensitive strains of *P. vulgaris* and *P. mirabilis* were screened by the method previously used (Coetzee, 1975*b*) as possible hosts for the phages (J. N. Coetzee, unpublished). One *P. mirabilis* strain, PM804, yielded transductants. Also available were *P. mirabilis* strain N, which appeared to restrict phage 34.PM13 without modifying it, and a restrictionless mutant of N, named NHI (Coetzee & Smit, 1969). Phage 5006M.PM5006 behaves like phage 34.PM13 in strain N (unpublished observations) and this paper describes results of transductions of antibiotic resistance markers to *P. mirabilis* strains PM804, N and NHI by phages 5006MHFTk and 5006MHFTak.

METHODS

Bacteria and phages. These are presented in Table 1.

Media. The sources of the nutrient broth, nutrient agar and MacConkey agar have been described (Coetzee *et al.* 1973). Minimal medium was that of Grabow & Smit (1967). Selection for drug resistance was made on MacConkey agar containing ampicillin and/or kanamycin at 50 µg/ml or streptomycin at 1 mg/ml. Incubation temperature was 37 °C.

General phage techniques. These were according to Adams (1956) and Coetzee (1974). Freeze-dried phage 34 antiserum (Coetzee & Sacks, 1960) with a K_m value of 180 min⁻¹ against phages 5006MHFTk and 5006MHFTak was used. Particular care was taken to ensure that phage lysates were sterile: after shaking thoroughly with 0.1 vol. chloroform the phage was decanted into sterile bottles; after overnight incubation, 10 ml samples were removed into 100 ml broth and incubated for 48 h to test for sterility.

Preparation of bacteria. Unless mentioned otherwise, bacteria in the stationary phase of growth were used. Overnight broth cultures were resuspended in 0.01 M-tris buffer pH 7.3 with 0.01 M-MgSO₄, and starved for 30 min. Exponentially-growing cells were obtained by diluting an overnight broth culture 1:5 in pre-warmed broth and incubating for 40 min.

Concentration of phage lysates. The method described for P22 by Yamamoto & Alberts (1970) was used.

Phage adsorption. The method of Kondo & Mitsunashi (1966) was used. Phage (0.1 ml) was added to 2 ml of the streptomycin-sensitive test strain prepared as above at different multiplicities of input (m.i.) of plaque-forming units (p.f.u.). At time zero and at intervals samples were removed, diluted in saline and titrated on PM5006 *str-r* using media which contained 1 mg streptomycin/ml. The viable count of the test strain was done concurrently

Table 1. *Bacteria and phages*

All strains were sensitive to ampicillin and kanamycin.

Bacteria	Properties	Reference
<i>Proteus mirabilis</i> PM804	Isolated locally from human faeces. Swarms form lines of demarcation with those of PM5006, strain N and NH1	Coetzee (unpublished) Dienes (1946, 1947)
<i>P. mirabilis</i> PM5006	Host for phages used. Cryptically lysogenic for phage 5006M. Not susceptible to bacteriocin or phage 13 <i>vir</i> liberated by strain N	Coetzee & Smit (1969, 1970) Coetzee (1974, 1975a) Krizsanovich (1973)
<i>P. mirabilis</i> PM5006 <i>str-r</i>	Spontaneous mutant resistant to 1 mg streptomycin/ml	Coetzee (1974)
<i>P. mirabilis</i> N	Adsorbs phages 34.PM13 and 5006M.PM5006 well but restricts without modifying the phage. Lysogenic for phage 13 <i>vir</i> and produces a phage tail-like bacteriocin. Forms Dienes demarcation line with PM5006	Coetzee & Sacks (1960) Coetzee & Smit (1969) Coetzee, De Klerk & Coetzee (1968)
<i>P. mirabilis</i> N <i>met-1</i>	Auxotrophic mutant of strain N	Coetzee & Smit (1969)
<i>P. mirabilis</i> NH1	Mutant of strain N which plates phage 34.PM13 and phage 5006M.PM5006 with an efficiency of 5×10^{-1}	Coetzee & Smit (1969)
<i>P. mirabilis</i> NH1(5006M)	Strain NH1 lysogenized with phage 5006M. Converted to homologous phage non-adsorption	Coetzee (unpublished) Coetzee (1961)
Bacteriophages		
5006M.PM5006 <i>str-r</i>	Cryptic prophage of PM5006. Lysate produced by lytic infection of PM5006 <i>str-r</i> . On lyso-genization converts hosts PM13 and PM5006 to non-adsorption of homologous phage	Krizsanovich (1973) Coetzee (1974) Coetzee & Sacks (1960)
5006MHFTk	High frequency transducing for kanamycin resistance. Produced by u.v. induction of PM5006(R394)*. Serologically identical to phage 5006M	Coetzee (1974, 1975b) Coetzee <i>et al.</i> (1973)
5006MHFTak	High frequency transducing phage for ampicillin and kanamycin resistance. Produced by u.v. induction of PM5006(R394)†. Serologically identical to phage 5006M	Coetzee (1975a, b)

* Superior line indicates that markers of R factor R394 were transduced to PM5006.

† Superior lines indicate that markers of R factor R394 were transduced to a derivative of PM5006(R394).

on MacConkey agar. Various concentrations of the test organisms in different phases of growth were used, and some tests were done at 15, 25, 30 and 37 °C.

Selection of auxotrophic mutants of PM804 and NH1. The *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine method of Adelberg, Mandel & Chen (1965) was used.

Ultraviolet irradiation of phage lysates. The method of Coetzee (1974) was used.

Phage induction. Ultraviolet light induction was according to Coetzee (1974) and cultures were treated with mitomycin C according to Altenbern & Stull (1965).

Transduction. The Millipore membrane filter technique (Coetzee, 1974) was used. The phage suspension was added to the bacterial culture and the mixture incubated for 15 min before dilution in saline and filtration. Membranes with impinged bacteria were placed directly on minimal medium (transduction to prototrophy), on pre-warmed nutrient agar for 1 h (transduction of ampicillin or kanamycin resistance) or 3 h (transduction of

streptomycin resistance) before transfer to selective media. In experiments where prevention of lysogenization of transductants by phage liberated during the experiment was critical, phage antiserum was added to the adsorption mixture 10 min after addition of phage. After a further 10 min the adsorption mixture was filtered with a large excess of saline. In experiments to exclude transfection, the adsorption mixture contained 6 µg deoxyribonuclease I (Sigma)/ml. Transductant clones were streaked for single colonies on selective media before further investigation. Some transductant clones were also suspended in phage antiserum for 1 h before streaking for single colonies.

Single cycle growth of phage. This was done according to the method of Coetzee & Smit (1969). Phage was adsorbed to about 2×10^9 organisms for 10 min at 37 °C. A sample was then diluted 1:100 in chloroform broth and titrated on PM5006 for unadsorbed phage. Another sample was diluted 1:2 in phage antiserum. After 5 min at 37 °C two samples were removed from the serum tube. One was diluted into chloroform broth for free phage assay while the other was diluted into nutrient broth and titrated for infective centres on a particular host.

Heat treatment of bacteria. This was done according to the methods of Cornelis & Colson (1975) and Lederberg (1965). A number of 3 ml samples of an overnight broth culture in thin-walled glass tubes were centrifuged and the bacteria from each sample resuspended in 1 ml of 0.85 % saline pre-heated to the chosen temperature. The tubes were maintained in a water bath at this particular temperature. At intervals, duplicate tubes were brought to 37 °C in a water bath for 2 min; the bacteria in one of the tubes were used immediately as recipients in transduction experiments, while single-cycle phage growth experiments were conducted with the cultures in the other tube.

RESULTS

Adsorption and plating efficiencies of phages

Strain PM804 and its derivatives did not adsorb phage as measured by any of the methods used (Table 2) and viable counts of test and control cultures were similar (not shown). Phage grown on PM804 transductants plated on PM5006 and NH1 but adsorption to PM804 could not be demonstrated. Strains N and NH1 adsorbed the phages to the same extent as PM5006. High titre phage formed areas of clearing when spotted on strain N but plaques were not observed. This was investigated in a single-cycle phage growth experiment (Table 3, line 1). Phage from strain N did not plate on it and the organism restricted the phage without modifying it. Strain NH1 plated the phages with an efficiency (e.o.p.) of about 5×10^{-1} (Table 2) and lysogenic derivatives were readily obtained. This small reduction in e.o.p. was unlikely to result from restriction (see Coetzee & Smit, 1969). Phages grown on strain NH1 plated with an e.o.p. of one on PM5006 but only formed areas of clearing and no plaques when spotted on strain N. Phage derived from NH1 transductants could not be shown to adsorb to NH1 lysogenic transductants or to NH1 lysogenized by phage 5006M, but adsorbed avidly to NH1 non-lysogenic transductants and plated with an efficiency of one on PM5006 (Table 2). Strain NH1 did not restrict or modify the HFT phages from PM5006 (see Table 3, line 2).

Transduction

A few transductants were obtained using PM804 as a recipient for the HFT phages (Table 4, lines 1 to 6). The phage lysates were sterile and uninfected recipient controls did not grow on selective media. Properties of the clones also ruled out a mutational origin and in experiments with phage 5006MHFTak lysates, it was unlikely that mutants resistant to two

Table 2. *Phage adsorption and plating efficiencies on P. mirabilis strains*

Phage lysate	Strain	Adsorption (%)	Efficiency of plating*
5006M.PM5006 <i>str-r</i>	PM5006	> 99	I
	PM804	< 10	NVE
	N	> 99	NPO
	NHI	> 99	5×10^{-1}
5006M.NHI	PM5006	> 99	I
	PM804	< 10	NVE
	N	> 99	NPO
5006MHFT <i>k</i>	PM5006	> 99	I
	PM804	< 10	NVE
	N	> 99	NPO
	NHI	> 99	5×10^{-1}
5006MHFT <i>k</i>	PM5006	> 99	I
	PM804	< 10	NVE
	N	> 99	NPO
	NHI	> 99	5×10^{-1}
PM804($\overline{K}$)†	PM5006	> 99	PP
	PM804	< 10	NVE
	N	> 99	NPO
	NHI	> 99	PP
NH($\overline{AK}$)†	PM5006	> 99	I
	PM804	< 10	NVE
	N	> 99	NPO
	NHI	> 99	I
	NHI($\overline{AK}$)‡	< 10	NVE
	NHI($\overline{AK}$)§	> 99	I
	NHI(5006M)	< 10	NVE

NPO, When spotted on lawns of N, the phage caused areas of partial clearing which disappeared with higher dilutions of the phage; no plaques observed; NVE, no visible effect; PP, plaques present but efficiency of plating not determined as phage did not plate on PM804; K, resistance to kanamycin; AK, resistance to ampicillin and kanamycin.

* Plaque-forming titre on strain/titre on last host.

† Superior line indicates that markers were transduced.

‡ Lysogenic transductant.

§ Non-lysogenic transductant.

Table 3. *Single cycle growth of a phage 5006MHFT*k* lysate in strains N and NHI*

Phage was adsorbed to 4×10^8 organisms of strain N and 9×10^8 strain NHI bacteria for 10 min at 37 °C. A sample was then diluted 1:100 in broth containing 0.1 vol. chloroform and titrated on strain PM5006 for unadsorbed phage. Another sample was diluted 1:2 in phage antiserum. After 5 min at 37 °C two samples were removed from the serum tube. One sample was diluted into chloroform broth for free phage assay. The other was diluted 1:100 in broth and titrated for infective centres on strains PM5006 and N or NHI.

Strain	M.o.i.	Adsorbed phage (%)	Free phage after antiserum	No. infective centres on:		Fraction of infected bacteria producing centres on PM5006
				PM5006	N	
N	0.4	99.6	< 10^2	1.3×10^4	< 10^2	10^{-5}
NHI	0.5	99.6	< 10^2	2.7×10^8	< 10^2	I

Table 4. *Transduction of antibiotic resistance by HFT phages to strains of P. mirabilis*

One ml of a stationary phase suspension of bacteria was mixed with 0.1 ml phage lysate and incubated for 15 min before dilution in saline and filtration through Millipore membranes. Membranes with impinged bacteria were placed directly on minimal medium for transductions to prototrophy or on nutrient agar for 1 h for transductions of ampicillin and kanamycin resistance and for 3 h on nutrient agar for streptomycin resistance transductions, before transfer to selective media.

Transducing lysate	Recipient	M.o.i.	Transduction frequency/p.f.u. adsorbed or no. of transductants			
			K	AK	<i>str-r</i>	Prototrophy
5006MHFTk	PM804	0.9*	2	—	—	—
5006MHFTk	PM804	7.1*	3	—	—	—
5006MHFTk	PM804 <i>met-I</i>	7.3*	2	—	—	0
5006MHFTak	PM804	0.8*	1	0	—	—
5006MHFTak	PM804	8.1*	2	1	—	—
5006MHFTak	PM804 <i>arg-I</i>	8.0*	1	3	—	0
5006MHFTk	N	0.8	10^{-5}	—	—	—
5006MHFTak	N	1.1	—	4×10^{-5}	—	—
5006MHFTk	M <i>met-I</i>	3.1	9×10^{-4}	—	—	10^{-8}
5006MHFTk		0.9				
+	N		3×10^{-5}	—	—	—
5006M.PM5006 <i>str-r</i>		3.0				
5006MHFTk	NH1	0.8	10^{-4}	—	—	—
5006MHFTak	NH1 <i>met-I</i>	2.8	2×10^{-4}	2×10^{-4}	—	2×10^{-7}
5006MHFTak		0.7				
+	NH1		—	6×10^{-4}	—	—
5006M.PM5006 <i>str-r</i>		3.0				
5006M.PM5006 <i>str-r</i>	NH1	3.0	—	—	8×10^{-7}	—
5006M.PM5006 <i>str-r</i>	PM5006	3.2	—	—	8×10^{-7}	—

M.o.i., multiplicity of infection; other symbols as in Table 2.

* Multiplicity input.

antibiotics would be selected. Transfection was excluded, by finding that the appearance of the transductants was not influenced by the presence of DNAase. Many experiments did not yield PM804 transductants but this erratic behaviour could often be overcome by increasing the m.i. to about 8. This procedure did not necessarily increase the number of transductants obtained. Experiments with recipient cells in the exponential phase of growth did not affect the predictability or the number of transductants obtained. Abortive transductants were not detected and phage 5006M.PM5006 *str-r* at various m.i. had no effect on the transductions. This was probably due to poor adsorption which prevented simultaneous infection. The HFT phage lysates are capable of general transduction at low frequencies (Coetzee, 1974, 1975a) but, as expected, could not transduce auxotrophs of PM804 to prototrophy (Table 4, lines 3 and 6).

Strain N was transduced to antibiotic resistance at frequencies of 1×10^{-5} to 4×10^{-5} /p.f.u. adsorbed (Table 4, lines 7 and 8) in comparison to frequencies of about 5×10^{-2} /p.f.u. adsorbed for transductions to PM5006 (Coetzee, 1974, 1975a). With strain NH1 as recipient, transduction frequencies were raised about tenfold to 1×10^{-4} to 2×10^{-4} /p.f.u. adsorbed (Table 4, lines 11 and 12) and transductants were obtained at multiplicities of infection (m.o.i.) as low as 5×10^{-5} . The general transducing phage 5006M transduced both strain N and PM5006 to streptomycin resistance at frequencies of 8×10^{-7} /p.f.u. adsorbed (Table 4, lines 14 and 15). Why the HFT lysates did not transduce NH1 to antibiotic resistance at frequencies similar to those at which they transduced PM5006 is not known. There was a small difference in the e.o.p. of the phages on PM5006 and NH1 (Table 2) but this could

Fig. 1. Effect of u.v. irradiation on a phage 5006MHFTak lysate. Quantitative transduction experiments were done with the irradiated lysates at a m.o.i. of 1.2 p.f.u. of the unirradiated lysate. Strain NH1 was the recipient. ●, Transducing activity; ○, plaque formation.

hardly explain the differences encountered, and abortive transductants were not found. Simultaneous injection with phage 5006M.PM5006 *str-r* increased transduction frequencies for HFT phages in strains N and NH1 about threefold (Table 4, lines 10 and 13). Maximal effects with the former phage were reached at a m.o.i. of about 3. Auxotrophic mutants of strain NH1 were readily obtained and these, together with a mutant of strain N, were transduced to prototrophy by the HFT phage lysates at low frequencies, while transduction of mutants of strain NH1 was about ten times the frequency in N *met-1* (Table 4, lines 9 and 12).

Ultraviolet irradiation of transducing lysates

Irradiation of the HFT lysates produced a simple exponential decline in the frequencies of ampicillin and/or kanamycin resistance transduction in strains N and NH1 as a function of time of irradiation (Fig. 1). This was taken to mean that transduction proceeded by lyso-genization and not by double cross-over events (Luria *et al.*, 1960; Coetzee, 1974) between phage markers and the chromosomes of strains N or NH1.

Lysogenic status of PM804

No phage which plated on PM5006 could be isolated from PM804 by u.v. or mitomycin C induction. Treated cultures did not undergo the clearing suggestive of phage or bacteriocin liberation. Also, spontaneous plaques were never observed on lawns of PM804 and there was thus no evidence for PM804 being cryptically or frankly lysogenic (Krizsanovich, 1973).

Properties of transductants

All transductants of PM804 by both HFT lysates were lysogenic, in that they spontaneously liberated phage which plated on PM5006 in low titre (Table 5, line 1). Ultraviolet induction raised the titre to about 10^7 p.f.u./ml. Phage liberation occurred even with the few transductants which arose from experiments at a phage m.i. of 10^{-2} in which secondary infection was prevented. This phage had no visible effect on PM804, and when grown on PM5006 it did not transduce antibiotic resistance to any of the strains used. Transductants of PM804 could not be tested for immunity to lysis by homologous phage because the HFT phages had no visible effect even on the wild-type strain (Table 2). Transductants of strains N and NH1, produced at a m.o.i. less than 10^{-4} , were non-lysogenic in that they did not liberate, either spontaneously or following u.v. induction, phage which plated on PM5006 (Table 5, lines 2 and 4). Strain N transductants were also susceptible to 'spot' formation (Table 5, line 2) by the original lysates as well as phage 5006M and adsorbed the phages to the same

Table 5. *Retransduction of kanamycin-resistant transductants by phage 5006MHFTak lysate*

Transductions were done as outlined in the legend to Table 4.

Recipient transductant	Susceptibility to 5006MHFTk lysate	Phage liberation*	M.o.i.	Transduction frequency† A
PM804($\overline{K}$)‡	NVE	+	2§	3
N($\overline{K}$)	NPO	—	1·4	4×10^{-5}
N($\overline{K}$)	NVE	+	1·2§	11
NH1($\overline{K}$)	+	—	1·2	2×10^{-4}
NH1($\overline{K}$)	NVE	+	1·4§	32

NPO, Areas of partial clearing which disappeared on dilution of the lysate, no plaques observed; NVE, no visible effect; other symbols as in Table 2.

* Plating on PM5006.

† Transduction frequency/p.f.u. adsorbed, or number of transductants to ampicillin resistance.

‡ Superior line indicates that marker was transduced.

§ Multiplicity input.

extent as strain N. Strain NH1 transductants plated the phages (Table 2, line 25). At higher m.o.i., lysogenic transductants were obtained (Table 5, lines 3 and 5). These liberated phage and were converted (Coetzee, 1974) to non-adsorption of homologous phage (Table 2, line 24), just as single infection of NH1 by phage 5006M converted it to non-adsorption of the phage or its HFT derivatives (Table 2, line 26).

At least 10 % of clone-forming bacteria present in overnight broth cultures of all transductants had segregated the transduced markers. Segregants of phage 5006MHFTak transductants selected for sensitivity to ampicillin were invariably sensitive to kanamycin, and vice versa. The frequency of segregation was indicative of transduction by lysogenization in all instances, and transductants were thus heterogenote-like (Luria *et al.*, 1960; Coetzee, 1974; see also Woods & Thomson, 1975). This conclusion was supported by the effect u.v. irradiation of the transducing lysates had on transduction frequencies (Fig. 1). Kanamycin-resistant lysogenic transductants of PM804 could sometimes be retransduced to ampicillin resistance by phage 5006MHFTak lysates (Table 5, line 1) but the number of transductants was similar to the original transduction with PM804 as recipient (Table 4, lines 1 to 6). Kanamycin-resistant lysogenic and non-lysogenic transductants of strains N and NH1 could be retransduced to ampicillin resistance by phage 5006MHFTak lysates (Table 5, lines 2 to 5). Retransduction frequencies could not be determined for transductions to the lysogens as phage adsorption could not be demonstrated but there were more ampicillin-resistant transductants with strain NH1($\overline{K}$) than with N($\overline{K}$) lysogens, in keeping with primary transductions to strains NH1 and N, respectively. The phage adsorbed avidly to non-lysogenic kanamycin-resistant transductants and transduction of ampicillin resistance proceeded at frequencies (Table 5, lines 2 and 4) comparable to those at which strains N and NH1 were transduced (Table 4, lines 8 and 12). At low m.o.i. of superinfecting transducing phage, double transductants of strain NH1 were obtained which adsorbed and plated phage 5006M.

Properties of phage derived from transductants

Ultraviolet-induced lysates of PM804 antibiotic-resistant transductants regularly produced many more PM804 transductants than phage 5006MHFTk or 5006MHFTak lysates (Table 6, lines 1 and 2; Table 4, lines 1 to 6) but transduced PM5006 to antibiotic resistance at much lower frequencies than lysates of the latter two phages (Table 6, line 3). Phage in the PM804

Table 6. *Transducing ability of u.v.-induced lysates from PM804, N and NHI transductants*

Transductions were done as outlined in the legend to Table 4.

Phage lysate	Recipient	M.o.i.	Transduction frequency/ p.f.u. adsorbed or no. of transductants	
			K	AK
PM804(K)*	PM804	3†	25	—
PM804(AK)	PM804	3†	17	24
PM804(AK)	PM5006	1.2	—	10 ⁻⁵
PM804(AK)	PM5006	3	—	3 × 10 ⁻⁵
+ 5006M.PM5006 <i>str-r</i>		2		
PM804(AK)	PM5006	3	—	3 × 10 ⁻⁵
+ PM804(K)		15		
PM804(AK)	PM804(K)	2†	—	36
N(AK)	N	0.9	—	5 × 10 ⁻⁵
N(AK)	NHI	0.8	—	1 × 10 ⁻⁴
NHI(K)	N	0.3	10 ⁻⁵	—
N(AK)	PM5006	1.2	—	5 × 10 ⁻²

For symbols, see Table 2.

* Superior line indicates that markers were transduced.

† Multiplicity input.

transductant lysates could not be demonstrated to adsorb to PM804, while > 99 % adsorption occurred to PM5006, N and NHI (Table 2, lines 16 to 19). Simultaneous infection with phage 5006M.PM5006 *str-r* at a m.o.i. of 2 raised the PM804 lysate transduction frequency of antibiotic resistance to PM5006 about threefold (Table 6, line 4). Increase in the m.o.i. of the non-transducing phage had no further effect, and simultaneous infection (Table 6, line 5) with phage which bore an incompatible modification pattern did not further increase the transduction frequency to PM5006.

Failure to demonstrate phage adsorption to PM804 made it impossible to determine whether PM804 transductants were converted to homologous phage non-adsorption. However, PM804 kanamycin-resistant transductants could be retransduced to ampicillin resistance (Table 6, line 6), consistently and in greater numbers, by phage from PM804 transductants than with phage from PM5006 (Table 5, line 1); presumably one factor involved was the absence of lysogenic conversion in recipients. In a single-cycle growth experiment with PM5006 and phage present in PM804 transductant lysates, transmission was severely curtailed despite the fact that the lysate still transduced the strain at a frequency of 10⁻⁵/p.f.u. adsorbed (Table 6, line 3). This meant that PM804 modified the original transducing phage so that phage produced by it was susceptible to a restriction system present in PM5006. The fact that phage from PM804 transductants consistently produced more transductants in PM804 (Table 6, lines 1, 2 and 6) than the original phage (Table 4, lines 1 to 6) implied that PM804 also possessed a restriction system which recognized unmodified phage that happened to adsorb.

Ultraviolet-induced phage lysates of strains N and NHI transductants transduced N or NHI at frequencies similar to those of the original HFT phage lysates (Table 6, lines 7 to 9; Table 4, lines 7, 8 and 12). Transduction to PM5006 also occurred at frequencies similar to

Table 7. *Transduction of segregants of transductants*

Phage lysate	Recipient	M.o.i.	Transducing frequency/ p.f.u. adsorbed or no. of transductants	
			K	AK
5006MHFTk	PM804($\overline{K}$)K ^{s*}	3†	3	—
5006MHFTak	PM804($\overline{AK}$)A ^s K ^s	3†	1	4
PM804($\overline{AK}$)	PM804($\overline{K}$)K ^s	3†	40	46
5006MHFTak	N($\overline{AK}$)A ^s K ^s	0.8	—	4×10^{-5}
5006MHFTak	NH1($\overline{K}$)K ^s	0.7	—	10^{-4}
5006MHFTak	NH1($\overline{K}$ $\overline{AK}$)A ^s	0.7	—	10^{-4}
N($\overline{AK}$)	N($\overline{K}$)K ^s	0.7	—	3×10^{-5}

For symbols, see Table 2.

* Superior line indicates that marker was transduced; superior s signifies segregant of particular marker.

† Multiplicity input.

those produced by these lysates (Table 6, line 10). These results were anticipated as the strains did not modify phage from PM13 (Coetzee & Smit, 1969) or PM5006 (Table 3, line 1).

The segregation properties of kanamycin-resistant transductants (PM804($\overline{K}$)) retransduced to ampicillin and kanamycin resistance (PM804($\overline{K}$ $\overline{AK}$)) by u.v.-induced lysates of PM804($\overline{AK}$) were investigated. While overnight broth cultures of these double transductants contained an average of 16.4 % ampicillin-sensitive segregants, the cultures only produced an average of 2.3 % kanamycin-sensitive clones. Thus the markers of each transducing phage segregated independently.

Properties of segregants

The lysogenic status of segregants was invariably similar to that of parent transductants. Segregants of PM804 transductants were transduced to antibiotic resistance by the phage lysates in similar numbers to those produced with PM804 as recipient (Table 7, lines 1 and 2; Table 4, lines 1 to 6). This was further evidence in favour of inability of the transducing phages to convert hosts to homologous phage non-adsorption. Just like transductants, segregants too were transduced by phage from PM804 transductants in greater numbers and more consistently (Table 7, line 3; Table 6, line 6) than PM804 was transduced by lysates 5006MHFTk or 5006MHFTak (Table 4, lines 1 to 6). The fact that lysates of PM804($\overline{AK}$) produced similar numbers of transductants with recipients PM804($\overline{K}$) as with segregant PM804($\overline{K}$)K^s (Table 6, line 6; Table 7, line 3) could have meant that phage 5006MHFTak did not integrate in the kanamycin resistance region of the resident prophage. Non-lysogenic segregants of strains N and NH1 transductants were transduced to antibiotic resistance at frequencies similar to those at which strains N and NH1 were transduced (Table 7, lines 4 and 5; Table 4, lines 8 and 12). Transduction of ampicillin resistance with phage 5006MHFTak to ampicillin-sensitive kanamycin-resistant single segregants of double non-lysogenic transductants of strain NH1 also occurred at frequencies similar to those of ampicillin plus kanamycin resistance transduction to NH1 by phage 5006MHFTak lysates (Table 7, line 6; Table 4, line 12). It is noteworthy that the homology offered by resident prophage 5006MHFTk did not affect transduction frequencies. Also, u.v.-induced lysates of strain N transductants transduced strain N non-lysogenic segregants at the same frequencies as phage 5006MHFTak (Table 7, lines 4 and 7). This was confirmation that strain N failed to modify phage from PM5006 (Table 3, line 1).

Heat treatment of strains

Due to poor adsorption, the effect of heat treatment of PM804 on phage transmission could not be tested. Treatment of strain N for periods of 5 and 20 min at various temperatures between 40 and 65 °C immediately before use in transmission experiments with a 5006MHFTak phage lysate did not increase transmission frequencies above that recorded in Table 3, line 1. Heat treatment of PM804 or strain N before use in transduction experiments with HFT lysates did not make results with PM804 more predictable or increase the number of transductants, nor were transduction frequencies of markers to strain N increased.

DISCUSSION

The selection provided by the high frequency antibiotic resistance transducing phages derived from *P. mirabilis* strain PM5006 has revealed additional hosts, *P. vulgaris* strain PV127 (Coetzee, 1975*b*) and *P. mirabilis* PM804, on which the phage lysates do not have macroscopic effects. These hosts have permitted transduction across a species barrier and the Dienes incompatibility line (Dienes, 1946, 1947; see Coetzee, 1972).

Use of strain NH1 provided transductants which for the first time (Coetzee, 1974, 1975*a, b*) proved to be non-lysogenic, and which were heterogenote-like and segregated markers at high frequency. In these properties they resembled the single particle transductants produced by specialized variants of phage P22 (Hoppe & Roth, 1974) but differed from the phage-sensitive transductants produced by single infection with HFT lysates of phage lamda (Campbell, 1957) which were stable rather than heterogenotic. Here then was evidence for a defect in a maturation function of the HFT phages in that they could not register as plaque-forming particles (see Coetzee, 1975*b*). Transductants of strain NH1 confirmed a suggestion (Coetzee, 1975*b*) that both the HFT phages were defective in conversion of hosts to non-adsorption of homologous phages. There is no evidence that NH1 carries a related prophage (see Coetzee & Smit, 1969) and single infection by the parent phage 5006M was clearly capable of converting NH1 to phage non-adsorption (Table 2, line 26). Defectiveness of the conversion gene(s) was supported by recovery of serial double transductants of NH1 which still adsorbed and plated homologous phage: provided the prophages were situated in tandem on the chromosome, this mode of integration clearly did not assist in reconstituting a functional gene.

The observation that HFT lysates transduced lysogenic transductants in the absence of demonstrable adsorption implied that measurement of phage adsorption was not as sensitive as transductant selection and that lysogenic conversion to phage non-adsorption did not constitute an absolute barrier to homologous phage. Despite the fact that phage 5006MHFTak carries an additional marker, no other difference was detected between it and phage 5006MHFTk.

The failure to detect abortive transductants of antibiotic resistance with all three recipients corresponds to results obtained with transductions of these markers to *P. vulgaris* strain PV127 (Coetzee, 1975*b*). If there was sufficient microhomology in a *P. vulgaris* strain for the phages to integrate, it could be assumed that this also held true for the *P. mirabilis* hosts studied here.

The reason why all PM804 transductants were lysogenic for phage plating on PM5006 is obscure. The absence of demonstrable phage adsorption to PM804, coupled with the use of low phage input, ensured that transductants arose from single phage infection. As there was no evidence for strain PM804 being cryptically lysogenic, the explanation given previously

(Coetzee, 1974) for all PM5006 transductants by phage 5006MHFTk being frankly lysogenic, does not apply.

The fact that lysates of PM804 transductants yielded transducing phage which adsorbed well to PM5006 but could not be shown (by adsorption tests) to adsorb to PM804, ruled out host range mutants of the phages as the cause of the original PM804 transductants. The phenotype of PM804 differed from that of *P. vulgaris* strain PV127 which did not modify the *P. mirabilis* transducing phages (Coetzee, 1975*b*). The transducing lysates of 5006MHFTk and 5006MHFTak thus not only adsorbed poorly to PM804 but had to contend with the restriction system of the latter organism. While the first obstacle remained, transducing phage derived from PM804 transductants possessed a compatible modification pattern. Taken in conjunction with the fact that the phage in single infection did not convert PM804 to homologous phage non-adsorption, this could explain why phage from PM804 transduced PM804 more readily than the original phages from PM5006. Due to the high potential transduction frequency, these lysates also retransduced appropriate lysogenic transductants despite possible lysogenic conversion to phage non-adsorption. Thus strain PM804, like PV127 (Coetzee, 1975*b*) was not an ideal host for determining the properties of the HFT phage lysates.

In contrast to PM804 were strain N and its restrictionless mutant NHI; both appear to be modification-deficient, and lysates of transductants of these strains transduced PM5006 at high frequency (see Table 6, line 10) while the parent strains were transduced at frequencies similar to those of the phage 5006MHFTk and 5006MHFTak lysates respectively (Table 4, lines 8 and 12; Table 6, lines 7, 8 and 9).

Despite the low transmission of the HFT phages in strain N, the transduction frequencies to strain N were never $< 10^{-1}$ of corresponding frequencies to strain NHI in which transmission was 100 % (Table 3, line 2). This phenomenon was noted with general transduction to these strains (Coetzee & Smit, 1969) and could mean that the particular markers transduced were protected from restriction enzymes, or possibly that transduced genetic material lacked sites susceptible to restriction enzymes of strain N.

The cause of the increase in transduction frequencies which simultaneous infection with non-transducing phage effected was most likely to have been the result of a helper effect rather than a multiplicity effect (Lederberg, 1965; Coetzee, 1974; see also Uetake, Toyama & Hagiwara, 1964). This was supported by the fact that non-transducing phage which bore an incompatible modification pattern did not have a greater effect than compatible phage and that maximal effects were attained at a m.o.i. of about 3. The fact that transduction frequencies to strain NHI (which is restrictionless) were increased to the same extent and at similar m.o.i. by this phage, also favoured a helper effect. The helper effect was small in comparison with that extended in transductions by the HFT phages to the homologous host PM5006 (Coetzee, 1974, 1975*a*). For strain N this result could (in the absence of a multiplicity effect) be due to the fact that phage 5006M was also restricted, but this would not apply to strain NHI. However, the latter strain was derived from strain N by nitroso-guanidine treatment and changes other than merely the selected mutation could be involved (see Coetzee & Smit, 1969). For instance, another discrepancy noted with strain NHI was that phage 34.NHI had an e.o.p. of 5×10^{-4} on strain N (Coetzee & Smit, 1969) while u.v.-induced lysates of lysogenic strain NHI transductants did not form plaques on the former strain (Table 2, line 23).

Another peculiarity of the systems investigated was that postulated restriction endonucleases could not be demonstrated to be heat susceptible.

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