Properties of the Temperate Actinophage SH10

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The temperate actinophage SH10 has a wide host-range. In size and morphology it is similar to phage λ . It has a latent period of 40 to 50 min. The average burst size depends on the host strain and varies between 60 and 100 at 28 °C. At 37 °C the burst size is diminished by a factor of ten. The adsorption constant varies between 1.55×10^{-9} and 8.8×10^{-10} ml min⁻¹ depending on the host strain. After mutagenesis by combined u.v.- and X-irradiation, clear and virulent mutants of SH10 were isolated. In addition, thermosensitive mutants of SH10 were obtained which fall into three complementation groups. The thermosensitive character was host-dependent. Phage SH10 is sensitive to sodium pyrophosphate and viable deletion mutants were isolated by virtue of their increased pyrophosphate resistance.

INTRODUCTION

Temperate phages have been useful tools in the genetic analysis of eubacteria, and are becoming of increasing interest in genetic studies of streptomycetes (for review, see Hopwood *et al.*, 1973). Previously, only two relatively narrow host-range temperate actinophages, both of which infect *Streptomyces coelicolor* A3(2), had been studied in detail. Phage ϕ C31 was isolated and characterized by Lomovskaya *et al.* (1972). This is the only actinophage for which a genetic map has been constructed (Chinenova & Lomovskaya, 1975). The other temperate phage, VP5, was partially characterized by Dowding & Hopwood (1973). More recently, the isolation and characterization of a wide host-range actinophage, R4, was reported by Chater & Carter (1979).

Here we describe some biological properties of another wide host-range actinophage, isolated from soil. This phage, SH10, infects numerous strains of different species of the genus *Streptomyces*. A relatively high yield, the isolation of viable deletion mutants, a small number of cleavage sites for some restriction endonucleases (Klaus *et al.*, 1979; Walter *et al.*, 1981) and the occurrence of transfection (Krügel *et al.*, 1980) make phage SH10 potentially useful as a phage vector for DNA cloning. The wide host-range of SH10 offers the possibility of phage-mediated transfer of genes between different strains of the genus *Streptomyces*. To obtain information about the host influence on phage functions, we used *S. lividans* 66 and different strains of *S. hygroscopicus* in this study.

METHODS

Bacterial strains. *Streptomyces hygroscopicus* JA6599 and 668 and the *S. griseus* strains 5142 and 107 were from the collection of the Zentralinstitut für Mikrobiologie und Experimentelle Therapie, Jena, D.D.R.; *S. hygroscopicus* 0477 was kindly provided by Y. I. Rautenstein, Institute of Microbiology of the Academy of Sciences, Moscow, U.S.S.R.; *S. lividans* 66 and *S. coelicolor* A3(2) were from N. D. Lomovskaya, Institute of Genetics and Selection of Industrial Microorganisms, Moscow, U.S.S.R. All other strains listed in Table 3 were

from Y. I. Rautenstein. The rifampicin-resistant mutants were isolated after treatment of *S. hygroscopicus* JA6599 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Phages. Phage SH10 was isolated from soil by the specific enrichment procedure of Welsch (1963) with minor modifications (Klaus *et al.*, 1979). Phage ϕ C31 (Lomovskaya *et al.*, 1972) was kindly provided by N. D. Lomovskaya; phage VP5 (Dowding & Hopwood, 1973) was from D. A. Hopwood, John Innes Institute, Norwich; phage SH3 (Rautenstein *et al.*, 1976) was from our own collection. Strains lysogenic for ϕ C31, VP5 and SH3 were obtained by infection of *S. coelicolor* A3(2), *S. lividans* 66 and *S. hygroscopicus* 0477, respectively.

Media. Complex media M79 and AL53 were described previously (Klaus *et al.*, 1978). Medium AL53 was used to obtain optimal sporulation of *Streptomyces* strains. Medium M79 was used for plating procedures and phage multiplication.

Preparation of phage stocks. The agar layer method (Adams, 1959) was used for phage titration and for stock preparation. About 10^8 spores or mycelial pellets from chemostat cultures (Roth *et al.*, 1978) of *S. lividans* 66 or *S. hygroscopicus* 0477 and 10^7 to 10^8 phage particles were added to the top layer and incubated for 24 h at 28 °C. The phage titre of the resulting lysate was usually between 5×10^9 and 5×10^{10} plaque-forming units (p.f.u.) ml⁻¹.

Morphological studies. Crude phage lysate was centrifuged at 90000 g for 90 min. The pellet was resuspended in 0.1 M-ammonium acetate followed by low-speed centrifugation (8000 rev. min⁻¹). The resulting supernatant was centrifuged at 150000 g for 90 min and the pellet was resuspended in 1 ml 0.1 M-ammonium acetate. Drops of phage suspension and phosphotungstic acid [2% (w/v) in distilled water, adjusted to pH 7.2 with 1 M-KOH] were mixed (1:1) and placed on carbon-Formvar-coated grids. Excess liquid was removed with filter paper and the grids were allowed to dry in air. The preparations were examined and photographed in a Siemens Elmiskop I electron microscope at a magnification of 40000.

Thin sections of *S. lividans* 66 infected with phage SH10 were prepared according to Kellenberger *et al.* (1958).

Isolation of stable lysogens. Phage SH10 was spotted on top layers inoculated with spores of *S. lividans* 66 or *S. hygroscopicus* JA6599. After 2 d incubation at 28 °C material from the lysis zones was transferred to medium M79 supplemented with phage-specific antiserum and cultivated at 28 °C for 6 h. The mycelial pellets were then plated on medium M79. After 4 d incubation single colonies were transferred, using sterile needles, to indicator plates to test for phage liberation. Phage-producing colonies were isolated and the clones were checked for immunity against superinfection by SH10⁺ and mutant SH10 c. Mutant SH10 *vir* was used as a control to test the colonies for resistance to SH10. Selected clones were grown for sporulation and again purified by the same procedure. In this way, stable lysogens were obtained.

Efficiency of lysogenization. Germinating spores were infected with phage SH10 at a multiplicity of 5 p.f.u. per colony-forming unit (c.f.u.). After 30 min incubation at 28 °C suitable dilutions were plated on medium M79 seeded with a mutant SH10 c1. Thus, the lysogenic colonies were identified by their immunity against superinfection.

Germination of spores for adsorption and growth studies. Spores were harvested from solid medium AL53 after 8 d incubation at 28 °C and incubated for about 6 h with shaking in medium M79 at 28 or 37 °C. The spore concentration was adjusted to 10^8 c.f.u. ml⁻¹. An equal volume of phage (at 10^8 p.f.u. ml⁻¹) was added when the spores germinated.

Adsorption. Adsorption rates were determined by free phage titration (Adams, 1959). Suspensions of germinated spores (5 ml, 10^8 c.f.u. ml⁻¹) and phages (5 ml, 10^8 p.f.u. ml⁻¹) were mixed and kept at 28 or 37 °C without shaking. Samples were withdrawn every 10 min, centrifuged at 5500 rev. min⁻¹ and free phage was determined on *S. lividans* 66 or on *S. hygroscopicus* JA6599 or 668. The adsorption constants, *K*, were calculated according to Adams (1959). In control experiments it was found that an increase in the concentration of divalent cations in liquid and solid medium M79 did not result in increased adsorption or a higher efficiency of plating.

Efficiency of plating (e.o.p.) at different temperatures. Spores were harvested from solid medium AL53 and immediately mixed with suitable dilutions of phage in top agar layers (medium M79). The plates were then transferred to different temperatures (between 28 and 37 °C) and incubated for 24 h. Thus, germination, adsorption and phage development up to plaque formation all took place at these temperatures. In the adsorption experiments it was found that SH10 particles adsorbed only to young germinated spores (after 4 to 6 h incubation). Control experiments demonstrated that pregermination of spores for more than 8 h or the use of mycelial pellets resulted in a lower e.o.p.

One-step growth experiments. These were performed according to Adams (1959) with minor modifications. The average phage input per spore was 0.1. SH10-specific antiserum was used to inactivate unadsorbed phages. The experiments were carried out with germinated spores of *S. hygroscopicus* JA6599 and *S. lividans* 66 at 28 or 37 °C.

Host-range. The host-range was determined by qualitative spot tests of phage SH10 on lawns of different strains. Lysate was transferred, using a sterile needle, to a lawn of the strain to be tested. If the lysis was transferable, the strain was considered to be a host for SH10. The formation of single plaques was followed only on strains of special interest.

U.v.- and X-irradiation. U.v.-irradiation was performed as described previously (Klaus *et al.*, 1978). For X-irradiation, phages were diluted 1:100 in TBT buffer (Klaus *et al.*, 1979); the X-ray tube delivered 136 krad min^{-1} at 50 kV.

Isolation of phage mutants. *Ts*, *c* and *vir* mutants were isolated after combined u.v.- and X-irradiation (u.v. dose, 5 J m^{-2} , followed by X-ray dose, 400 krad) resulting in a surviving fraction of 0.4%. The *c* mutants were selected on *S. lividans* 66 at 37 °C; *vir* mutants were selected on *S. lividans* 66 (SH10) at 37 °C. The *ts* mutants were isolated on *S. hygroscopicus* JA6599 according to Edgar & Lielausis (1964) with minor modifications (Klaus *et al.*, 1978). For sodium pyrophosphate treatment and isolation of deletion mutants, the methods of Parkinson & Huskey (1971) were used.

Complementation test. Best results were obtained by a modification of the normal technique. One phage mutant (5×10^8 p.f.u.) was added to 10^8 spores of the indicator strain *S. hygroscopicus* JA6599 in the top-layer agar, and the other mutants were spotted on to the surface of the plates (10^7 p.f.u. per drop). Plates were incubated at 37 °C. The appearance of lysis was taken as indicative of complementation between a pair of mutants. The complementation procedure was repeated several times. Separate plates with single mutants were incubated at 37 °C to exclude the possibility of revertant formation.

RESULTS

Morphological studies

A phage SH10 preparation, containing particles with full and empty heads, is shown in Fig. 1(a). The heads were hexagonal (64 ± 3 nm both lengthways and laterally) and the tails were long, flexible and non-contractile (180 ± 18 nm long, about 10 nm wide). All measurements given are the means for 20 particles.

A thin section of *S. lividans* 66 fixed 90 min after infection with phage SH10 is shown in Fig. 1(b). Particles with full and empty heads were relatively widely distributed throughout the hyphae. The small number of particles observed and the prolonged latent period may be due to suboptimal growth conditions in agar blocks, possibly due to limitation of oxygen.

Temperate behaviour of phage SH10

After infection of *S. lividans* 66, *S. hygroscopicus* JA6599 and mutants of the latter strain with phage SH10, stable lysogenic derivatives were isolated. After several cycles of sporulation and repeated incubation in SH10-specific antiserum we failed to obtain non-lysogenic clones. The stable lysogenic clones were immune against superinfection with SH10⁺ (wild-type) or SH10 *c* (mutant forming clear plaques on alysogenic *S. lividans* 66 at 37 °C, see below), but sensitive to SH10 *vir* (mutant forming clear plaques on strains lysogenic for SH10). Moreover, the lysogenic strains were able to liberate free phage by spontaneous induction and the prophage was inducible by u.v. light (H. Krügel *et al.*, unpublished results). Phage SH10 was able to grow on strains which are lysogenic for phages ϕ C31, VP5 and SH3, and so it belongs to a different immunity group. In contrast, a strain lysogenic for phage R4 was not a host for phage SH10 and vice versa, so they were probably of the same immunity group (K. F. Chater, personal communication).

Temperature sensitivity of phage SH10 development

The plaque morphology and efficiency of plating (e.o.p.) of phage SH10 depended on the indicator strain and the temperature of incubation. On *S. hygroscopicus* JA6599, phage SH10 formed turbid plaques at all temperatures between 28 and 37 °C. On *S. lividans* 66, clear-centred plaques surrounded by a turbid halo were formed at 28 °C; the turbidity of the plaque centre increased with temperature, and at 37 °C all plaques were turbid. On *S. hygroscopicus* 0477, the plaques were clear at all temperatures between 28 and 37 °C.

The e.o.p. decreased with increasing temperature for all strains tested (Table 1). This temperature effect was less marked with *S. lividans* 66 than with the *S. hygroscopicus* strains. The host influence on the e.o.p. suggested that phage particles were not thermally inactivated

Fig. 1. (a) Electron micrograph of a purified preparation of phage SH10, negatively stained with phosphotungstate. The bar marker represents 100 nm.

(b) Thin section of *S. lividans* 66, 90 min after infection with phage SH10 (fixation according to Kellenberger *et al.*, 1958). The bar marker represents 200 nm.

during plating at 45 °C. Control experiments indicated that heat inactivation of the plaque-forming ability took place at above 50 °C.

Phage lysates resulting from single plaques were picked from the 37 °C plates and propagated on *S. lividans* 66. They showed the same temperature sensitivity as the original lysate, indicating that the phage population was genetically homogeneous with respect to the temperature effect.

The effect of temperature on the adsorption of phage SH10 was studied. For each strain tested, the adsorption constant was the same at 28 and 37 °C (Table 2), indicating that adsorption in medium M79 was not affected at the higher temperature.

Table 1. *Effect of temperature of incubation on the efficiency of plating (e.o.p.) of phage SH10 (propagated on S. lividans 66) on different indicator strains*Results are expressed relative to the e.o.p. on *S. lividans* 66 at 28 °C.

Indicator strain	Relative e.o.p.			
	28 °C	31 °C	34 °C	37 °C
<i>S. lividans</i> 66	1.00	0.91	0.76	0.60
<i>S. hygroscopicus</i> JA6599	0.11	0.08	0.04	0.01
<i>S. hygroscopicus</i> 0477	0.18	0.14	0.07	0.04
<i>S. coelicolor</i> A3(2)	0.37	0.03	0.01	—

—, Relative e.o.p. < 0.001.

Table 2. *Effect of host strain and temperature of incubation on the adsorption of phage SH10*

Adsorption constants (K) were calculated according to Adams (1959): $K = (2.3/Bt) \log(p_0/p)$, where B is the number of c.f.u. in the spore suspension, t is the time of adsorption (20 min), p_0 is the number of p.f.u. at time 0 and p is the number of p.f.u. after 20 min adsorption.

Host strain	Adsorption constant, K (ml min ⁻¹)	
	28 °C	37 °C
<i>S. hygroscopicus</i> 668	1.55×10^{-9}	1.68×10^{-9}
<i>S. hygroscopicus</i> JA6599	1.56×10^{-9}	1.59×10^{-9}
<i>S. lividans</i> 66	4.4×10^{-10}	8.8×10^{-10}

Fig. 2

Fig. 2. One-step growth curves of phage SH10 on *S. lividans* 66 at 28 °C (Δ) and 37 °C ($\blacktriangle$) and on *S. hygroscopicus* JA6599 at 28 °C ($\circ$) and 37 °C ($\bullet$).

Fig. 3

Fig. 3. Effect of temperature on the number of p.f.u. of phage SH10 on different indicator strains: $\bullet$, *S. hygroscopicus* JA6599; $\circ$, JA6599 *rif*-1; $\blacktriangle$, JA6599 *rif*-2; $\triangle$, JA6599 *rif*-3. For each indicator strain, the numbers of p.f.u. at different temperatures are expressed as a percentage of the number of p.f.u. on the same strain at 28 °C.

In addition, one-step growth experiments were performed at 28 and 37 °C using *S. lividans* 66 and *S. hygroscopicus* JA6599. The results are summarized in Fig. 2. Surprisingly, the latent and rise periods were not influenced by the temperature of incubation, but at 37 °C

Table 3. *Host-range of phage SH10*

<i>Streptomyces</i> species	Strain no.	Transferable lysis
<i>S. hygroscopicus</i>	JA6599	+
<i>S. hygroscopicus</i>	668	+
<i>S. hygroscopicus</i>	0129	+
<i>S. hygroscopicus</i>	0245	+
<i>S. hygroscopicus</i>	0477	+
<i>S. hygroscopicus</i>	0439	+
<i>S. hygroscopicus</i>	0485	—
<i>S. hygroscopicus</i>	33X	—
<i>S. lividans</i>	66	+
<i>S. coelicolor</i>	123	+
<i>S. coelicolor</i>	124	+
<i>S. coelicolor</i>	A3(2)	+
<i>S. humidus</i>	9	+
<i>S. sichawensis</i>	10	—
<i>S. endus</i>	0009	—
<i>S. endus</i>	0010	—
<i>S. endus</i>	0014	+
<i>S. endus</i>	0184	+
<i>S. endus</i>	0215	+
<i>S. endus</i>	0582	+
<i>S. erythraeus</i>	154	+
<i>S. scabies</i>	1072	+
<i>S. scabies</i>	10246	+
<i>S. olivochromogenes</i>	3332	+
<i>S. verna</i>	3353	+
<i>S. streptomycinii</i>	B-6	+
<i>S. fluorescens</i>	2161	+
<i>S. orientalis</i>	134	—
<i>S. globisporus</i>	425	+
<i>S. globisporus</i>	91	—
<i>S. tinorans</i>	2703	+
<i>S. platensis</i>	15	—
<i>S. levoris</i>	1331	+
<i>S. griseus</i>	20	+
<i>S. griseus</i>	5142	+
<i>S. griseus</i>	107	+

the burst sizes were about tenfold smaller in both strains. This was confirmed by single burst experiments (results not shown). The temperature effect was less marked with some rifampicin-resistant mutants of *S. hygroscopicus* JA6599, particularly mutant *rif-3* (Fig. 3).

Host-range

Phage SH10 has a wide host-range; it lysed 28 out of 36 *Streptomyces* strains tested belonging to 18 species (Table 3). No lysis was observed with strains of the genera *Oerskovia*, *Promicromonospora*, *Nocardioideis*, *Rhodococcus*, *Nocardia*, *Micropolyspora*, *Actinomadura* and *Micromonospora* (H. Prauser, personal communication). Thus, SH10 appears to be specific for the genus *Streptomyces*.

In addition to the spot test, the formation of single plaques was observed on *S. levoris* 1331, *S. lividans* 66, *S. coelicolor* A3(2), *S. hygroscopicus* JA6599, 668 and 0477 and *S. griseus* 5142 and 107.

Survival after u.v.- and X-irradiation

Survival of phage SH10 after u.v.- and X-irradiation was studied to find the optimal conditions for mutagenesis. Semi-logarithmic plots of the surviving fraction against u.v. or X-ray dose both showed a linear decrease in the surviving fraction indicating single-hit kinetics for the inactivation of the plaque-forming ability.

Table 4. Complementation of phage SH10 *ts* mutants

Complementation tests were carried out as described in Methods. The appearance of lysis spots (+) on *S. hygroscopicus* JA6599 at 37 °C was taken as indicative of complementation between a pair of mutants. Failure of lysis (—) indicated no complementation.

	<i>ts1</i>	<i>ts2</i>	<i>ts3</i>	<i>ts4</i>	<i>ts5</i>	<i>ts6</i>	<i>ts20</i>
<i>ts1</i>	—	+	—	—	—	—	+
<i>ts2</i>	+	—	+	+	+	+	+
<i>ts3</i>	—	+	—	—	—	—	+
<i>ts4</i>	—	+	—	—	—	—	+
<i>ts5</i>	—	+	—	—	—	—	+
<i>ts6</i>	—	+	—	—	—	—	+
<i>ts20</i>	+	+	+	+	+	+	—

Genetic studies

For mutagenesis, SH10 was u.v.- and X-irradiated as described in Methods. After mutagenesis, no clear plaques were detected among 4.4×10^5 plaques on *S. hygroscopicus* JA6599, but on *S. lividans* 66 two types of clear plaques appeared at 37 °C at a frequency of 1.2×10^{-3} (*c*) and 1.8×10^{-4} (*vir*). One class (*c*) was able to grow only on the sensitive indicator, and not on the lysogenic one. The second class (*vir*) was also able to form clear plaques on the lysogenic strain *S. lividans* 66 (SH10). Both *c* and *vir* mutants of SH10 formed turbid plaques on *S. hygroscopicus* JA6599 which could not be distinguished from those of SH10⁺ (wild-type).

After infection of *S. lividans* 66 with different SH10 *c* mutants the frequency of lysogenization was two orders of magnitude lower than with SH10⁺. Thus, the clear plaque phenotype on *S. lividans* 66 was correlated with a defect in lysogenization and was not simply a result of a higher burst size mutation.

Using the method of Edgar & Lielausis (1964), 56 *ts* mutants were isolated. After three cycles of lytic growth on *S. lividans* 66, always followed by single plaque isolation steps on *S. hygroscopicus* JA6599, we obtained only seven stable *ts* mutants of phage SH10 able to form plaques on *S. hygroscopicus* JA6599 and 0477 at 28 °C but not at 37 °C. (The alternate hosts were used because we were interested in SH10 mutants which were temperature-sensitive on *S. hygroscopicus* JA6599; *S. lividans* was normally used for phage propagation because higher phage titres were obtained with this host.) As shown in Table 4, the mutants fell into three complementation groups. Group I contains the mutants *ts1*, *ts3*, *ts4*, *ts5* and *ts6*, group II is represented by mutant *ts2* and group III by mutant *ts20*.

Most of the *ts* mutants had altered plaque morphology on *S. hygroscopicus* JA6599. Mutant *ts3* was apparently affected in adsorption: at 28 °C the adsorption rate was higher than with SH10⁺, whereas at 37 °C the mutant adsorbed more slowly. Temperature sensitivity in plaque formation and adsorption efficiency may be independent mutations. The other mutants have not been characterized.

In most of the mutants the *ts* character was expressed only on *S. hygroscopicus* JA6599 and 0477. Using *S. lividans* 66 as indicator, there was no difference between the mutants and the wild-type phage. A typical example of this kind of host-dependent temperature sensitivity is shown in Fig. 4.

In control experiments, one representative mutant from each complementation group (*ts1*, *ts2* and *ts20*) was propagated on *S. hygroscopicus* JA6599 and on 0477 and plated on the indicator strains *S. lividans* 66 and *S. hygroscopicus* JA6599 and 0477 at 28 and 37 °C. Again, the *ts* character of the phage mutants was expressed on both *S. hygroscopicus* strains, but not on *S. lividans* 66.

Inactivation of phage SH10 by sodium pyrophosphate

Parkinson & Huskey (1971) isolated viable deletion mutants of phage λ by selecting mutants resistant to chelating agents or heat treatment. As shown in Fig. 5, phage SH10 was

Fig. 4

Fig. 5

Fig. 4. Effect of temperature on the number of p.f.u. of phage mutant SH10 *ts2* on different indicator strains: ○, *S. lividans* 66; ▲, *S. hygroscopicus* JA6599; △, *S. hygroscopicus* 0477; ●, phage SH10 wild-type on *S. lividans* 66 (control). For each indicator strain, the numbers of p.f.u. at different temperatures are expressed as a percentage of the number of p.f.u. on the same strain at 28 °C.

Fig. 5. Survival in sodium pyrophosphate solution at 37 °C of phage SH10 wild-type (▲, 0.01 M-pyrophosphate; △, 0.03 M) and of its deletion mutants SH10 d1 (○, 0.03 M) and SH10 c1 (●, 0.03 M). Survival was estimated as p.f.u. on *S. lividans* 66.

also sensitive to the chelating agent sodium pyrophosphate, the surviving fraction depending on the pyrophosphate concentration. Stable resistant clones were obtained. Most of these showed a clear plaque phenotype on the indicator strain *S. lividans* 66 at 37 °C, suggesting that mutation to pyrophosphate resistance in some cases altered or deleted genes involved in the regulation of lysogeny.

In separate experiments (results not shown) it was found that survivors of the pyrophosphate treatment were also more resistant to EDTA (0.01 M), sodium citrate (0.01 M) and heating at 55 °C. As a control, DNA of one representative pyrophosphate-resistant mutant (SH10 *c1*) was used in physical mapping studies with restriction endonucleases (Walter *et al.*, 1981). It was found that a small deletion corresponding to a molecular weight of 0.5×10^6 was located on the right side of the physical map.

DISCUSSION

From a collection of actinophages isolated from soil, SH10 was chosen for detailed study because of its favourable plaque morphology, its relatively wide host-range and the ease with which high titre preparations were obtained. As revealed by electron microscopic observation, phage SH10 belongs to group B of Bradley's morphological classification (Bradley, 1967) and resembles phage λ in appearance and dimensions (Kellenberger & Edgar, 1971). The same is true for the actinophages φC31 (Lomovskaya *et al.*, 1972), VP5 (Dowding & Hopwood, 1973), VP11 (Dowding, 1973), S2 (Klaus *et al.*, 1978), φSV1 (Stuttard, 1979), R4 (Chater & Carter, 1979) and numerous actinophages isolated in our laboratory (unpublished results). Thus, SH10 belongs to the most common morphological type of actinophages.

Despite the low lysogenization rate, SH10 was found to be a temperate phage. Lysogenic derivatives of *S. hygroscopicus* JA6599 and of *S. lividans* 66 did not segregate non-lysogenic clones as measured by the immunity test and by the liberation of free phages produced by spontaneous induction of single colonies. The lysogenic strains were also stable through many cycles of growth and sporulation. In addition, the prophage SH10 is inducible by u.v.-irradiation.

The fact that the plaque morphology and lytic development of SH10 were affected by temperature is in accordance with similar reports on other actinophages (Kuroda & Bradley, 1967; Lomovskaya *et al.*, 1972; Chater & Carter, 1979). The cause of this effect was not investigated in detail and might be different in different systems. However, colonies of *S. hygroscopicus* JA6599 and of *S. lividans* 66 grow faster at 37 °C and the synthesis of protein, RNA and DNA is more rapid (B. Frunder *et al.*, unpublished results); thus, the synthesis of macromolecules of the host cannot be the temperature-sensitive target. The temperature effect did not appear to be due to differences in phage adsorption. The adsorption constants were in the same range as reported for ϕ C31 (Lomovskaya *et al.*, 1972), VP11 (Dowding & Hopwood, 1973), ϕ SV1 (Stuttard, 1979), RP2 (Hranueli *et al.*, 1979) and R4 (Chater & Carter, 1979). In general, the adsorption constants of actinophages are an order of magnitude lower than those for coliphages (Adams, 1959).

One-step growth experiments showed that the latent periods and the rise periods of SH10 in both host strains were not influenced by the incubation temperature, but at 37 °C the burst sizes were diminished by a factor of ten. This result is in contrast to the situation in *Escherichia coli*: using T-phages, Ellis (1966) found that the temperature had no effect on the burst size, but strongly influenced the latent and rise periods.

The finding that plating of SH10 on rifampicin-resistant mutants of *S. hygroscopicus* JA6599 influences the temperature effect might be interpreted in terms of lower efficiency of transcription of the SH10 genome at higher temperatures, presumably due to promoter-specific effects.

The efficiency of plating of SH10 at 28 °C is tenfold lower on *S. hygroscopicus* JA6599 than on *S. lividans* 66. This difference cannot be explained in terms of restriction-modification because SH10 propagated on JA6599 again has an e.o.p. of 0.1 on JA6599, in contrast to 1.0 on strain 66.

In contrast to the temperate phages VP5 (Dowding & Hopwood, 1973), RP2 (Hranueli *et al.*, 1979), or phage SH3 of *S. hygroscopicus* (Rautenstein *et al.*, 1976), SH10 has a remarkably wide host-range within the streptomycetes. A similar wide host-range was found for temperate phage R4 (Chater & Carter, 1979).

For mutagenesis of phage SH10, we used a combination of u.v.- and X-irradiation. Such a treatment was effective in creating stable auxotrophic and thermosensitive mutations in the host organism (B. Frunder & D. Noack, unpublished results). Clear mutants of SH10 were selected at 37 °C, because plaques of the wild-type phage were clear at 28 °C and turbid at 37 °C on *S. lividans* 66. The two types of clear mutants obtained phenotypically resembled *c* and *vir* mutations of phage λ . We have not yet been able to identify heat-inducible mutants of SH10, because the clear-plaque phenotype of SH10⁺ at 28 °C complicates the selection of this useful mutant type. The observation that the clear mutant *c*1 bears a deletion near the right end of the physical map indicates that the genetic elements involved in the control of lysogeny are located near one end of the SH10 genome. Virulent mutants of phage ϕ C31 often have deletions close to one end of the physical map (Lomovskaya *et al.*, 1979).

In contrast to the *c* and *vir* mutations of SH10, the thermosensitive mutants were very unstable, even after repeated single plaque isolation steps. This is in accordance with the properties of many *ts* mutants of ϕ C31 (Chinenova & Lomovskaya, 1975) and of the virulent actinophage VP11 (Dowding, 1973). Thus, only a limited number of *ts* mutants have been used in complementation experiments. These *ts* mutants have not been further characterized but in most cases their temperature sensitivity was found to be host dependent; they behaved

as *ts* mutants after plating on *S. hygroscopicus* JA6599 and 0477, but behaved like the wild-type phage on *S. lividans* 66. This phenomenon does not depend on the host on which the mutants were previously grown.

A similar behaviour was reported for *ts* mutants of the virulent actinophage S2 (Klaus *et al.*, 1978). Since nearly all of our *ts* mutations were suppressed in *S. lividans* 66, it seems that a suppressor gene of *S. lividans* 66 is not involved in this phenomenon. It seems more likely that transcription of SH10 is more effective in *S. lividans* 66, resulting in a higher concentration of the mutant protein which can function if the concentration is increased. Such an effect was found for *ts* mutations in the *i*-gene of the lac operon (Müller-Hill *et al.*, 1968). In another streptomycete, *S. coelicolor* A3(2), suppressor genes were identified using polyauxotrophic derivatives (Alikhanian *et al.*, 1976).

The isolation of viable deletion mutants by virtue of their increased resistance to sodium pyrophosphate will facilitate the study of inessential regions of the SH10 genome. The frequent appearance of clear plaques among the survivors of pyrophosphate-treatment suggests that genes involved in the regulation of lysogeny are located in the deleted regions, as shown for phage λ (Parkinson & Huskey, 1971).

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