Characterization of B278, a Phage Different from Mu That Also Produces Auxotrophic Mutations in *Escherichia coli* K12

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Bacteriophage B278 has been characterized and compared with Mu, the only phage known to produce random mutations in *E. coli*. Although both phages are morphologically indistinguishable and have a similar host range, they clearly differ at both the protein and the DNA level. B278 apparently possesses a DNA protection mechanism that is different from the *mom* system described for Mu.

INTRODUCTION

Bacteriophage Mu, the first prokaryotic transposable element to be described, took its name from 'mutator' because of the new phenotypes acquired by cells of *Escherichia coli* K12 after being infected by the phage (Taylor, 1963; Howe & Bade, 1975). Since its isolation, only one other phage able to produce random mutations in *E. coli* has been described (Mise, 1971). This phage, named D108, has been shown to be 95% homologous to Mu at the DNA level (Hull *et al.*, 1978; Faelen & Toussaint, 1980; Gill *et al.*, 1981). Phages with similar properties have also been described in *Vibrio cholerae* (Johnson & Romig, 1981) and in *Pseudomonas aeruginosa* (Krylov *et al.*, 1980; Rehmat & Shapiro, 1983; Akhverdian *et al.*, 1984, 1985).

In this paper we describe B278, an *E. coli* phage, first isolated by Fusté *et al.* (1980), which like Mu and D108 also produces a high number of auxotrophic mutants among the survivors of its infection. Nevertheless, our results clearly show that B278 differs from Mu when analysed at the DNA and the protein level.

METHODS

Bacterial and phage strains. Throughout the work B278 was grown in *E. coli* K12 C600 (F⁻ *thr-1 thi-1 leuB6 lacY1 tonA21 supE44 λ*⁻). Bacteriophage Mu_{cts} was obtained by induction of the lysogenic strain *E. coli* K12 LC31 (provided by M. Chandler, Toulouse, France). The other bacterial strains used in this work were from the American Type Culture Collection (ATCC) or from the Collection of the Facultat de Farmàcia, Universitat de Barcelona, Spain (CFB).

Isolation of phages and phage DNA. Phages B278 and Mu were prepared according to the methods described by Maniatis *et al.* (1982). For DNA extraction the phage particles were purified on CsCl block gradients according to Davis *et al.* (1980).

Restriction endonucleases. These were purchased from Boehringer, Amersham or Biolabs and were used as specified by the manufacturers.

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Agarose gel electrophoresis. DNA fragments, generated by restriction endonucleases, were separated by electrophoresis on agarose gels (Sigma type II; 0.8%, w/v) in TBE (90 mM-Trizma base, 90 mM-boric acid, 2.5 mM-Na₂EDTA, pH 8.3) or TAE (40 mM-Trizma base, 18 mM-acetic acid, 2 mM-Na₂EDTA, pH 8.1) and visualized by ethidium bromide fluorescence.

SDS-PAGE. Virion proteins were prepared by adding 0.2 ml of a solution containing 0.5 M-Tris/HCl, 1% (w/v) SDS, 1% β -mercaptoethanol, 10% (v/v) glycerol and 0.001% bromophenol blue to 0.1 ml of phage suspension (10^{10} – 10^{11} p.f.u. ml⁻¹). Before loading the gel, the mixture was boiled (5 min). Polyacrylamide gels were prepared as described by Bravo (1984). Molecular mass markers (Sigma MW-SDS-70L kit) were used and silver staining was as described by Wray *et al.* (1981).

Diagnosis of auxotrophs. Survivors of phage infection were plated out in both LB and M9 minimal medium agar plates (Davis *et al.*, 1980). The diagnosis was done according to the method of Holliday (1956), as adapted by Clowes & Hayes (1968) and Davis *et al.* (1980).

Electron microscopy. A drop of concentrated phage suspension ($>10^{11}$ p.f.u. ml⁻¹) was placed on a Formvar-carbon coated copper grid and negatively stained with 2% (w/v) uranyl acetate. Stained virions were observed with a Philips EM 200 electron microscope operating at 60 kV.

One step growth curve. This experiment was done as described by Clowes & Hayes (1968).

Determination of phage adsorption rates. Adsorption rates were determined by measuring the unadsorbed phages (Adams, 1959). Exponential bacterial cultures were infected at a m.o.i. of 0.1 p.f.u. per c.f.u. and kept at 37 °C without shaking. Samples were periodically withdrawn and treated with chloroform.

RESULTS AND DISCUSSION

General characterization of phage B278

Electron microscopy of purified phages revealed that the B278 virion consists of a polyhedral head connected to a contractile tail (Fig. 1). These data indicate that B278 is of Type A in the Bradley classification (Bradley, 1967). The head is 58 nm in diameter, and the tail is 102 nm long and 16 nm wide. The buoyant density of phage particles estimated by centrifugation in a continuous CsCl gradient was 1.450 g cm⁻³.

The adsorption kinetics and one step growth experiments showed that 90% of the bacteriophage population was adsorbed to *E. coli* K12 C600 after incubation (10 min, 37 °C), that the latent period was 28 min and that the burst size was about 70 p.f.u. per infected bacterium.

The morphological and kinetic characteristics of B278 closely resemble those reported for bacteriophage Mu (Howe & Bade, 1975).

Host range and immunity properties

B278 and Mu were tested for their ability to form plaques on different bacteria. Using the agar-overlay method and spot lysis test, it was determined that B278 was able to grow on *E. coli* (strains ATCC 27325, ATCC 25947, ATCC 23725, K12 C600 and K12 W3110), *Citrobacter freundii* (ATCC 11606), *Acinetobacter calcoaceticus* CF7 (CFB) and on two species of *Salmonella* (*S. typhimurium* V577 and *S. typhi* ser. London, CFB). No plaque formation was observed on *Klebsiella pneumoniae* (ATCC 10031 and ATCC 13883), *Proteus vulgaris* (ATCC 8427), *Proteus mirabilis* (ATCC 7002), *Enterobacter aerogenes* (ATCC 15038), *Azotobacter chroococcum* CF17 (CFB), *Pseudomonas acidovorans* CF102 (CFB), *Pseudomonas aeruginosa* CF103 (CFB), *Bordetella bronchiseptica* (ATCC 10580) and *Alcaligenes faecalis* (ATCC 8750). The principal difference between B278 and Mu was that the latter was not active on the *Salmonella* strains, in agreement with the host range reported in the literature (Howe & Bade, 1975; Faelen *et al.*, 1981). Other differences were observed when clinical isolates of *E. coli* were used as hosts. In this case, most of the strains were either sensitive or resistant to both phages, although some strains were infected only by B278 or by Mu.

Another approach to compare the infectivity of both phages was to study their interaction with cell surface receptors. By analysing the cell surface lipopolysaccharide (LPS) structures of several *E. coli* K12 C600 strains resistant to MuG(+), Sandulache *et al.* (1984) identified the MuG(+) primary receptor site in the outer end of the LPS. According to the carbohydrate composition of the LPS in the resistant strains and by phage typing, Sandulache *et al.* (1984)

Fig. 1. Electron micrograph of bacteriophage B278. (a) Phage showing the contracted tail; (b) typical structure of the virion. Bar, 0.1 μ m.

Fig. 2. Proposed structure of the LPS of different *E. coli* K12 mutants resistant to bacteriophage Mu (according to Sandulache *et al.*, 1984).

classified these mutants strains, resistant to Mu, into six groups, each one with a different carbohydrate composition in the LPS. We have tested the ability of B278 to infect mutants belonging to groups 1, 2 and 5 of this classification. The LPS structures of these mutants are shown in Fig. 2. B278 is able to infect mutants of groups 1 and 2, but not group 5 mutants. These results show that the structural requirements of the LPS receptor for B278 are clearly different from those described for Mu. From the results of typing the Mu-resistant strains with different phages (D108, P1, P7 and T4), the adsorption spectrum of B278 closely resembles that of phage T4.

B278 was shown to be heteroimmune to Mu and D108 since it was able to infect the Mu lysogens UQ129, LC31, MAL103 and AT861, and a C600 strain containing a D108 prophage.

Induction of auxotrophic mutations

When *E. coli* K12 C600 was infected by bacteriophage B278, resistant clones appeared at a mean frequency of about $1-2 \times 10^{-5}$. Of these clones 1-2% were found to have acquired new nutritional requirements. These results are in agreement with those previously obtained by Fusté *et al.* (1980).

Table 1 shows the distribution of the auxotrophic mutations in 35 new mutants. Out of 25 nutrient requirements tested using the Holliday test we found auxotrophies for 10 of them.

Fig. 3

Fig. 4

Fig. 3. SDS-PAGE of Mu (lane 1) and B278 (lane 2) proteins. The migration of protein standards is indicated on the left.

Fig. 4. Agarose gel electrophoresis of restriction fragments generated by digestion of: B278 DNA with *Hind*III (lane 1); Mu DNA with *Hind*III (lane 2); B278 DNA with *Xba*I (lane 3); and Mu DNA with *Xba*I (lane 4). The positions of DNA standards are indicated on the left.

Table 1. *Number of auxotrophic mutants found among survivors of E. coli K12 (strain C600) after infection with bacteriophage B278*

Metabolite required	No. of mutants	Metabolite required	No. of mutants
Proline	17	Glutamic acid	1
Cysteine	6	Aspartic acid	1
Isoleucine	3	Uracil	1
Valine	2	Tryptophan	1
Methionine	2	Tyrosine	1

Despite the low number of auxotrophic strains studied, the results obtained show a bias towards mutations affecting proline and cysteine metabolism.

Protein components of the virions

Our objective was to compare the protein components of B278 and Mu virions. The electrophoretic pattern obtained after the separation of the virion proteins on an SDS-

polyacrylamide gel is shown in Fig. 3. B278 virions contained two major polypeptides of 41 and 14 kDa, as well as other minor polypeptides. The pattern obtained for Mu, which is consistent with published data (Giphart-Gassler *et al.*, 1981), clearly differs from that found for B278.

DNA restriction analyses

B278 and Mu DNAs were digested with several restriction endonucleases. The results of the electrophoretic separation of the DNA fragments produced by *Hind*III and *Xba*I are shown in Fig. 4. Other restriction enzymes able to cleave Mu DNA, like *Bam*HI, *Bgl*II, *Bgl*II, *Kpn*I and *Sal*I, were inactive on B278 DNA. In all cases, Mu DNA restriction patterns were consistent with previous data (Allet *et al.*, 1977; Kahmann *et al.*, 1977; Marrs & Howe, 1983; Toussaint, 1985). These results indicate that B278 and Mu are clearly different at the DNA level.

In addition, it is interesting to note that the genome length of B278 DNA, estimated from the fragments produced by digestion with *Xba*I, is about 87 kb, more than twice the value reported for the Mu genome. Although there is no strict correlation between the head size and the genome length among different bacteriophages characterized so far (Birge, 1981), it should be pointed out that the genome length estimated for B278 is much higher than those described for other phages with similar head sizes. Nevertheless, several lines of evidence indicate that the fragments generated by *Xba*I from B278 DNA, and from which the B278 genome size was estimated, correspond to a complete digestion pattern. Thus the pattern was very reproducible and was not modified either by incubation with higher concentrations of *Xba*I or by longer incubation times. Also, when λ DNA was introduced in the digestion reaction as an internal control, it was completely digested. Additional information supporting the complete digestion of B278 DNA with *Xba*I was obtained after the controlled digestion of B278 DNA with nuclease BAL-31 and further digestion with *Xba*I. Under these conditions, only two specific fragments were shortened in size.

Another interesting feature of B278 DNA is its refractoriness to cleavage by many restriction enzymes (eleven out of sixteen tested). This suggests the possibility that B278 might possess a protection mechanism to evade the host restriction systems. Such mechanisms have been widely described for different phages (Warren, 1980; Sharp, 1986). Nevertheless, the protection mechanism should be different from the *mom* system described for bacteriophage Mu (Kahmann, 1984) since most of the enzymes that do not cleave B278 DNA are not affected by this system (Kahmann *et al.*, 1985).

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