Transduction of Leucine Auxotrophs of
Proteus mirabilis to Prototrophy or Antibiotic Resistance by
P. mirabilis High Frequency Transducing Bacteriophages

By J. N. COETZEE AND KATHRYN KRIZSANOVICH-WILLIAMS
South African Medical Research Council Microbial Genetics Research Unit,
Department of Microbiology, University of Pretoria, South Africa
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SUMMARY
High frequency transducing (HFT) phages 5006MHFTk and 5006MHFTak for
kanamycin or ampicillin plus kanamycin resistance, derived from Proteus mirabilis
strains PM5006(R394) and PM5006(R394) respectively, transduced (at low multiplicities of infection, m.o.i.) antibiotic resistance and prototrophy to PM5006 leu-I
at high frequency. Simultaneous transduction of these markers occurred at very
much lower frequencies. The latter result was correlated with the proportion of
multiply-infected bacteria which, due to the great transducing potential of the
phage, could register as transductants. Each HFT lysate was thus heterogenous
with regard to high frequency transducing phage. Apart from the additional anti-
biotic resistance marker carried by one phage, no other difference between the two
lysates was detected. High segregation frequencies of antibiotic-resistant or proto-
trophic transductants indicated transduction by lysogenization. Although anti-
biotic-sensitive segregants of antibiotic-resistant prototrophic transductants
occurred at high frequency, no auxotrophic segregants of these transductants were
found. This suggests transduction by a double cross-over event in the leucine region.
Most transductants, even at low m.o.i., were lysogenically converted to homologous
phage non-adsorption as a result of interaction between the transducing phage
genome and the resident cryptic prophage. They could, however, be retransduced
by appropriate phage lysates; thus, lysogenic conversion to non-adsorption was
not absolute. Some prototrophic transductants were non-lysogenic although their
seggregants liberated low-titre phage. The latter anomaly, and the fact that the
leucine marker and antibiotic resistance were not cotransduced, are explained by
the mode of integration of the phage into the host chromosome in relation to the
resident cryptic prophage and the leucine region.

INTRODUCTION
Proteus mirabilis strain PM5006 is cryptically lysogenic for prophage 5006M (Krizsanovich,
1973; Coetzee, 1974). When strain PM5006 is relysogenized with phage 5006M it no longer
adsorbs homologous phage. This is due to lysogenic conversion by phage 5006M (Krizsanovich,
1973; Coetzee, 1961, 1974). To account for the absence of lysogenic conversion to
non-adsorption in the cryptic lysogen PM5006, it was suggested that the responsible gene is
inactivated by the manner in which the cryptic prophage is integrated (Bertani, 1970). In
the double lysogen, the second phage integrates either at a different episite so maintaining
the integrity of the conversion gene, or at the original episite in tandem to the cryptic prophage
to reconstitute this gene (Krizsanovich, 1973).
Coetzee (1974, 1975a) described the formation of two derivatives of strain PM5006,
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namely PM5006(R394) and PM5006(R394), which in addition to the cryptic prophage also contain the phage used to transduce R factor R394. Ultraviolet induction of these strains yields lysates 5006MHFTk and 5006MHFTak, capable of high frequency transduction of kanamycin resistance and of ampicillin plus kanamycin resistance respectively. Although phages 5006MHFTk and 5006MHFTak are defective in lysogenic conversion (J. N. Coetzee, unpublished), strains PM5006(R394) and PM5006(R394) are converted to non-adsorption of homologous phage (Coetzee, 1974, 1975a). This suggested that phages 5006MHFTk and 5006MHFTak have partially-functional conversion genes which interact in some way with the corresponding gene(s) of the resident cryptic prophage to yield an active product. This supports the hypothesis that in strain PM5006(5006M) the gene for lysogenic conversion is reconstituted by tandem lysogenization.

Ultraviolet induction of strain PM5006(5006M) yielded lysates capable of transducing PM5006 leu-I to prototrophy at frequencies of $1 \times 10^{-4}$ to $4 \times 10^{-5}$/plaque-forming unit (p.f.u.) adsorbed (Krizsanovich-Williams, 1975). The same lysates transduced other auxotrophs of strain PM5006 at frequencies $1 \times 10^{-2}$ to $1 \times 10^{-3}$/p.f.u. adsorbed. The leucine transductants were heterogenotes and segregated the marker. These findings suggested that the prophages were integrated in close proximity to the leucine region on the host chromosome. Lysates of phages 5006MHFTk and 5006MHFTak are also capable of general transduction (Coetzee, 1974, 1975a) and this paper describes their ability to transduce leucine auxotrophs to prototrophy and the properties of the transductants.

METHODS

Organisms. Bacteria and phages are listed in Table 1.

Media and antibiotics. These have been described (Coetzee, 1974, 1975a). Incubation temperature was 37°C.

General phage techniques. These were according to Adams (1956). Phage 5006M antiserum with a $K_v$ value of 180 min$^{-1}$ was that previously used (Krizsanovich-Williams, 1975).

Phage adsorption. The methods of Coetzee (1975b) were used.

Transduction. This was done by the methods of Coetzee (1974). In critical experiments phage antiserum was added to the adsorption mixture after 10 min. After a further 10 min incubation the adsorption mixture was filtered with a large excess of saline. Except for transductions to streptomycin resistance (Coetzee & Sacks, 1960) membranes with impinged cells were placed directly on appropriate selective media, as resistance to 50 μg kanamycin and ampicillin/ml is expressed immediately after the adsorption period (Coetzee, 1974, 1975a). For streptomycin resistance, transduction membranes were incubated on nutrient agar for 3 h before transfer to streptomycin-containing media.

Segregation. The methods of Coetzee (1974) and Krizsanovich-Williams (1975) were used.

RESULTS

Transduction of PM5006 leu-I by high frequency transducing lysates

Lysates of phages 5006MHFTk and 5006MHFTak were capable of transducing PM5006 leu-I to antibiotic resistance [PM5006 leu-I(K), PM5006 leu-I(AK)] at high frequencies, prototrophy [PM5006 leu-I(proto)] at frequencies about three times lower, and both prototrophy and antibiotic resistance [PM5006 leu-I(protoK), PM5006 leu-I(protoAK)] at very much lower frequencies (Table 2). Homologous non-transducing phage at a multiplicity of infection (m.o.i.) of 3, increased the transduction frequency of all markers about threefold
Leucine and antibiotic resistance transduction

Table 1. Bacteria and phages

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Properties*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus mirabilis</em> PM5006</td>
<td>Host for phages used. Cryptically lysogenic</td>
<td>Coetzee &amp; Smit (1970)</td>
</tr>
<tr>
<td></td>
<td>for phage 5006M. Sensitive to ampicillin</td>
<td>Krizsanovich (1973)</td>
</tr>
<tr>
<td></td>
<td>and kanamycin</td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em> PM5006(5006M)</td>
<td>Relysogenized with phage 5006M</td>
<td>Krizsanovich (1973)</td>
</tr>
<tr>
<td><em>P. mirabilis</em> PM5006 leu-1</td>
<td>Leucine auxotroph of PM5006</td>
<td>Krizsanovich-Williams (1975)</td>
</tr>
<tr>
<td>Bacteriophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5006MHFTk</td>
<td>Derivative of phage 5006M capable of high</td>
<td>Coetzee (1974, 1975b)</td>
</tr>
<tr>
<td></td>
<td>frequency transduction of K. Produced by</td>
<td>Coetzee <em>et al.</em> (1973)</td>
</tr>
<tr>
<td></td>
<td>u.v. induction of PM5006(R394)</td>
<td></td>
</tr>
<tr>
<td>5006MHFTak</td>
<td>Derivative of phage 5006M capable of high</td>
<td>Coetzee (1975a, b)</td>
</tr>
<tr>
<td></td>
<td>frequency transduction of A and K.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Produced by u.v. induction of PM5006(R394)</td>
<td></td>
</tr>
<tr>
<td>5006M PM5006 str-r</td>
<td>Lysate of phage 5006M lytically prepared on</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PM5006 str-r</td>
<td></td>
</tr>
<tr>
<td>5006M PM5006 leu-1</td>
<td>Lysate of phage 5006M lytically prepared on</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PM5006 leu-1</td>
<td></td>
</tr>
</tbody>
</table>

* K, A and str-r signify resistance to kanamycin, ampicillin and streptomycin respectively. Superior line indicates that markers of R factor R394 were transduced to PM5006. Double superior line indicates that markers of R factor R394 were transduced to a derivative of PM5006(R394).

Table 2. Transduction of PM5006 leu-1 by lysates of high frequency transducing phages

One ml organism was incubated with 0.1 ml phage suspension for 15 min. Dilutions of the adsorption mixture were then filtered with an excess of saline and membranes were placed directly on appropriate media for 48 h.

<table>
<thead>
<tr>
<th>Lysate</th>
<th>M.o.i.</th>
<th>Prototrophy</th>
<th>K</th>
<th>A and K</th>
<th>Prototrophy and A or K</th>
</tr>
</thead>
<tbody>
<tr>
<td>5006MHFTk</td>
<td>0.1</td>
<td>4 x 10^3</td>
<td>1 x 10^-2</td>
<td>1 x 10^-6</td>
<td></td>
</tr>
<tr>
<td>5006MHFTk</td>
<td>0.01</td>
<td>4 x 10^3</td>
<td>1 x 10^-2</td>
<td>1 x 10^-8</td>
<td></td>
</tr>
<tr>
<td>5006MHFTk</td>
<td>0.001</td>
<td>4 x 10^3</td>
<td>1 x 10^-2</td>
<td>&lt; 1 x 10^-9</td>
<td></td>
</tr>
<tr>
<td>5006MHFTk</td>
<td>0.0001</td>
<td>4 x 10^3</td>
<td>1 x 10^-2</td>
<td>&lt; 1 x 10^-9</td>
<td></td>
</tr>
<tr>
<td>5006MHFTk</td>
<td>0.1</td>
<td>1 x 10^-2</td>
<td>3 x 10^-1</td>
<td>5 x 10^-6</td>
<td></td>
</tr>
</tbody>
</table>

+ 5006M, PM5006 leu-1  3.0  6 x 10^3  2 x 10^-2  3 x 10^-6

+ 5006M, PM5006 leu-1  3.0  6 x 10^-3  2 x 10^-2  1 x 10^-8

+ 5006M, PM5006 leu-1  3.0  2 x 10^-2  5 x 10^-2  8 x 10^-6

M.o.i., multiplicity of infection.

* Symbols as in Table 1.

(Table 2, lines 5 and 10). This helper effect was less marked than that recorded previously (Coetzee, 1974, 1975a) but corresponded to the effect encountered with phage 5006M transductions of PM5006 leu-1 to prototrophy (Krizsanovich-Williams, 1975). Abortive transductants were not found. At lower m.o.i., while the frequencies of transduction to prototrophy and to antibiotic resistance remained unchanged, there was a dramatic reduction in the frequency of simultaneous transduction to antibiotic resistance and prototrophy. This
Table 3. Stability of transduced markers

Appropriate dilutions of overnight broth cultures of transductants were plated on MacConkey agar and the growth replicated to selective media.

<table>
<thead>
<tr>
<th>Transductant*</th>
<th>Lysogenic</th>
<th>Kanamycin-sensitive</th>
<th>Auxotrophic</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM5006 leu-1(K)</td>
<td>+</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>PM5006 leu-1(proto)</td>
<td>+</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>PM5006 leu-1(proto)</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>PM5006 leu-1(proto K)</td>
<td>+</td>
<td>7</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

* K, resistance to kanamycin; proto, prototrophy; superior line indicates that the marker was transduced.

result was due to the proportion of multiply-infected bacteria even at low m.o.i., and implied that for transduction to antibiotic resistance plus prototrophy multiple infection was required. Most experiments were done with both phages but since results were similar, mainly those with phage 5006MHFTk lysates are presented.

Properties of transductants

Overnight broth cultures of auxotrophic and prototrophic kanamycin-resistant transductants contained 5 and 7% kanamycin-sensitive segregants [PM5006 leu-1(K)K*, PM5006 leu-1(protoK)K*], respectively (Table 3, lines 1 and 4). Whereas lysogenic prototrophic transductants gave rise to 6% auxotrophs [PM5006 leu-1(proto)Leu-1], non-lysogenic prototrophic transductants segregated the leucine marker at much higher frequencies (Table 3, lines 2 and 3). Kanamycin-resistant prototrophic transductants, in contrast, yielded no auxotrophic segregants (Table 3, line 4). The high segregation frequencies of both kanamycin-resistant transductants and prototrophic transductants indicated transduction by lysogenization with the formation of heterogenotes and heterogenote-like transductants (Luria, Adams & Ting, 1960; Coetzee, 1974). Transductants with markers of both phages 5006MHFTk and 5006MHFTak (J. N. Coetzee, unpublished) segregate the markers of the two phages independently at high frequencies. Apart from invoking a double cross-over in the leucine region, no explanation can be given for the failure to detect auxotrophic segregants of kanamycin-resistant prototrophic transductants.

All antibiotic-resistant transductants and most prototrophic transductants at low m.o.i. were lysogenic and spontaneously liberated phage which plated on PM5006. These transductants were lysogenically converted (Coetzee, 1974) and no adsorption of homologous phage could be demonstrated by phage adsorption experiments. Some prototrophic transductants were non-lysogenic in the above sense but their auxotrophic segregants could be induced with u.v. light to liberate phage of low titre active on PM5006.

Lysogenic and non-lysogenic transductants could be retransduced by lysates of phages 5006MHFTk and 5006MHFTak. Whereas non-lysogenic transductants were retransduced at the same frequencies as the initial transductions of PM5006 leu-1 (not shown), lysogenic recipients produced relatively few retransductants (Table 4). Strain PM5006(5006M) as a recipient for the transducing phages also produced a similar number of transductants to that yielded by lysogenic transductants (Table 4, line 5).
Leucine and antibiotic resistance transduction

Table 4. Retransduction of lysogenic transductants and segregants by high frequency transducing lysates

Transductions were done as outlined in Table 2. For streptomycin resistance, membranes were incubated on nutrient agar for 3 h before transfer to selective medium.

<table>
<thead>
<tr>
<th>Lysate</th>
<th>Recipient*</th>
<th>Multiplicity of input</th>
<th>Prototrophy</th>
<th>K</th>
<th>A and K</th>
<th>str-r</th>
</tr>
</thead>
<tbody>
<tr>
<td>5006 MHFTk</td>
<td>PM5006 leu-1(proto)</td>
<td>0:1</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5006 MHFTk</td>
<td>PM5006 leu-1(K)K</td>
<td>0:1</td>
<td>11</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5006 MHFTk</td>
<td>PM5006 leu-1(proto)Leu</td>
<td>0:1</td>
<td>17</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5006 MHFTak</td>
<td>PM5006 leu-1(K)K</td>
<td>0:1</td>
<td>25</td>
<td></td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>5006 MHFTak</td>
<td>PM5006(5006M)</td>
<td>0:1</td>
<td>2.0†</td>
<td></td>
<td>35</td>
<td>124</td>
</tr>
<tr>
<td>5006 M, PM5006 str-r</td>
<td>PM5006</td>
<td>2:0</td>
<td>2.0</td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

K, resistance to kanamycin; K*, kanamycin sensitive segregant; proto, prototrophy; Leu−, leucine auxotrophic segregant.

* Superior line indicates that the marker was transduced.
† M.o.i.

DISCUSSION

*Proteus mirabilis* strains PM13 (Coetzee, 1961) and PM5006 (Krikanovich, 1973), lysogenized by serologically identical phages 34 and 5006M, fail to adsorb homologous phage as determined by phage adsorption experiments. Lysogenic transductants of strain PM5006 leu-1 could be retransduced by lysates of phages 5006 MHFTk and 5006 MHFTak (Table 4). A possible explanation is that these transductants were not fully converted to phage non-adsorption. However, since a wild-type lysogen which was lysogenically converted could also be transduced, a more probable explanation is that lysogenic conversion to phage non-adsorption is not as absolute as was thought. The fact that phage 5006 M could not be shown to adsorb to phage 5006 MHFTk transductants (Coetzee, 1974) discouraged attempts to transduce those transductants to ampicillin resistance with phage 5006 MHFTak lysates (Coetzee, 1975a). These results emphasize the superiority of high frequency transducing phages in the demonstration of phage adsorption (Kondo & Mitsuhashi, 1966; Goldberg, Bender & Streicher, 1974; Coetzee, 1975b).

Another unique situation created by the potential of these phage lysates to transduce at high frequencies was the seemingly linked transduction of the leucine marker with antibiotic resistance. The rapid reduction in the frequency of prototrophic antibiotic-resistant transductants with decreasing m.o.i., however, indicated that these transductants were the result of multiple infection (Table 2). At a m.o.i. of 0.01 the proportion of bacteria multiply-infected is 10−1. With the high frequency transducing phage, this proportion of bacteria simultaneously infected with antibiotic resistance as well as leucine transducing phages could still be detected as transductants. Here then is evidence for heterogeneity among high frequency transducing phage particles present in the HFT lysates. An explanation for the fact that antibiotic resistance and leucine prototrophy were not co-transduced is that if the antibiotic resistance transducing phage had integrated in tandem to the cryptic prophage on the side distal to the leucine gene, the distance between the antibiotic resistance and leucine markers was too great for both to be incorporated in the same capsid.

The reason why antibiotic-resistant transductants and some prototrophic transductants were lysogenic and lysogenically converted may be similar to that previously given (Coetzee,
1974, 1975a), namely an interaction between the resident cryptic prophage and the genome of the transducing particle. The observation that some kanamycin-sensitive prototrophic transductants were non-lysogenic while their segregants proved lysogenic is a contradiction which may be explained in terms of the mode of integration of the transducing phage. The leucine transducing phage may presumably integrate in the cryptic prophage or in the contiguous leucine region as it has homology with both. On the assumption that with the transductants in question integration occurred in the leucine region, placing the functional leucine marker between the two prophages, recombination between homologous regions of the phages could result in loss of the leucine marker while a complete prophage genome was maintained. Resulting segregants would have a similar prophage constitution to the cryptically lysogenic \textit{PM}5006 \textit{leu-1} strain. This interpretation is supported by the high segregation frequency of these transductants and the observation that the phage lysates obtained after u.v. induction of their segregants did not have the high titres of lysates usually obtained by induction of double lysogens. Lysates induced from these segregants had the low titres associated with phage 5006M which is sporadically liberated by cryptic lysogens (Krizsanovich, 1973). The fact that the low titre phage was obtained more consistently from these segregants points to some difference between them and the wild-type cryptic lysogen.

Apart from the ampicillin resistance marker transduced by phage 5006MHFTak, no other phenotypic difference between lysates of this phage and phage 5006MHFTk has been detected (see Coetzee, 1975a, b).

**REFERENCES**


