Isolation and Characterization of Actinophages Infecting *Streptomyces* Species and Their Interaction with Host Restriction–Modification Systems

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Nine different phages, ϕ A1 to ϕ A9, were isolated from soil samples on *Streptomyces antibioticus* ATCC 11891, a strain which produces the macrolide antibiotic oleandomycin. Each phage displayed a different host-range which did not extend beyond *Streptomyces* species. Host-range was mainly limited by adsorption specificity and host-controlled restriction–modification systems. All the phages except ϕ A3 and ϕ A9 formed turbid plaques on *S. antibioticus*, but did not lysogenize this host. However, three of the phages (ϕ A5, ϕ A7 and ϕ A8) were identified as temperate, since they were able to lysogenize other *Streptomyces* strains. All of the phages were morphologically similar and belonged to group B of Bradley's classification. They had polyhedral heads and long, non-contractile tails. ϕ A5, ϕ A6 and ϕ A7 had a base plate at the terminal end of the tail. Analysis with restriction endonucleases indicated that the nine phages contained double-stranded DNA. Hybridization studies between the phage genomes, together with results on genome structure, allowed classification of the phages into five groups: (I) ϕ A2, ϕ A4 and ϕ A9, (II) ϕ A3 and ϕ A8, (III) ϕ A7, (IV) ϕ A5 and ϕ A6, and (V) ϕ A1.

INTRODUCTION

Streptomyces phages are of interest for a variety of reasons, including (1) the problems they cause in fermentation industries, (2) their value for typing streptomycetes in taxonomic studies (Bradley *et al.*, 1961; Korn *et al.*, 1978), (3) their use for the detection and understanding of host-controlled restriction–modification systems, (4) their utilization as tools for genetic exchange and analysis in *Streptomyces*, and (5) the study of their general and molecular biology (for reviews on 3, 4 and 5 see Lomovskaya *et al.*, 1980; Chater, 1986).

Several temperate actinophages (ϕ C31, R4, SH10, TGI, ϕ SFI; see Chater, 1986) have been developed into cloning vectors, as alternatives to plasmids in the genetic manipulation of *Streptomyces* species. ϕ C31 is the best characterized and has been used successfully for the cloning and functional analysis of *Streptomyces* DNA (Chater *et al.*, 1985). Although ϕ C31 shows a broad host-range, including about half of the 137 strains on which it has been tested (Voeykova *et al.*, 1979; Chater, 1986), it does not infect *S. antibioticus* ATCC 11891, a strain which produces the macrolide antibiotic oleandomycin. In this paper we report the isolation from soil of nine new phages able to form plaques on this strain and their preliminary characterization. We have analysed the interaction of these phages with other strains of *Streptomyces*, some of which produce well-characterized restriction–modification systems, and have assessed their potential for development into cloning vectors.

METHODS

Bacteria, phages and their propagation. *Streptomyces* and other actinomycete strains used in this work are listed in Table 3. Phages ϕ C31 (Lomovskaya *et al.*, 1972) and R4 (Chater & Carter, 1979) were kindly provided by K. F. Chater (John Innes Institute, Norwich, UK), and SH10 (Klaus *et al.*, 1981) by S. Klaus (Zentralinstitut für Mikrobiologie und Experimentelle Therapie, Jena, DDR). Other phages were isolated from soil as described

below. General bacterial and phage techniques were as in Hopwood *et al.* (1985) but R2YE medium (Thompson *et al.*, 1980) was only used for culture of *S. lividans* 1326 and *S. coelicolor*. All other strains were grown and sporulated on GAE (Hardisson *et al.*, 1978).

Phage isolation. Phages infecting *S. antibioticus* ATCC 11891 were isolated from soil following essentially the enrichment method of Dowding (1973). Samples (1 g) of commercially available garden soil (of unknown origin and characteristics) were added to nutrient broth (10 ml) supplemented with glucose (1%, w/v), MgSO_4 (10 mM), $\text{Ca}(\text{NO}_3)_2$ (8 mM), oleandomycin ($50 \mu\text{g ml}^{-1}$) and spores (about 10^6) of *S. antibioticus*. After overnight incubation at 30 °C with shaking, samples were centrifuged and supernatants were sterilized by passage through Millipore filters (0.45 μm). Phages were detected by plating in soft agar overlays (Hopwood *et al.*, 1985), using *S. antibioticus* as indicator strain. Phages were distinguished by differences in plaque morphology, size and turbidity, and were purified by serial replating from single plaques.

Morphological studies. Phage particles from high-titre lysates were stained with 2% (w/v) potassium phosphotungstate, pH 7, and observed under a Zeiss EM109 electron microscope, as described by Suárez *et al.* (1984).

Preparation and in vitro manipulation of phage DNA. Phage DNA was extracted from high-titre lysates by hot-SDS disruption of the particles, as described by Suárez & Chater (1981). Standard procedures (Hopwood *et al.*, 1985) were used for restriction enzyme digestions, ligation and agarose gel electrophoresis. Cohesive ends were detected by comparison of the gel patterns corresponding to unheated and heated (70 °C, 10 min), or to ligated and unligated, phage DNA, digested with appropriate restriction enzymes. Homology between phage genomes was investigated by Southern hybridization. Digested phage DNAs were electrophoresed, transferred to nitrocellulose filters and probed with ^{35}S - or ^{32}P -labelled phage DNA. Hybridization and washing of Southern blots was done in conditions which would allow detection of DNA sequences of about 500 bp with > 84% base sequence identity to the probe (Hopwood *et al.*, 1985; hybridization procedure 'B').

Host-range determinations. Plaque formation by serial dilutions of each phage was used rather than qualitative spot tests (which frequently gave false positive results) to determine the host range of the phages.

Identification of temperate phages and determination of immunity. Phages (5–10 μl of a suspension containing about 10^6 p.f.u. ml^{-1}) were spotted on GAE (Hardisson *et al.*, 1978) plates inoculated with spores of the appropriate strain. Plates were incubated at 30 °C until cells growing within the lysis zone sporulated. Spores were transferred into 1 ml of 0.1 M-tetrasodium pyrophosphate and left at 37 °C for 30 min. Then they were filtered through a 0.45 μm filter, washed three times with 5 ml tetrasodium pyrophosphate and finally resuspended in 1 ml sterile distilled water. Spores were serially diluted and spread on GAE plates. After sporulation they were replicated to lawns of an appropriate indicator strain and tested for the liberation of phage. Spores prepared from phage-producing colonies were checked for resistance against superinfection by the same phage using the aforementioned soft agar overlay method. Putative lysogens for one phage were also subjected to infection by other lysogenic phages in order to establish immunity relationships.

RESULTS AND DISCUSSION

Isolation of phages infecting S. antibioticus

Phage plaques were obtained on *S. antibioticus* ATCC 11891 from different garden soil samples, enriched by pre-incubation with the host in the presence of oleandomycin. Using differences in plaque morphology, size and degree of turbidity as initial criteria, nine different phages, termed ϕA1 to ϕA9 , were identified. ϕA3 and ϕA9 produced clear plaques on lawns of *S. antibioticus*. All the other phages formed turbid plaques. The uniqueness of each phage was confirmed by morphological studies of the phage particles, analysis of the genome and host-range determination.

Morphology of the virions

Electron microscope observations (Fig. 1) showed that the nine phages had polyhedral heads and long, apparently non-contractile tails (like nearly all previously characterized *Streptomyces* phages). Phages ϕA5 , ϕA6 and ϕA7 had a baseplate at the end of the tail. The dimensions of the phages are shown in Table 1. The largest phage, ϕA1 , had a head of 79 nm from apex to apex and a tail 327 nm long. The heads of the other phages ranged from 50 to 62 nm but the length of the tails showed a greater variation (100 to 230 nm).

Characterization of phage genomes

The genomes of all the phages consisted of double-stranded DNA, as deduced from analysis with restriction endonucleases. This is also the case for all other actinophages reported so far and

Fig. 1. Electron micrographs of *Streptomyces* phages. (Note that the phages are grouped by similarity.) Bars, 50 nm.

Table 1. *Phage dimensions*

Phage	Head*		Tail*		Base-plate†
	Apex to apex	Side to side	Length	Width	
$\phi A2$	52 ± 3	48 ± 3	228 ± 12	8	—
$\phi A4$	61 ± 3	58 ± 3	233 ± 8	7	—
$\phi A9$	62 ± 4	59 ± 4	227 ± 6	8	—
$\phi A3$	55 ± 3	53 ± 3	150 ± 9	7	—
$\phi A8$	56 ± 4	50 ± 3	145 ± 8	7	—
$\phi A7$	52 ± 3	48 ± 4	102 ± 5	7	+
$\phi A5$	50 ± 2	46 ± 3	110 ± 3	6	+
$\phi A6$	57 ± 3	51 ± 3	115 ± 3	6	+
$\phi A1$	79 ± 6	73 ± 5	327 ± 35	9	—

* Values are given in nm, and are means $\pm$ SD of at least 10 independent measurements.

† +, presence, —, absence of basal plate.

for all bacteriophages with complex symmetry. The number and size of fragments obtained after digestion with each of 20 restriction enzymes (Table 2) confirmed the uniqueness of each phage. The phages were also distinct from $\phi C31$ (Harris *et al.*, 1983), R4 (Morino *et al.*, 1983), SH10 (Walter *et al.*, 1981), TG1 (Foor *et al.*, 1985) and other reported actinophages.

Fig. 2. Detection of cohesive ends by agarose gel electrophoresis of ligated (L) and unligated (U) phage DNAs, digested with restriction enzymes. S, *Pst*I-digested λ (size standards); 1, *Eco*RI-digested ϕ A2; 2, *Bgl*/II-digested ϕ A3; 3, *Eco*RI-digested ϕ A4; 4, *Eco*RV-digested ϕ A7; 5, *Bgl*/II-digested ϕ A8; 6, *Bst*EII-digested ϕ A9. Arrows indicate the fragments carrying the cohesive ends and asterisks the *cos* fragment.

Table 2. *Restriction enzyme analysis of phage DNA*

Restriction endonuclease	Number of fragments obtained after digestion								
	ϕ A2	ϕ A4	ϕ A9	ϕ A3	ϕ A8	ϕ A7	ϕ A5	ϕ A6	ϕ A1
<i>Ava</i> I	≥ 15	≥ 15	≥ 15	≥ 15	≥ 15	≥ 15	≥ 15	≥ 20	≥ 15
<i>Bam</i> HI	—	≥ 15	≥ 15	—	—	—	—	—	—
<i>Bcl</i> I	≥ 15	≥ 15	≥ 15	≥ 10	12	12	6	≥ 10	—
<i>Bgl</i> I	≥ 15	≥ 15	≥ 15	≥ 15	≥ 15	≥ 15	≥ 10	≥ 10	≥ 15
<i>Bgl</i> II	10	≥ 10	≥ 15	7	11	—	3	—	≥ 15
<i>Bst</i> EII	8	≥ 10	≥ 10	≥ 15	≥ 15	≥ 20	≥ 10	7	≥ 15
<i>Clal</i>	11	≥ 10	≥ 10	2	3	5	—	3	≥ 10
<i>Eco</i> RI	6	13	12	3	3	8	4	2	≥ 15
<i>Eco</i> RV	3	8	9	—	—	11	5	5	≥ 15
<i>Hind</i> II	NT	NT	NT	NT	≥ 10	≥ 10	—	NT	NT
<i>Hind</i> III	—	≥ 10	9	—	2	—	—	—	≥ 15
<i>Hpa</i> I	6	—	—	—	—	3	—	—	≥ 15
<i>Kpn</i> I	≥ 10	9	P	7	P	P	8	P	≥ 15
<i>Pst</i> I	≥ 10	≥ 10	≥ 15	—	—	—	—	—	—
<i>Pvu</i> I	≥ 10	≥ 15	≥ 15	—	—	7	≥ 10	≥ 15	—
<i>Pvu</i> II	≥ 10	≥ 10	≥ 10	—	—	—	—	≥ 15	—
<i>Sac</i> I	≥ 10	≥ 20	≥ 15	≥ 15	≥ 15	—	—	—	—
<i>Sac</i> II	≥ 15	≥ 15	≥ 15	—	—	2	≥ 10	≥ 10	—
<i>Sal</i> GI	≥ 10	≥ 15	≥ 15	P	≥ 10	—	≥ 10	≥ 15	3
<i>Sma</i> I	NT	NT	NT	NT	—	—	≥ 10	NT	NT
<i>Xba</i> I	2	3	3	—	—	7	—	—	P
<i>Xho</i> I	—	—	≥ 10	—	—	—	—	—	—
<i>Xho</i> II	NT	NT	NT	NT	≥ 15	≥ 10	—	NT	NT

—, No cleavage sites; NT, not tested; P, partial digest.

Fig. 3. Hybridization between phage DNAs. (a) Restriction enzyme digests of different phage DNAs. Lanes: S, *Pst*I-digested λ (size standards); 1, *Eco*RV-digested ϕ A1; 2, *Eco*RI-digested ϕ A2; 3, *Eco*RI-digested ϕ A3; 4, *Eco*RI-digested ϕ A4; 5, *Eco*RV-digested ϕ A5; 6, *Eco*RV-digested ϕ A6; 7, *Eco*RV-digested ϕ A7; 8, *Bgl*II-digested ϕ A8; 9, *Eco*RI-digested ϕ A9; 10, *Eco*RI-digested ϕ C31; 11, *Bgl*II-digested SH10. (b, c, d, e) Southern blots of gels similar to the one shown in (a), probed with (b) 35 S-labelled ϕ A4 DNA, (c) 35 S-labelled ϕ A5 DNA, (d) 32 P-labelled ϕ A7 DNA, and (e) 35 S-labelled ϕ A8 DNA.

By summation of restriction fragments for each of several endonucleases, the lengths of the double-stranded DNA molecules were calculated for ϕ A1 (100 kbp), ϕ A2 (43 kbp), ϕ A3 (51 kbp), ϕ A4 (49 kbp), ϕ A7 (46 kbp), ϕ A8 (50 kbp) and ϕ A9 (53 kbp). All of these except ϕ A1 had cohesive ends (Fig. 2), as commonly found among *Streptomyces* phages.

In the cases of ϕ A5 and ϕ A6, disagreements in the length of the genome obtained from different enzyme digests were observed, and sub-stoichiometric bands were always present in gels. Such bands were not due to partial digestions and they did not correspond to fused and unfused cohesive ends. These observations could be explained by the occurrence in the population of heterogeneous phage molecules present as a result of packaging of the DNA by the headful mechanism (Botstein *et al.*, 1973; Jackson *et al.*, 1978; Sternberg & Hoess, 1983).

Table 3. *Bacteriophage plaque formation on Streptomyces and other actinomycetes*

None of the bacteriophages formed plaques on *Saccharopolyspora erythraea* NRRL B-2338, *Micromonospora chalybeata* ATCC 12452, *M. purpurea* ATCC 15835, *Micromonospora* sp. IMET 8002 or *Nocardia mediterranea* ATCC 13685.

Strain*	ϕ A2	ϕ A4	ϕ A9	ϕ A3	ϕ A8	ϕ A7	ϕ A5	ϕ A6	ϕ A1
<i>Streptomyces antibioticus</i> ATCC 11891	+	+	+	+	+	+	+	+	+
<i>S. antibioticus</i> CECT 3224	—	+	—	+	+	+	+	+	NT
<i>S. antibioticus</i> CECT 3225	—	—	—	+	+	—	+	—	—
<i>S. antibioticus</i> CECT 3232	+	+	+	+	+	—	—	+	+
<i>S. antibioticus</i> CECT 3234	—	+	+	+	—	+	—	+	+
<i>S. antibioticus</i> ETHZ 7451	—	—	—	—	—	—	—	—	—
<i>S. antibioticus</i> KCC S-0680	+	—	—	+	+	—	—	—	+
<i>S. antibioticus</i> KCC S-0707	—	—	—	—	+	—	+	—	—
<i>S. antibioticus</i> KCC S-0868	—	—	—	—	—	—	—	—	—
<i>S. achromogenes</i> ATCC 12767	—	+	—	—	—	—	—	—	—
<i>S. albus</i> G†	—	—	—	—	—	+	—	—	—
<i>S. albus</i> J1074	+	+	—	+	+	+	+	+	+
<i>S. albus</i> P (CMI 52766)	+	—	—	+	+	+	—	+	+
<i>S. clavuligerus</i> ATCC 27064	—	—	—	+	+	—	—	—	NT
<i>S. coelicolor</i> CECT 3079	+	+	—	+	+	+	—	+	+
<i>S. coelicolor</i> J1501	—	—	—	—	—	—	—	—	—
<i>S. fradiae</i> NRRL B-3357	—	—	—	—	—	+	—	+	—
<i>S. glaucescens</i> CECT 3175	—	—	—	+	+	+	+	+	NT
<i>S. glaucescens</i> ETHZ 22794	—	—	—	—	—	—	—	—	—
<i>S. griseus</i> ATCC 11429	—	—	—	—	—	+	—	—	—
<i>S. hygroscopicus</i> UC 5208	—	—	+	+	+	+	—	—	+
<i>S. lavendulae</i> NRRL B-1871	—	—	—	+	+	+	—	—	—
<i>S. lavendulae</i> NRRL B-2036	—	—	—	+	+	+	—	—	—
<i>S. lavendulae</i> NRRL B-2343	—	—	—	+	+	+	—	—	—
<i>S. lividans</i> ATCC 19844	—	—	—	—	—	—	—	—	—
<i>S. lividans</i> 66 (JII strain 1326)	—	—	—	—	—	—	—	—	—
<i>S. lydicus</i> UC 2056	—	—	+	+	+	+	+	+	+
<i>S. orchidaceus</i> NRRL B-2454	—	—	—	+	+	+	—	—	—
<i>S. scabies</i> CMI 99049	+	+	+	+	+	+	+	+	+
<i>S. showdoensis</i> Z-452	+	+	+	+	+	+	+	+	+
<i>S. spectabilis</i> UC 2472	—	—	—	—	—	—	—	—	—

NT, Not tested; +, plaque formation (variations occurred in both e.o.p. values and size of the plaques, data not shown); —, e.o.p. $< 10^{-5}$, assuming an e.o.p. of 1 on *S. antibioticus* ATCC 11891.

* ATCC, American Type Culture Collection; CECT, Colección Española de Cultivos Tipo; CMI, Commonwealth Mycological Institute; ETHZ, Institutes der Eidgenössischen Technischen Hochschule Zürich; IMET, Institut für Mikrobiologie und Experimentelle Therapie, Jena; JII, John Innes Institute; KCC, Kaken Culture Collection; NRRL, Northern Regional Research Laboratories; UC, Upjohn Company; Z, Shionogi and Co.

† Leyh-Bouille *et al.* (1970).

This mechanism gives rise to phage genomes which are partially circularly permuted and terminally redundant. Actinophages with this type of organization have already been reported (Chung, 1982; Sladkova, 1982; Grund & Hutchinson, 1987) and ϕ A5 and ϕ A6 could be new examples. The structure of the genome of ϕ A1 remains unknown but it does not seem to correspond to any of the cited models; sub-stoichiometric bands were not detected after electrophoresis of digested DNA and cohesive ends were not demonstrated.

Genetic interrelation among phages

Possible homology between the genomes of the different phages was investigated by Southern hybridizations. DNA from the nine newly isolated phages and also from ϕ C31 and SH10, digested with appropriate restriction enzymes (Fig. 3a), was hybridized to ϕ A4, ϕ A5, ϕ A7 and ϕ A8 DNA, used independently as radioactive probes (Fig. 3b, c, d, e).

ϕ A4 DNA gave a strong hybridization signal with nearly all *Eco*RI-generated fragments of

Table 4. Efficiency of plating of the phages on several endonuclease-producing strains

Strain	Enzyme produced	Efficiency of plating*								
		ϕ A2	ϕ A4	ϕ A9	ϕ A3	ϕ A8	ϕ A7	ϕ A5	ϕ A6	ϕ A1
<i>S. albus</i> G	<i>Sal</i> GI	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-7}$	$< 10^{-5}$	$< 10^{-5}$	5	$< 10^{-5}$	$< 10^{-6}$	$< 10^{-4}$
<i>S. albus</i> J1074		10^{-1}	10^{-1}	$< 10^{-7}$	6	1	7	10^{-3}	5×10^{-2}	1
<i>S. albus</i> P	<i>Sal</i> PI	10^{-1}	$< 10^{-5}$	$< 10^{-6}$	1	2	4	$< 10^{-5}$	10^{-1}	10
<i>S. achromogenes</i> ATCC 12767	<i>Sac</i> I <i>Sac</i> II <i>Sac</i> III	$< 10^{-7}$	6×10^{-1}	$< 10^{-6}$	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-7}$	$< 10^{-5}$	$< 10^{-6}$	$< 10^{-4}$
<i>S. lividans</i> 1326		$< 10^{-4}$	$< 10^{-4}$	$< 10^{-5}$	$< 10^{-4}$	$< 10^{-4}$	$< 10^{-4}$	$< 10^{-4}$	$< 10^{-4}$	$< 10^{-4}$

* The e.o.p. was calculated with respect to plating efficiency on *S. antibioticus* ATCC 11891.

ϕ A9 DNA and a very weak signal with two fragments of ϕ A2 DNA digested with the same enzyme (Fig. 3b). ϕ A5 DNA strongly hybridized with all *Eco*RV-generated fragments of ϕ A6 DNA and more weakly with some fragments of ϕ A8 DNA digested with *Bgl*II; very faint bands were observed in lanes corresponding to ϕ A3 and SH10 DNAs respectively digested with *Eco*RI and *Bgl*II (Fig. 3c). ϕ A8 DNA showed strong homology with all the fragments of ϕ A3 and SH10 DNAs, digested with *Eco*RI and *Bgl*II respectively (Fig. 3e). In our experimental conditions ϕ A7 DNA did not hybridize with the DNA of any of the other phages used in these experiments (Fig. 3d). None of the four phage genomes used as probe hybridized with ϕ A1 or ϕ C31 DNA.

The results from the Southern hybridization experiments, together with the characteristics of the phages reported above (Tables 1 and 2, Fig. 1), allowed their classification into five groups: (I) ϕ A2, ϕ A4 and ϕ A9, (II) ϕ A3 and ϕ A8, (III) ϕ A7 (phages belonging to these three groups having linear double-stranded DNA molecules with cohesive ends), (IV) ϕ A5 and ϕ A6 (possibly having terminally redundant and partially permuted genomes) and (V) ϕ A1 (which appears to be very different from all the other phages with respect to both the size and structure of its genome and the dimensions of its phage particle). Despite differences in genome structure, phages in groups II and IV are genetically related, since ϕ A5 DNA of group IV hybridized, albeit weakly, with DNA of phages in group II.

Host-range and interaction with host-specified restriction-modification systems

The host-range of the phages was examined by plaque formation on 31 strains of *Streptomyces*, belonging to 16 species. Each phage displayed a different host-range (Table 3), which varied from relatively narrow, as in the case of ϕ A9 (7 out of the 31 strains positive), to relatively broad, as in ϕ A3 and ϕ A8 (19 out of 31 strains positive). In addition to *S. antibioticus* ATCC 11891, the host in which the phages were originally isolated, all the phages formed plaques on *S. showdoensis* and *S. scabies*, an observation which could have taxonomic implications.

None of the nine phages was able to infect strains of the actinomycete genera *Micromonospora*, *Nocardia* and *Saccharopolyspora*. This could reflect the absence of receptors for *Streptomyces* phages in actinomycetes with different cell wall types. Thus, while *Streptomyces* has a type I cell wall composition, *Micromonospora* has type II and both *Nocardia* and *Saccharopolyspora* have type IV (Lechevalier & Lechevalier, 1981; Labeda, 1987). Failure to infect genera with different cell wall composition might be a general feature of actinophages, as has already been reported (Grund & Hutchinson, 1987; McHenney & Baltz, 1988).

The plating efficiencies of the nine phages were tested on three strains of *Streptomyces* known to produce type II restriction endonucleases (*S. albus* G, *S. albus* P and *S. achromogenes*), on a restrictionless mutant of *S. albus* G (*S. albus* J1074; Chater & Wilde, 1980), and on *S. lividans* 1326, a strain which seems to lack any restriction activity (Table 4). The numbers of

recognition sites in each of the phages for the relevant restriction enzymes are shown in Table 2.

The presence of *Sal*GI sites in a given phage genome correlated with an inability of the phage to form plaques on *S. albus* G, the strain which produces the *Sal*GI restriction endonuclease (Arrand *et al.*, 1978). Thus, only ϕ A7, which contains no restriction sites for *Sal*GI, gave plaques on *S. albus* G. ϕ A1, ϕ A3 and ϕ A8, although unable to infect the wild-type strain, plated with high efficiency on the restrictionless mutant *S. albus* J1074, demonstrating that the restriction enzyme was the only barrier preventing development of these phages on *S. albus* G. This result corroborates the important role of *Sal*GI endonuclease in protecting the host against phage infection, as was originally reported by Chater & Wilde (1976). Other phages either gave no plaques (ϕ A9) or formed very small plaques with low efficiency of plating (ϕ A2, ϕ A4, ϕ A5 and ϕ A6) on *S. albus* J1074, indicating that factor(s) other than restriction by *Sal*GI endonuclease could prevent infection of *S. albus* G by actinophages.

Among the six phages which lacked *Pst*I sites in their DNA (ϕ A1, ϕ A3, ϕ A5, ϕ A6, ϕ A7 and ϕ A8) only ϕ A5 was unable to infect *S. albus* P, producer of the restriction enzyme *Sal*PI, which is an isoschizomer of *Pst*I (Chater, 1977, 1980). ϕ A4 and ϕ A9, two of the three phages with many *Pst*I sites in their genome, gave no plaques on *S. albus* P and therefore, as in the case of *Sal*GI, a correlation was found between the reduction in plating efficiency and the presence of restriction enzyme recognition sites. However, since restrictionless mutants of *S. albus* P are not available, the possibility could not be ruled out that factor(s) other than restriction are responsible for reduced plating efficiency on *S. albus* P by phages with *Pst*I sites. ϕ A2, a third phage also containing many *Pst*I sites in its DNA, could infect *S. albus* P with relatively high efficiency of plating (10^{-1}), but with very small plaques.

S. achromogenes ATCC 12767 produces at least three type II restriction endonucleases (J. R. Arrand, P. A. Myers & R. J. Roberts, unpublished observations, cited by Roberts, 1987): *Sac*I, *Sac*II (commercially available) and *Sac*III. All the phages except ϕ A1 had *Sac*I and/or *Sac*II recognition sites in their DNA, and all except ϕ A4 were unable to infect this strain. Surprisingly ϕ A4, which has many *Sac*I and *Sac*II sites in its DNA, gave normal-sized plaques, with an efficiency of plating close to 1 on *S. achromogenes*. Mechanism(s) used by ϕ A4 to avoid restriction by the *Sac* enzymes and by ϕ A2 to escape the *Sal*PI enzyme are currently under study.

None of the phages was able to form plaques on *S. lividans* 1326, despite the fact that no restriction endonucleases have been detected in this strain. In the cases of phages ϕ A5, ϕ A7 and ϕ A8 at least, adsorption specificity was the barrier preventing infection of this host. This was demonstrated by adsorption experiments and also by transfection of *S. lividans* protoplasts with DNA of these phages, using *S. antibioticus* ATCC 11891 as indicator strain (L. A. Diaz & M. R. Rodicio, unpublished). The inability of ϕ A5 to form plaques on *S. albus* P, despite the absence in its genome of recognition sites for the enzyme produced by the host, could be also due to an inability to adsorb to this strain.

Identification of temperate phages

Seven of the nine phages isolated from soil on *S. antibioticus* ATCC 11891 formed turbid plaques in this host. However, cells growing within the plaques did not release phages and they were not immune to superinfection. Therefore none of the phages appeared to produce stable lysogenic derivatives in their original host.

The temperate character of the nine phages was tested in other *Streptomyces* strains in which they produced turbid plaques. At least three phages (ϕ A5, ϕ A7 and ϕ A8) formed stable lysogens in some of the strains tested, as judged by the liberation of free phages by spontaneous induction and by immunity to superinfection. Thus ϕ A5 was temperate in *S. antibioticus* KCC S-0707, ϕ A8 in *S. antibioticus* KCC S-0707 and *S. orchidaceus* NRRL B-2454 and ϕ A7 in *S. albus* P, *S. albus* J1074, *S. orchidaceus* NRRL B-2454 and in three strains of *S. lavendulae* (NRRL B-1871, NRRL B-2036 and NRRL B-2343). Since ϕ A5, ϕ A7 and ϕ A8 contain all the genetic information required for the establishment and maintenance of lysogeny, failure to lysogenize *S. antibioticus* ATCC 11891 could be due to absence of an *attB* integration site in this host.

Phages ϕ A5, ϕ A7, ϕ A8 and also ϕ C31 belong to different immunity groups, since each was

able to lyse strains which were lysogenic for the others. SH10 and R4, two phages which are in the same immunity group (Chater, 1986) were unable to infect ϕ A8 lysogens.

From the results reported in this paper it would appear that ϕ A7 and ϕ A8 are good candidates for the development of cloning vectors: (i) they are temperate, a characteristic which would allow selection of cloned genes during the phage lysogenic cycle, (ii) they have cohesive ends, offering the possibility of isolating deletion mutants among phages resistant to treatment with chelating agents, and (iii) they have a relatively wide host-range, respectively infecting 18 and 19 out of 31 *Streptomyces* strains tested. If the failure of these phages to lysogenize *S. antibioticus* ATCC 11891 is due to the absence of an *attB* site, prophage formation could be achieved by the insertion of a fragment of host DNA into the phage genome, thereby conferring the homology required for integration.

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