Assignment of Two New Host Range Types to the P2 Family of Temperate Coliphages

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SUMMARY

Six non-inducible coliphages which grow on Escherichia coli C but not on K12 (C-specific) were shown to be antigenically related to P2. All six were shown to be P4 helpers and some of them could also recombine with P2.

All known temperate coliphages of the P2 family share the following characteristics. They have the same host range, they serologically cross-react with all corresponding antisera, and their prophages are not readily inducible by u.v. radiation (Bertani & Bertani, 1971). Coliphages possessing these attributes have been recovered from the natural habitats of Escherichia coli and their distribution patterns described (Dhillon \textit{et al.}, 1976). We have also isolated phages that are not inducible by u.v. but can grow on P2-resistant strains of \textit{E. coli} (Dhillon & Dhillon, 1981). In this communication, we describe properties of some of these phages which have enabled us to assign them to the P2 family.

Including P2, a total of nine phages were used for this comparative study. The P2 host range phage HK239 has been shown to be closely related to P2 (Dhillon & Dhillon, 1973a). Its exclusion-defective mutant \textit{excl} (Dhillon & Dhillon, 1973b) was used in the present study but will be referred to as HK239. The third phage which also has the host range of P2 is HK321 (Dhillon \textit{et al.}, 1982). All three phages can grow on both the C and the K12 strains of \textit{E. coli}.

The remaining six phages which are the principal object of this study were recovered from naturally occurring lysogenic bacteria. Lysogens isolated from human rectum yielded HK122 and HK124 and a coliform bacterium from a bovine rectal swab was the source of HK180. HK162, HK166 and HK167 were recovered from bacteria isolated from sewage. Like phage P2, the isolates HK239, HK 321 and HK122 can grow on both the C and the K12 strains of \textit{E. coli} but the other five grow only on \textit{E. coli} C. These five will be referred to as the C-specific phages.

Clear plaque mutants of most of the above phages were selected after \textit{N}-methyl-\textit{N}'-nitro-\textit{N}-nitrosoguanidine treatment but some were of spontaneous origin. Those which formed plaques on the lysogens harbouring the homologous prophage were designated as virulent (\textit{vir}). HK166, HK167, HK122, HK239 and HK321 readily yielded \textit{vir} mutants. Clear plaque phage mutants were used for the selection of phage-resistant bacteria.

Bacteria selected for resistance to P2, HK239 or HK321 were found to be resistant to all three but were sensitive to HK122 and to the five C-specific phages. Conversely, mutants selected for resistance to any one of the C-specific phages were found to be resistant to all five but were invariably sensitive to HK122 and the three P2 host range phages. Hence, the P2 host range phages and the C-specific phages were considered to possess different adsorption specificities. HK122 is clearly quite different in its host range from phages of the other two groups.

Bacteria selected for resistance to HK122 were found to be resistant to all nine phages. Some but not all of them were also found to be incapable of growth on MacConkey agar or on nutrient agar containing 0.15% bile salts. These bile salts-sensitive mutants were also found to be resistant to T7 and \textit{\lambda}. The lipopolysaccharide core mutants (\textit{lpc}) of \textit{E. coli} K12 have been reported to be resistant to T7 and \textit{\lambda} (Tamaki \textit{et al.}, 1971). We have tested the \textit{lpc}A2 mutant of...
Tamaki et al. and have found it to be bile salts-sensitive. To map the gene conferring pleiotropic resistance to T7, λ and HK122, and sensitivity to bile salts, an HK122-resistant, bile salts-sensitive mutant of the donor bacterial strain HfrH was selected. It was mated with an F′ thr-leu gal str r strain. One-hundred thr+leu+str r recombinants were tested but none was found to be either sensitive to bile salts or resistant to HK122 although 26% were gal+ . Thus, the mutant allele conferring resistance to HK122 and sensitivity to bile salts is not located between the origin of HfrH and the gal locus.

Serological cross-reactions were tested using antisera against phages HK239 and HK124. With anti-HK239 serum, the inactivation rate constants (k) of the P2 host range phages were 169, 246 and 252, for P2, HK239 and HK321, respectively, while the values of k for the five C-specific phages ranged from 47 to 117; for HK122 the value of k was 99. In contrast to this were the results with anti-HK124 serum. With the exception of HK166, which gave a k value of only 28, the C-specific phages gave k values ranging from 233 to 402, while for the three P2 host range phages k values of 44 to 47 were found. All nine phages are thus antigenically related; however, phages of the same host range group are antigenically more closely related to one another than to phages of the other host range group.

Lysogenic bacteria harbouring a given prophage were tested with lysates of all nine phages to identify their immunity specificities. Virulent mutants were also used to confirm the homoiimmune relationships and to rule out the possibility of exclusion of the superinfecting phage. Four distinct immunity specificities were recognized: P2, HK162, HK180; HK122, HK124; HK166, HK321; HK167, HK239 (groups of homoiimmune phages are separated by semicolons). These observations reveal the existence of wild-type phages which may be looked upon as recombinants. For example, phages HK162 and HK180 showed the P2 immunity specificity but belong to the C-specific host range group.

The defective (helper-dependent) phage P4 can grow only on bacteria harbouring P2 or a related helper phage (Six & Klug, 1973). Tests were therefore carried out to see whether HK122 and some of the C-specific prophages support the growth of P4.

P4 is usually amplified on lysogens harbouring the cox mutant of P2. P2 cox is defective in excision (Bertani & Bertani, 1971) but can furnish all the functions that are needed for the synthesis of P4 virions. The resulting lysates contain very few P2 cox particles. P4 lysates prepared on P2 cox lysogens were plated on lysogenic bacteria harbouring a given prophage. The titre of P2 virions was determined by plating on the non-lysogenic parental strain and was found to be 10^3 p.f.u./ml. The same lysate, however, when plated on the lysogenic bacteria registered titres which were two to three orders of magnitude higher. The increased titres were clearly due to the presence of P4 virions which did not form plaques on the non-lysogenic strain but were able to do so on the lysogenic strain. The prophages of the lysogenic strains were thus characterized as P4 helpers.

Lysates were made from plaques of P4 on various helper phage lysogens. The host range and the serological properties of these P4 virions were identical with the corresponding properties of the helper phage. For example, P4 particles grown on a strain lysogenic for HK122 could form plaques on strains resistant to P2 or HK124 if these strains harboured another P4 helper prophage (Table 1). Also, a P4 lysate prepared on strain C(HK321) gave a k value of 284 with anti-HK239 serum but a k value of only 42 with anti-HK124 serum. Conversely, P4 grown on an HK124 lysogen gave k values of 50 and 243 for anti-HK239 and anti-HK124 sera, respectively. We are thus able to confirm the results of Six & Klug (1973) who first showed that the P4 virions reflect the phenotype of the helper prophage.

A crucial test for establishing phylogenetic kinship between two life forms is their ability to recombine under normal conditions. Attempts were therefore made to detect virions of recombinant genotypes displaying the immunity specificity of one parent and the host range determinant of the other. Bacterial strains selective for the detection of recombinant phages were prepared. Mixtures of parental phage strains were placed on agar overlay plates of strain E. coli C1a and the progeny phage were eluted in broth containing chloroform. Aliquots of the resulting phage suspensions were plated on a non-lysogenic indicator strain and on strains selective for the recombinant particles. From phage cross P2 × HK124, the frequency of
**Table 1. P4 virions of different host range**

<table>
<thead>
<tr>
<th>Indicator strain phenotype*</th>
<th>HK124-resistant (prophage)</th>
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<tbody>
<tr>
<td></td>
<td>N.L. (HK124)</td>
</tr>
<tr>
<td>HK122 phage-resistant (prophage)</td>
<td>$3 \times 10^3$</td>
</tr>
<tr>
<td>HK2oxy phage-resistant (prophage)</td>
<td>$1 \times 10^3$</td>
</tr>
<tr>
<td>HK321 phage-resistant (prophage)</td>
<td>$1 \times 10^3$</td>
</tr>
<tr>
<td>HK124 phage-resistant (prophage)</td>
<td>$1 \times 10^3$</td>
</tr>
<tr>
<td>HK167 phage-resistant (prophage)</td>
<td>$1 \times 10^3$</td>
</tr>
</tbody>
</table>

* Indicator strains are phage-resistant. N.L. is the non-lysogenic strain. The prophages of the lysogenic derivatives are shown in parentheses. No plaques were observed on lysogenic or non-lysogenic bacteria resistant to phage HK122.

† P4 lysates were prepared on lysogens harbouring prophage shown in this column.

‡ From the number of plaques observed on the non-lysogenic strain, C1a.

§ NP, No plaques.

ImmP2h124 particles appearing on a P2-resistant, HK124 lysogenic strain was found to be $4 \times 10^{-2}$ and that of imm124hP2 particles on the HK124-resistant, P2 lysogenic strain was found to be $2 \times 10^{-5}$. By similar means successful genetic recombination between P2 on the one hand and HK122 or HK167 on the other was readily demonstrable. These and other data presented above fully justify the assignment of HK122 and the five C-specific phages to the P2 family.

We have attempted to isolate stable, doubly lysogenic bacteria harbouring phenotypically distinguishable prophages. For some pairs of phages this has been possible but for the others not. For example, upon superinfection of P2 lysogens, double lysogens of prophage constitution P2, HK122 or P2, HK124 were only infrequently recoverable; when further analysed, they gave rise to variable numbers of single lysogenic segregants. Similar behaviour was shown by HK239, HK321 double lysogens. In contrast to the above, double lysogens of constitution P2, HK239 or P2, HK321 were readily obtained and upon subculturing evinced no loss of either prophage. From these and other similar results we tentatively consider prophages P2, HK239 and HK321 as compatible with one another and prophages HK122 and HK124 as incompatible with P2. It is very likely that the compatibility is a reflection of heterologous attachment specificities and that incompatibility indicates homology of prophage attachment specificity. This, however, remains to be verified by the use of integration-negative phage mutants.

In conclusion, our results have uncovered an additional genetic polymorphism in the P2 family of phages. Specifically, we have shown the existence of phages which are antigenically related to P2 and can recombine with it, but have a different host range. All of them can help the growth of P4 and can be used to obtain P4 virions which possess adsorption specificities different from that of P2.

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**REFERENCES**


Short communication


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