Characterization of Temperate Bacteriophage Ba Isolated from *Streptomyces lavendulae*

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(Received 16 April 1980; revised 21 July 1980)

An actinophage designated Ba was isolated from *Streptomyces lavendulae* S283. Phage Ba produced turbid plaques characteristic of temperate phage on *S. lavendulae* and other *Streptomyces* strains. Electron microscopic observation showed that phage Ba belongs to group B of Bradley's morphological classification. Surviving cells from the plaques were lysogenic: they liberated phages during growth and were not lysed by the phage. Phage Ba was immediately inactivated on dilution into NaCl unless Ca²⁺ was also present. The cleavage pattern of the phage DNA was studied using restriction endonucleases.

INTRODUCTION

We have studied a pleiotropic mutation affecting secondary metabolism in *Streptomyces lavendulae* S55-B1 (Matsubara-Nakano *et al.*, 1980; Nakano & Ogawara, 1980). Although a transposition or an insertion of unstable genetic elements may be involved in pleiotropic mutations in *Streptomyces* species (Redshaw *et al.*, 1979; Sermonti *et al.*, 1978, 1980), they have not yet been identified. There are reports of the transposition of DNA to phages in other bacteria (Kleckner *et al.*, 1977). Once a genetic element is transposed to a phage, it is easily identified by restriction enzyme cleavage analysis or by heteroduplex analysis. We have searched for temperate phages infecting *S. lavendulae* S55-B1. In this paper some characteristics of phage Ba isolated from another strain (S283) of *S. lavendulae* are described.

METHODS

Bacterial strains. *Streptomyces lavendulae* strains used were S55-B1 (ATCC 8664), S57, S263 (IFO 3146), S283, S328, S329 (IN 309T), S589 (IFO 12789), S664 (IFO 12903) and S985 (DSM 40), kindly provided by Dr A. Seino, Kaken Chemical Co. They were maintained on agar slants of GAA medium [containing (% w/v): glucose, 1.0; asparagine, 0.05; sodium asparaginate, 0.1; MgSO₄, 0.05; KH₂PO₄, 0.1; CaCO₃, 0.05 agar, 2.0; pH 7.0 (Ogawara, 1975)] or on Bennett's medium (Matsubara-Nakano *et al.*, 1980).

Media. Nutrient broth contained 0.8% (w/v) Difco Bacto-nutrient broth. Nutrient agar and soft agar were 0.8% (w/v) Difco Bacto-nutrient broth solidified by adding 1.5 and 0.6% (w/v) agar, respectively. After autoclaving, nutrient broth and nutrient agar were supplemented with sterile solutions of glucose (final concentration 0.5%), Ca(NO₃)₂ and MgCl₂ (final concentrations 10 mM each). Soft agar was also supplemented with Ca(NO₃)₂ and MgCl₂.

Phage assay. A spore suspension (0.1 ml) was mixed in 2.5 ml soft agar with 0.1 ml of the phage sample. Phage lysates were prepared and diluted with nutrient broth. The mixture was maintained at 47 °C. A preliminary experiment showed that incubation at 47 °C for up to 1 h did not reduce the phage titre. The mixture was poured on to a 20 ml nutrient agar base layer. Plates were incubated at 27 °C overnight.

Isolation of phage. Nine strains of *S. lavendulae* were cultured for 2 d in 100 ml nutrient broth at 27 °C with shaking. After centrifugation the supernatant from each strain was tested for phages capable of infecting strain

S55-B1. A temperate phage Ba was isolated from the culture broth of strain S283. Phage Ba was purified by three successive single plaque isolation steps and a confluent lysate was prepared. Confluent lysis plates were extracted by soaking at room temperature for 1 h with 5 ml nutrient broth. The slurry was collected in tubes, and agar and cellular debris were removed by centrifugation at 1200 g for 10 min and by membrane filtration (Millipore, pore size 0.22 μm).

Electron microscopy. Confluent lysis plates were extracted by the method described above, but using 5 ml 10 mM-Tris/HCl containing 10 mM-MgCl₂ (pH 7.5). A drop of phage suspension was negatively stained with uranyl acetate on carbon-coated copper grids for electron microscopy.

Concentration and purification of phage. Phage lysates were prepared from 20 plates (13.5 $\times$ 9.5 cm) as described above and incubated for 20 min at 37 °C with DNAase I (from bovine pancreas; Worthington Biochemical Corp.; 1 $\mu\text{g ml}^{-1}$) to remove the host DNA. The phage lysates were centrifuged at 40 000 g for 2 h. The phage pellets were then resuspended in 5 ml TBT buffer [0.1 M-Tris/HCl containing 0.1 M-NaCl, 10 mM-MgCl₂ and 10 mM-Ca(NO₃)₂, pH 7.5] and stored overnight at 4 °C. The phage suspension was centrifuged again at 8000 g for 10 min and the supernatants were overlaid on to a preformed CsCl gradient (densities of 1.3, 1.4 and 1.7 g cm⁻³). After centrifugation at 80 000 g for 4 h, the phage fraction was isolated and dialysed against TBT buffer.

Isolation of phage DNA. The purified phage suspension was mixed gently with 0.01 vol. 0.2 M-EDTA (pH 7.5) and 0.01 vol. 20% sodium dodecyl sulphate. After the addition of 1 vol. phenol presaturated with 0.1 M-Tris/HCl containing 0.1 M-NaCl (pH 7.5), the solution was mixed at 0 °C for 15 min by gentle rotation using the motor of a rotary evaporator. The aqueous phase was removed after low-speed centrifugation and dialysed against 10 mM-Tris/HCl containing 10 mM-NaCl and 1 mM-Na₂EDTA (pH 7.6).

Cleavage of phage DNA with restriction enzymes. Restriction enzymes *EcoRI*, *BamHI*, *HindIII* and *SalI* were from Bethesda Research Laboratories, Md, U.S.A., and λ DNA was from Boehringer. DNA (0.6 to 1.0 μg in 40 μl) was digested at 37 °C for 30 min according to the assay conditions described in the manufacturer's directions. The reaction was stopped by adding 10 μl of a solution containing 50% (w/v) sucrose, 100 mM-Na₃EDTA and 0.025% (w/v) bromophenol blue and heating the mixture at 70 °C for 5 min.

Agarose gel electrophoresis of DNA fragments. Agarose gels (0.7%, w/v) were prepared by the method of Aaij & Borst (1972), except that a vertical slab gel apparatus (13.5 $\times$ 13.5 $\times$ 0.3 cm) was used. Electrophoresis was carried out at 40 mA for 4 h. The gels were stained with ethidium bromide (1 $\mu\text{g ml}^{-1}$ in water) and DNA bands were visualized by fluorescence under ultraviolet light.

RESULTS AND DISCUSSION

Isolation and host range of phage Ba

Phage Ba was isolated from a broth culture of strain S283. It produced turbid plaques on a lawn of strain S55-B1. Among nine *S. lavendulae* strains, however, S985 and S57 had increased susceptibilities to Ba. The efficiency of plating (e.o.p.) on S985 and S57 was several fold higher than on other strains. Moreover, the two strains showed different plaque morphology. Ba produced plaques 0.5 to 1 mm in diameter on most strains of *S. lavendulae* but on S985 and S57 the plaques were 2 to 3 mm in diameter and were clearer. After further incubation, colonies appeared in the centre of the plaques.

The host range of phage Ba was examined by plaque formation and was shown to be relatively wide. Of 11 strains tested, eight (*S. flavus* IFO 3359, *S. microflavus* ETH 24414, *S. somaliensis* ATCC 19017, *S. kasugaensis*, *S. parvullus* ATCC 12434, *S. lipmanii* ISP 5070, *S. lavendulae* and *S. cattleya* NRRL 8057) were sensitive to Ba and the rest [*S. californicus* ATCC 3312, *S. felleus* NRRL 2251 and *S. coelicolor* A3(2)] were resistant.

Electron microscopic observation of Ba lysates showed phage particles with hexagonal heads and long, flexible, non-contractile tails (Fig. 1). The phage belongs to group B of Bradley's morphological classification.

Requirement of Ca²⁺ and Mg²⁺ for stability of phage Ba and plaque formation

The effect of Ca²⁺ and Mg²⁺ on the e.o.p. was assayed by plaque formation on strain S985. Appropriate dilutions of the phage lysate were mixed with suspensions of spores of S985 and plated on nutrient agar containing a range of concentrations of Ca(NO₃)₂ and MgCl₂. The concentration of Ca²⁺ and Mg²⁺ in the soft agar was the same as that in the base layer. In the

Fig. 1. Electron micrograph of a Ba particle negatively stained with uranyl acetate. Bar marker represents 100 nm.

Table 1. *Effects of Ca²⁺ and Mg²⁺ on infection by phage Ba*

Cation concns (mM)		Relative e.o.p.
Ca ²⁺	Mg ²⁺	
10	10	1.00
5	5	1.20
10	25	1.23
25	10	0.82
25	25	0.33
10	0	0.29
0	10	0.55
0	0	0.00

Table 2. *Effects of Ca²⁺ and Mg²⁺ on the stability of phage Ba*

Phage Ba was suspended in 0.85% NaCl with various concentrations of Ca²⁺ and Mg²⁺ and assayed immediately or after 2 d storage at 4 °C.

Cation concns (mM)		Relative e.o.p.	
Ca ²⁺	Mg ²⁺	At 0 d	After 2 d
10	10	1.00	0.56
1	1	1.36	0.49
10	0	0.98	0.58
0	10	0.04	0.12
0	0	0.00	0.00

absence of both Ca²⁺ and Mg²⁺, no plaques were detected even if 10³ plaque-forming phages were added (Table 1). The absence of either of the cations decreased the e.o.p. In the presence of excess Ca²⁺, the outline of the plaques was difficult to discern because the agar became turbid. We decided to use 10 mM-Ca²⁺ and 10 mM-Mg²⁺ routinely for the plaque assay.

The influence of Ca²⁺ and Mg²⁺ on the stability of the phage was also examined. The phage lysate in nutrient broth was diluted 1000-fold with various liquid media and assayed immediately. Infection with diluted phage was performed in the presence of Ca²⁺ and Mg²⁺. Surprisingly, 0.85% NaCl without Ca²⁺ inactivated phage quickly and irreversibly (Table 2). In the presence of Ca²⁺, the phage titre remained more than 50% after storage at 4 °C for 2 d.

Isolation of a lysogen

Surviving clones of strain S985 in plaques after infection with phage Ba were isolated and streaked on a plate of Bennett's medium. A single clone (S985a) was isolated and the production of Ba from a shaken culture of S985a was examined. Strain S985a was suspended

Fig. 2. Agarose gel electrophoresis of the DNA of phages Ba and λ digested with restriction endonucleases. A, *EcoRI*-digested λ DNA; B, *EcoRI*-digested Ba DNA; C, *HindIII*-digested Ba DNA; D, *BamHI*-digested Ba DNA; E, *SaII*-digested Ba DNA. Electrophoresis was carried out in 0.7% agarose gels as described in Methods.

in 0.85% NaCl to inactivate intact phages and cultured in nutrient broth with shaking. The culture broth of S985a formed plaques on a lawn of strain S985. Strain S985a itself was insensitive to infection by phage Ba. These results showed that S985a was lysogenic. Other clones isolated from plaques were also shown to be lysogenic.

Cleavage of phage Ba DNA by restriction enzymes

The molecular mass of phage Ba DNA was approximately 20 to 30 megadalton, as determined by agarose gel electrophoresis. Treatment of Ba DNA with *EcoRI* produced two fragments. A short fragment was estimated to be about 3.4 megadalton, using fragmented λ DNA digested by *EcoRI* as a molecular mass standard. Phage Ba DNA had no cleavage site for *HindIII* or *BamHI*. It was, however, fairly susceptible to *SaII* (Fig. 2).

Several temperate actinophages have been isolated from soil samples (Dowding & Hopwood, 1973) and cultures of *Streptomyces* strains (Lomovskaya *et al.*, 1972; Hranueli *et al.*, 1979). Phage Ba might be suitable for gene transfer experiments because of its wide host range and single cleavage site for *EcoRI*. Recently several cleavage studies of phage DNA of *Streptomyces* species have been reported. Phages R4 (Chater, 1979; Chater & Carter, 1978, 1979) and SH10 (Klaus *et al.*, 1979) have been shown to have relatively wide host ranges and have the same numbers of cleavage sites for *EcoRI*, *BamHI* and *HindIII* as phage Ba.

suggesting that they may be closely related (K. F. Chater, personal communication). The *Sal*I digest bands in Fig. 2 were almost identical to those of phage R4 (Chater & Carter, 1979). Phages R4 and SH10 were isolated independently from soil samples and phage Ba from a *Streptomyces* strain. The frequent isolation of this type of phage might be due to its wide host range.

We are grateful to Professor H. Umezawa for his encouragement throughout the present work, to Professor D. A. Hopwood and Dr K. F. Chater for their invaluable discussions and to Dr T. Shiba for his helpful advice. We thank the Institute of Microbial Chemistry for its support of this work.

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