Bacteriophage Ec1-resistant Mutants of Enteric Bacteria

By S. B. PRIMROSE

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL

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INTRODUCTION

Strains C-M7 and C-M9 are His⁺ Nif⁺ *Escherichia coli* hybrids derived from a cross between a *hisD* strain of *E. coli* and a prototrophic nitrogen-fixing strain, M5a1, of *Klebsiella pneumoniae* (Dixon & Postgate, 1972). In strain C-M7, a segment of the *E. coli* genome, which includes the *hisD* gene has been replaced by a corresponding segment of the *K. pneumoniae* genome (Cannon *et al.*, 1974a). In strain C-M9, a plasmid carrying the *K. pneumoniae his* and *nif* genes is present as well as the original *hisD* mutation (Cannon *et al.*, 1974b). Both strains are sensitive to phage Ec1 whereas the two parent strains are resistant. In addition, both the hybrids and the *K. pneumoniae* parent are resistant to phages λ , T7 and ϕ X174 whereas the *E. coli* parent is sensitive to these phages. Since the *rfb* genes, which determine the biosynthesis of the O-repeat units of lipopolysaccharides, are located close to the histidine operon in *Salmonella typhimurium* (Sanderson, 1972) and *E. coli* K12 (Taylor & Trotter, 1972), it was assumed that the hybrids had acquired similar genes from *K. pneumoniae*. Laboratory strains of *E. coli* are rough (Jones, Koeltzow & Stocker, 1972) and lack both the *rfb* and *rfc* loci. Transfer of the *rfb* genes to *E. coli*, and their expression there, would make the strains semi-rough. Thus, Cannon *et al.* (1974a) argued that if *K. pneumoniae* were smooth, and phage Ec1 specific for semi-rough strains, the sensitivity of the hybrids and the resistance of the parents would be explained. Evidence is now presented to show that this explanation cannot be correct.

METHODS

Bacteria. *Escherichia coli* strain C603 is *hisD arg* Str^R Ec1^R λ ^S T7^S ϕ X174^S. *K. pneumoniae* M5a1 is prototrophic, nitrogen-fixing and resistant to Ec1, λ , T7 and ϕ X174. *Escherichia coli* C-M7 is a hybrid in which the *nif* genes and at least the *hisD* gene from M5a1 have been integrated into the chromosome of C603; it is Ec1^S λ ^R T7^R ϕ X174^R. These strains have been described previously (Cannon *et al.*, 1974a). *Salmonella typhimurium* LT7 is Ec1^S and P22^S and was supplied by C. Colson. *Salmonella typhimurium* UNF602, which carries the FN68 plasmid (Dixon, 1974), is *hisD* Str^R F' *his*⁺ *nif*⁺ *rfb*⁺ and was supplied by J. Postgate.

Media. Nutrient broth (Oxoid no. 2) was used as a complete medium. Minimal medium was the E mineral salts medium of Vogel & Bonner (1956) with amino-acid requirements supplied at a concentration of 25 μ g ml⁻¹. Nitrogen-free medium consisted of MgSO₄, 0.1 g; Na₂MoO₄.2H₂O, 25 mg; FeSO₄.7H₂O, 25 mg; K₂HPO₄, 12.06 g; KH₂PO₄, 3.4 g; glucose, 20 g; in 1 litre distilled water. All media were solidified by the addition of Difco Special Noble agar to a final concentration of 1.4 % (w/v).

Tests for nitrogen fixation. Growth in nitrogen-free liquid medium and ability to reduce acetylene were tested by the Parkhurst tube technique (Postgate, 1972).

Selection of bacteriophage-resistant bacteria. An overnight broth culture of bacteria (0.1 ml) was added to molten nutrient agar (2 ml) held at 45 °C, and the mixture poured over the

surface of a nutrient agar plate. When this overlay had set, one drop of phage suspension (titre about 7×10^8 plaque forming units (p.f.u.)/ml) was placed in the centre of the plate. One colony from the area of lysis was chosen from each plate and purified by restreaking.

Phage-sensitivity tests. Agar overlays of bacteria were prepared as described above and small drops (about 0.03 ml) of phage suspension implanted on the surface. After incubating overnight, the plates were examined for lysis.

Detection of O antigens. The presence of O or R antigens was determined by slide agglutination in 0.3 % auramine solution (Schmidt *et al.*, 1969).

Gluconate 6-phosphate dehydrogenase (gnd). Bacteria were treated with chloroform and the gnd product was assayed by the colorimetric method of Peyru & Fraenkel (1968).

Infection of spheroplasts. Spheroplasts were infected with viral DNA as described by Guthrie & Sinsheimer (1963).

RESULTS AND DISCUSSION

Strains of *S. typhimurium* LT7 were sensitive to Ec1 but they cannot be semi-rough for they were also sensitive to P22 which is known to be smooth-specific (Naide *et al.*, 1965). Either the receptor for Ec1 does not lie in the O-repeat unit of the lipopolysaccharide (LPS) or else some of the cores have only one O-repeat unit attached while others have more than one. To distinguish between these possibilities, 100 independent Ec1-resistant and 100 independent P22-resistant mutants were isolated. None of the Ec1-resistant mutants was P22-resistant; and none of the P22-resistant mutants was Ec1-resistant, even though some of the latter were rough, as indicated by their agglutination with auramine. Clearly the receptors for the two phages have nothing in common and, since the P22 receptor is located in the LPS, the Ec1 receptor, at least in *S. typhimurium*, must lie elsewhere.

On the basis of the results with *S. typhimurium*, it is unlikely that there is a relationship between the Ec1 receptor and the *rfb* locus in *E. coli*. Further proof of this came from studies of Gal⁻ mutants. Mutants of *E. coli* which were either *galE* or *galU* are phenotypically rough because they are unable to synthesize complete LPS cores. As such they were resistant to T7 which requires complete LPS cores for attachment (Lindberg, 1973). When the *his nif* element FN68 was transferred to such strains they became sensitive to Ec1 even though there was not a complete LPS core for attachment of the O-repeat units. Cells of *K. pneumoniae* and *E. coli* failed to adsorb phage Ec1 and this was the sole basis of their resistance, because spheroplasts of both could be infected with phage DNA with an efficiency of 10^{-6} p.f.u./DNA molecule. Since sensitivity to Ec1 could be conferred upon *E. coli* by transfer of the *his* and *nif* genes from *K. pneumoniae*, the receptor for Ec1 must not only be present in *K. pneumoniae* but must be coded by a *his-nif* linked gene(s). The phenotypic resistance of *K. pneumoniae* was probably due to other wall components. Indeed, some mutants resistant to the phage ϕ Kp4, which plates on *K. pneumoniae* M5a1, were sensitive to Ec1.

The *rfb* genes of *K. pneumoniae* were probably expressed in the *E. coli* hybrids C-M7 and C-M9 because these strains were not only resistant to the rough-specific phages T7 and ϕ X174 but, unlike the parental *E. coli* C603, they were not agglutinated by auramine. In addition, hybrids C-M7 and C-M9 were resistant to bacteriophage λ . When the phage sensitivities of 216 independent Ec1^R derivatives of C-M7 were examined, five different classes were discerned (Table 1). Strains in classes I and II were resistant to all four bacteriophages, provided low titre phage stocks were used. When high titre stocks were employed, host range mutants of Ec1, capable of plating on members of class I, were readily isolated. These host range mutants failed to plaque on members of class II. Class I probably contains those

Table 1. *Properties of the different classes of Eci^R mutants of E. coli C-M7*

Class	Number of strains	Properties
I	30	His ⁺ Gnd ⁺ Nif ⁺ Eci ^R λ ^R T7 ^R φX ^R
IIA	12	His ⁺ Gnd ⁺ Nif ⁺
IIB	20	His ⁺ Gnd ⁺ Nif ⁻
IIC	34	His ⁺ Gnd ⁻ Nif ⁻
IID	10	His ⁻ Gnd ⁻ Nif ⁻
IIIA	14	His ⁺ Gnd ⁺ Nif ⁺
IIIB	3	His ⁺ Gnd ⁺ Nif ⁻
IIIC	1	His ⁺ Gnd ⁻ Nif ⁻
IVA	46	His ⁺ Gnd ⁺ Nif ⁺
IVB	2	His ⁺ Gnd ⁻ Nif ⁻
VA	4	His ⁺ Gnd ⁺ Nif ⁺
VB	2	His ⁺ Gnd ⁺ Nif ⁻
VC	2	His ⁺ Gnd ⁻ Nif ⁻
VD	36	His ⁻ Gnd ⁻ Nif ⁻

strains with a point mutation in the gene controlling Eci sensitivity, whereas class II contains those strains with a deletion in this gene. Strains grouped in class III were sensitive to λ but resistant to Eci, T7 and φX174. Class IV contains those strains which were sensitive to λ and T7 but not Eci or φX174, and class V those strains which were resistant only to Eci. Unlike members of the first three classes, all strains in class IV and V agglutinated in the presence of 0.3 % auramine, indicating that they were rough.

Theoretically, eight sensitivity patterns could be obtained with three phages but, since only four of these were found in Eci^R derivatives of C-M7, the resistance determinants, whatever their nature, must have been lost in an ordered fashion. Sensitivity to T7 and ability to be agglutinated by auramine were acquired simultaneously, so the resistance to T7 in C-M7 was probably a consequence of the presence of the *rfb* gene cluster. Presumably other wall components, coded by Klebsiella-derived *his*-linked genes, were responsible for the resistance of C-M7 to λ and φX174. Because of the limited number of phage-sensitivity patterns observed, these genes would have to be arranged to one side of the Eci-sensitivity gene. Furthermore, since His⁻ Gnd⁻ Nif⁻ segregants could be found which were still resistant to λ, T7 and φX174, these genes must lie on the other side of the Eci-sensitivity gene.

Since Eci could be used to select His⁻, Gnd⁻ and Nif⁻ derivatives of *E. coli* C-M7, it should be possible to select similar mutants in other Eci-sensitive strains. However, none of 500 Eci-resistant mutants of *S. typhimurium* examined were either His⁻ or Gnd⁻. Similarly, 500 Eci^R mutants of a nitrogen-fixing clinical isolate of *K. pneumoniae*, UW66, were still His⁺ Gnd⁺ Nif⁺. These results suggest that the Klebsiella-derived genes in *E. coli* C-M7 are inherently unstable.

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