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Isolation and Characterization of a Temperate Bacteriophage from *Streptomyces galilaeus*

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A new temperate actinophage from *Streptomyces galilaeus* ATCC 31133 was purified after that strain was crossed with *S. peucetius* ATCC 29050. Sensitive hosts became lysogenized and yielded turbid plaques of 2 to 3 mm in diameter. Host-range analysis indicated that 16 of 27 *Streptomyces* strains tested were sensitive to infection on solid medium. *S. lividans* and *S. coelicolor* A3(2) were among those not infected by this new actinophage. The new actinophage, designated ϕSPK1, belongs to the Bradley group B morphological type, the pH optimum for infection is 6.75 to 7.0, it is not efficiently induced by mitomycin C or UV irradiation, it has a circular chromosome of 35.8 ± 0.5 kilobase pairs in length containing overlapping (cohesive) ends, and the G+C content of its DNA was calculated from the buoyant density of 1.7240 to be 69 mol%. The DNA of phage ϕSPK1 was cleaved by the restriction endonucleases *Apa*I, *Alu*II, *Eco*RI, *Pvu*II, and *Sal*I, but, in all cases except that with *Eco*RI, treatment yielded greater than 20 restriction fragments. No sites were detected for *Bam*HI, *Bcl*I, *Bgl*II, *Cla*I, *Hin*dIII, *Mlu*I, *Pst*I, *Sma*I, *Sph*I, *Sst*I, *Xba*I, or *Xho*I.

Bacteriophages isolated from *Streptomyces* spp. are quite common, and a variety of them have been characterized (2, 14). These actinophages frequently are isolated from soil samples with or without the use of enrichment techniques. Many new actinophages are of interest in *Streptomyces* genetics because of their potential use in the construction of cloning vectors (2, 7, 14, 16). The phage ϕC31 from *S. coelicolor* A3(2) and its derivatives constructed by Chater (2) are excellent examples of the cloning vectors that can be constructed from naturally occurring lysogenic actinophages.

During studies designed to detect indigenous plasmids and phages in anthracycline-producing streptomycetes, we detected an actinophage that was temperate in the genome of *S. galilaeus*. We have since purified and characterized this new actinophage from *S. galilaeus* ATCC 31133, a producer of the anthracycline antibiotics aclacinomycins A and B. Here we describe the morphological and structural characteristics of this new actinophage.

MATERIALS AND METHODS

Organisms and maintenance conditions. The streptomycetes used in this study, along with strain designations and sources, are listed in Table 1. These strains were maintained at room temperature in the dark on plates of yeast malt agar (6) which were sealed with Parafilm. The cultures stored in this manner were transferred every 3 to 4 weeks.

Media and buffers. Mycelia for plasmid isolation were grown in yeast malt broth (YMB) at 30°C on a rotary shaker at approximately 220 rpm. Phage studies were carried out with AC medium, which consisted of 1% glucose–1% yeast extract–0.075% $CaCl_2 \cdot 2H_2O$ (pH 7.0 [18]). For solid medium, 1.5% agar was added, and 0.6% agar was added for soft agar overlays. R2YE medium has been described previously (3).

The phage buffer (SM buffer) contained 0.02 M Tris hydrochloride (pH 7.5), 0.001 M $MgSO_4$, 0.1 M NaCl, and 0.1% gelatin. TE buffer was made as described previously (13). The carbonate buffer for electron microscopy contained 0.4 ml of H_2O, 40 μl of 1 M sodium carbonate, and 20 μl of 0.2 M EDTA.

Plasmid analyses. Several methods were used in efforts to extract indigenous plasmids from anthracycline-producing streptomycetes. These included the rapid boiling method described by Holmes and Quigley (10), the polyethylene glycol (PEG) procedure described by Okanishi and Manome (17), the salt precipitation methods of Ikeda et al. (11), the alkaline denaturation method described by Yugisawa et al. (20), the procedure used by Xue et al. (19) for *S. griseus*, and the procedure described by Eckhardt (9).

Initial isolation of phage from *S. galilaeus*. Cultures of *S. peucetius*, *S. peucetius* subsp. *carneus*, *S. peucetius* subsp. *caesius*, *S. coeruleorubidus*, *S. galilaeus*, and *Streptomyces* sp. strain C5 were grown in YMB for 24 to 48 h, mixed with R2YE soft agar, and spread as lawns on R2YE medium. Each lawn then was spot-inoculated with 5 μl of the liquid cultures of each strain. A set of plates was incubated at 30°C, and another set of plates was irradiated with UV light (58 erg s^{-1} for 15 s) before similar incubation. Control plates of lawns which were not spotted with test cultures also were irradiated and incubated as described above. When lysis zones or turbid plaques were noticeable after 24 h, independent of UV irradiation, agar plugs were removed from the lysis zones and plaques, soaked in SM buffer, and then respotted onto lawns of the indicator organisms.

Because *S. galilaeus* appeared to cause plaque formation at rates much higher than those of the other organisms, it was tested for spontaneous release of phages. *S. galilaeus* was streaked out on AC agar plates, incubated for 3 days, and then overlayed with 0.1 ml of *S. peucetius* ATCC 29050, the indicator organism, in 2.5 ml of soft agar. Plaques observed within 36 h of incubation were suggestive that phage particles were released from the test strain.

To enrich suspected phage particles, *S. galilaeus* was grown at 30°C for ca. 48 h in YMB, after which the mycelia were pelleted by centrifugation at 15,000 × *g* for 15 min. Phage particles were precipitated out of the supernatant by the addition of NaCl and PEG (0.5 M and 10% final concentrations, respectively), followed by incubation overnight at

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TABLE 1. Host range for φSPK1 purified from *S. galilaeus* ATCC 31133

Streptomyces species	Strain designation	Sensitivity
S. pactum	ATCC 27456	+
S. fradiae	948[a]	+
S. coeruleorubidus	ATCC 31276, ATCC 23900, NRRL B-2569	+
S. peucetius	ATCC 29050, ATCC 27952, ATCC 21354, ATCC 31428, ATCC 31052	+
Streptomyces sp.	C5[b]	+
S. achromogenes	2397[a]	+
S. albus G	2396[a]	+
S. griseus	NRRL B-2682	+
S. viridochromogenes	NS[c]	+
S. lincolnensis	ATCC 25466	+
S. rimosus	ATCC 10970	−
S. coelicolor	A3(2) [M110][d]	−
S. parvulus	2266[a]	−
S. alboniger	ATCC 12461	−
S. puniceus	NS	−
S. bamburgiensis	737[a]	−
S. lucensis	ATCC 17804	−
S. lividans	1326[d]	−
S. aureofaciens	ATCC 10762i	−
S. venezuelae	ATCC 10595	−
Saccharopolyspora erythraea	Cd 340[e]	−

[a] Strain designations are from the Ohio State University Culture Collection.
[b] Obtained from the Frederick Cancer Research Center, Frederick, Md.
[c] NS, No strain designation. Obtained from the Ohio State University Culture Collection.
[d] Obtained from D. A. Hopwood, John Innes Institute, Norwich, United Kingdom.
[e] Obtained from L. Katz, Abbott Laboratories, North Chicago, Ill.

4°C. The phages-salt-PEG mixture was pelleted at 10,000 × *g* for 10 min and suspended in 1 to 2 ml of SM buffer. The phage preparation was amplified by spotting 5 μl of phage in SM buffer, obtained as described above, onto lawns of indicator strains and incubating for 24 h. The phage particles were soaked out of turbid plaques by using SM buffer as described above, and plated onto indicator strains to obtain complete plate lysis. Complete plate lysates were eluted from nutrient broth as described by Chater et al. (3). Phages obtained from the nutrient broth elution were collected by high-speed centrifugation (30,000 rpm, 90 min; Ti 60 rotor; Beckman Instruments, Inc., Fullerton, Calif.) and observed by transmission electron microscopy.

Methods for obtaining large quantities of phages. Small quantities of phage were enriched initially by the PEG-NaCl method described above. They were then propagated by mixing 1.2 ml of the phages (10^{10} PFU ml^{-1}) with 3.0 ml of concentrated, homogenized mycelia of *S. peucetius* ATCC 29050 and 30 ml of AC soft agar. These were placed into sterile bioassay dishes (243 mm^2; Nunc, Roskilde, Denmark) containing ca. 100 ml of AC solid medium as a base. The sensitive strain was incubated overnight with the phages, after which 100 ml of nutrient broth was added and the mixture was reincubated for 2 h. The nutrient broth and soft agar were removed and pelleted at a low speed to remove the cells and agar. The phages were precipitated and concentrated by one of the following methods: (i) 2.92% NaCl and 10.0% PEG were added to precipitate the phages, followed by overnight incubation at 4°C and then centrifugation at 10,000 × *g*; the pellet was then suspended in SM buffer; (ii) high-speed centrifugation at 30,000 rpm (Ti 60 rotor, 75 min at 4°C); the pellet was suspended in SM buffer; (iii) 45.0% NH_4SO_4 was added for precipitation, followed by incubation overnight at 4°C and centrifugation at 5,000 × *g*; the precipitate was suspended in 0.1 M ammonium acetate and then dialyzed overnight against ammonium acetate buffer. The phages were pelleted at 30,000 rpm in a Ti 60 rotor, and then suspended in SM buffer.

Electron microscopy of phage. A drop of fluid containing phage particles was suspended on top of a carbon-coated Formvar grid, and the phages were allowed to settle for 2 min. The grid was then washed with 2.0% aqueous uranyl acetate, washed again with water, and allowed to dry. Phage particles were observed with a transmission electron microscope (EM-9; Zeiss). Phage dimensions were calculated from 16 phage particles at two different magnifications. The microscope was calibrated at each magnification by using a catalase specimen (15).

Determination of pH optimum for infection. *S. peucetius* ATCC 29050 was grown in AC liquid medium at 30°C for 24 h and was used as the indicator strain. AC solid medium, which was adjusted with NaOH or HCl to pH values of 5.0 to 8.0, was used as the base. Homogenized *S. peucetius* mycelia (0.2 ml) were mixed with 5 ml of AC medium soft agar (0.6% agar) and adjusted to the pH values of the AC base agar medium and 0.1 ml of phage preparations (10^{-2} to 10^{-8} dilutions of an original 10^{10} PFU ml^{-1} stock). The phages, indicator strain, and soft agar mixture were spread over the base medium; and the number of plaques was determined after 48 h of incubation.

Determination of host range and lysogeny. Streptomycete test strains were grown in liquid AC medium for 24 h, homogenized as described above, and plated with AC soft agar onto AC solid medium. Phages were spotted (10^{10} PFU ml^{-1}) onto the overlays, and plaque formation was determined after 24 h. Presumptively lysogenic organisms, streaked out from turbid plaques obtained from the spot assay, were streaked out on AC medium and were grown for 72 h. They were then overlayed with a sensitive, phage-free indicator strain in a lawn of soft agar, and after 24 h plaque formation was observed. By using a similar technique, the lysogens also were assayed for the ability to be reinfected. *S. galilaeus* ATCC 31133 was grown for 72 h on plates of AC medium and then overlayed with the presumptive lysogen. Superinfection immunity was determined by the lack of plaque formation in the lysogenic indicator strains.

Induction of phages with mitomycin C. Six replicate cultures of *S. galilaeus* were grown at 30°C for 16 h in 100 ml of YMB, at which time mitomycin C was added to yield final concentrations of 0.0, 0.5, 0.75, 1.0, 2.0, and 5.0 μg ml^{-1}. The mitomycin C-treated cultures and the control cultures were incubated with the mitomycin C at 30°C for 24 h, at which time the mycelia were pelleted by centrifugation; and the supernatant was precipitated overnight at 4°C with PEG and NaCl as described above. The induced phages were pelleted by centrifugation, suspended in SM buffer, and assayed by using a sensitive host for plaque-forming titers.

Purification of phage DNA. Phage particles (4 ml; 10^{10} PFU ml^{-1}) were incubated with RNase (40 μg ml^{-1}) for 15 min at 37°C. A lysis mixture containing 0.25 M EDTA (pH 7.4), 0.5 M Tris hydrochloride (pH 9.6), and 2.5% sodium dodecyl sulfate then was added; and the suspension was incubated at 37°C for 30 min. Ice-cold 8 M potassium acetate was added to a final concentration of 0.28 M, and the suspension was incubated on ice for 15 min. The lysate was pelleted at 17,000 rpm in a Ti 60 rotor for 30 min, and the supernatant was dialyzed against TE buffer (10 mM Tris hydrochloride, 1 mM EDTA [pH 8.0]). The dialyzed DNA was precipitated over-

night at −20°C with 1 M sodium acetate (final concentration, 0.28 M) and 2 volumes of 95% ice-cold ethanol. The DNA was collected by centrifugation, suspended in TE buffer, and used for restriction endonuclease digests and electron microscopy.

Analysis of phage DNA by restriction endonuclease digestion. Purified phage DNA was incubated with restriction endonucleases as described in the information sheets accompanying the enzymes. The endonuclease-digested phage DNA was analyzed by standard agarose gel electrophoresis with 0.6% gels.

Phage DNA was analyzed for cohesive ends by treating with 50% formamide for 15 min at 37°C immediately after digestion with *Pvu*II or by heat treating the *Pvu*II-digested DNA at 80°C for 15 min. To promote the formation of the annealed cohesive ends, the phage DNA was ligated with T4 DNA ligase as described previously (13), followed by digestion with *Pvu*II. φX174 DNA, which was digested with *Hae*III, was used as linear DNA size standard. Molecular masses of the linear phage DNA fragments were estimated from the mobility of the fragments relative to those of the linear size standards after electrophoresis on a 5% polyacrylamide gel (40 mA, 3 h).

Buoyant density determinations. Phages suspended in SM buffer were mixed with 0.85 g of CsCl per ml to give a final density of 1.39 g cm^{-3}. Phage DNA in TE buffer was mixed with 0.922 g of CsCl per ml to give a final density of 1.6778 g cm^{-3}. *Escherichia coli* DNA used as a control was mixed with 1 g of CsCl per ml to give a final density of 1.54 g cm^{-3}. Gradients were spun in a VTi 65 rotor (Beckman) at 44,000 rpm for 16 to 24 h using a L8-70 ultracentrifuge (Beckman) and fractionated by displacement of the CsCl with oil. Samples of 5 μl were removed from the 100-μl fractions for refractive index readings with a refractometer (Bausch & Lomb, Inc., Rochester, N.Y.). Aliquots of each fraction were examined spectrophotometrically at 260 nm on a spectrophotometer (2000; Bausch & Lomb) to determine DNA content.

Chemicals. Restriction endonucleases were obtained from International Biotechnologies, Inc. (New Haven, Conn.) or Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). CsCl was obtained from U.S. Biochemicals, Inc. (Cleveland, Ohio), and Seakem ME agarose was obtained from FMC Corp., Marine Colloids Div. (Rockland, Maine). All other chemicals were of the highest grade available.

RESULTS AND DISCUSSION

Isolation of actinophage φSPK1. Because of our intentions of developing cloning systems in anthracycline-producing streptomycetes (13), it was important for us to determine if those strains contained endogenous plasmids or phages. Several attempts were made to extract endogenous plasmids from the anthracycline-producing strains, including *S. peucetius* ATCC 29050, ATCC 27952, and ATCC 21354; *S. coeruleorubidus* ATCC 23900 and ATCC 31276; *S. galilaeus* ATCC 31133; and *Streptomyces* sp. strain C5. Occasionally, a faint band was observed on gels after plasmid extraction procedures, but these were never reproducible. Control cultures of *S. coelicolor* A3(2) and *S. lividans* containing SCP2* and pIJ101, respectively, yielded those plasmids by these procedures. Therefore, the anthracycline-producing streptomycetes do not contain easily extractable plasmids. The sporadic bands did raise the possibility, however, that a defective prophage like that observed by Chung (4) might be present. Thus, the anthracycline-producing streptomycetes were tested for the presence of temperate phages.

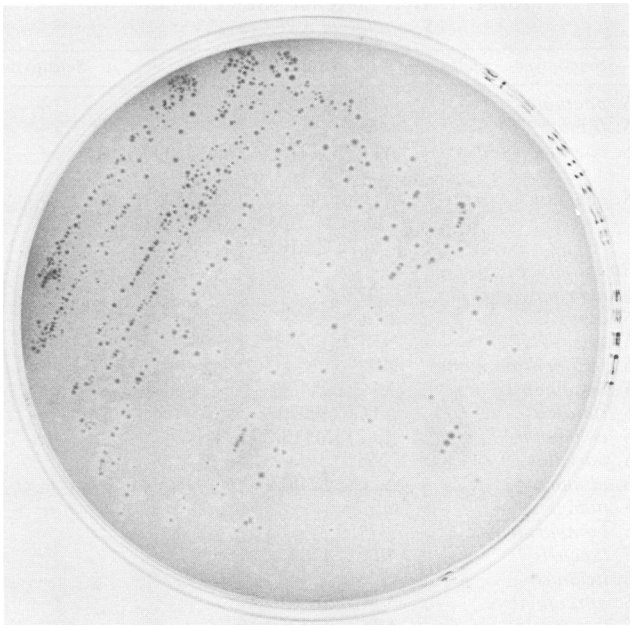

FIG. 1. Formation of plaques in overlayed *S. peucetius* ATCC 29050 by phages liberated from *S. galilaeus* ATCC 31133 which was streaked onto R2YE medium.

The anthracycline producers *S. galilaeus* ATCC 31133; *S. peucetius* ATCC 29050, ATCC 21354, and ATCC 27952; *S. coeruleorubidus* ATCC 31276; and *Streptomyces* sp. strain C5 were concentrated from liquid AC medium and spotted onto lawns of each other to determine if crossing of the strains caused any pock or plaque formation. Plaques were observed only when *S. galilaeus* was spotted onto lawns of the other anthracycline-producing streptomycetes, but not vice versa. Plaques of about 2 to 3 mm in diameter also were observed when *S. galilaeus* was streaked out on solid AC medium and, after 72 h, overlayed with soft agar containing any of the indicator strains (Fig. 1). Early experiments showed that *S. peucetius* ATCC 29050 was the strain most sensitive to the plaque-forming ability of *S. galilaeus*.

A phage was isolated from turbid plaques after *S. galilaeus* was plated on *S. peucetius* ATCC 29050. This phage was able to infect *S. peucetius* and has been designated as φSPK1. Even though it is apparent that *S. galilaeus* liberates particles of φSPK1 (Fig. 1), this liberation was not altered by incubation of *S. galilaeus* with mitomycin C. Incubation of *S. galilaeus* for 24 h with up to 5 μg of mitomycin C per ml resulted in no effect on the ability of the streptomycete to liberate phage particles. UV irradiation or raised temperature (37°C) also did not result in increased induction of the phage. In *Bacillus* spp., lysogens can be induced to liberate free phage particles by using both mitomycin C and UV irradiation (5), and actinophage SAt1 from *S. azureus* was induced by both mitomycin C and UV irradiation (16). Most other investigators also have found that streptomycetes do not liberate phages as a result of mitomycin C or UV treatment (8, 12, 14). The ability of *S. galilaeus* to liberate phage particles at random is a good indication that φSPK1 lysogenized the host strain.

Host range of actinophage φSPK1. φSPK1 has a relatively wide host range (Table 1). Of the 27 streptomycete strains tested, 16 were susceptible to infection by this phage. Although the titer of phage used (10^{10} PFU ml^{-1}) might cause

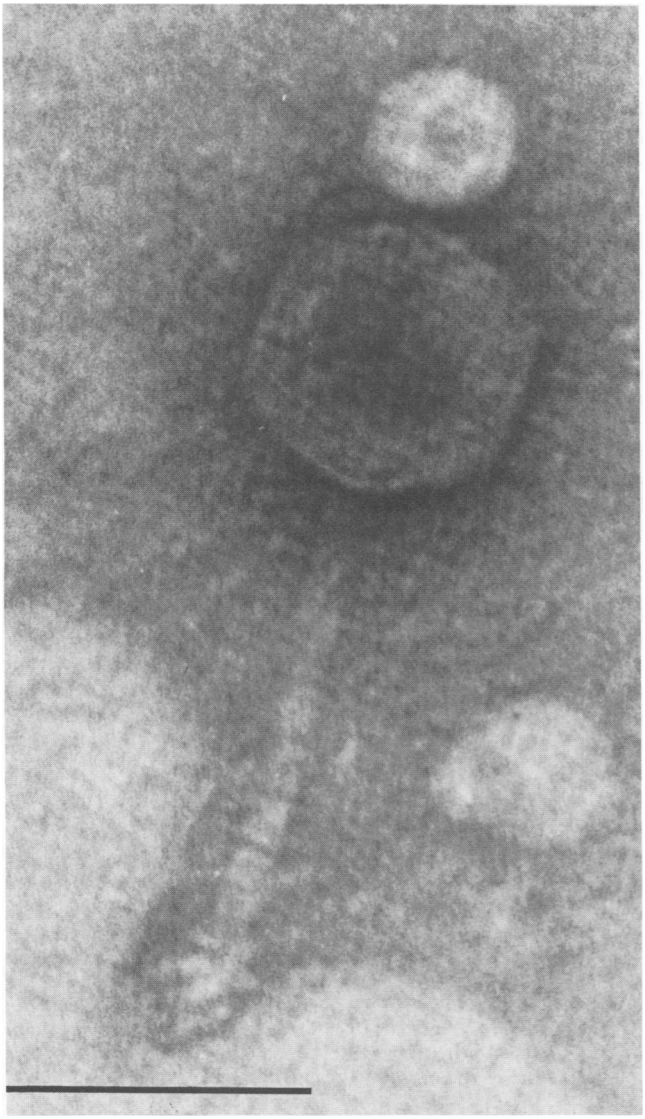

FIG. 2. Actinophage ϕSPK1 from lysates of infected *S. peucetius* ATCC 29050. The phage particles were negatively stained with 2% aqueous uranyl acetate for 2 min. Bar, 0.1 μm.

lysis from without, this probably did not occur here. Of the four susceptible strains subsequently tested for lysogeny, all were lysogens that were able to cause plaque formation on *S. peucetius*. Furthermore, *S. galilaeus*(ϕSPK1) and *S. peucetius*(ϕSPK1) were not able to be lysed with 10^{10} PFU of phage per ml, indicating that lysogeny protected the organisms from lysis, another indication that lysis from without did not occur.

Strains of *S. lividans* and *S. coelicolor*, the best characterized streptomycetes, were not included in the host range for this new actinophage. Strains that were tested showed resistance to superinfection, which also was an indication that ϕSPK1 lysogenized its hosts. The optimum pH for infection by ϕSPK1 of *S. peucetius* was found to be between 6.75 and 7.0.

Physical characteristics of actinophage ϕSPK1. Phage particles of ϕSPK1 had icosahedral heads and noncontractile tails (Fig. 2). From a total of 16 measurements the following sizes were calculated (with standard deviations): the head width was 94 ± 9 nm, the head length was 95 ± 7 nm, the tail length was 210 ± 20 nm, and the tail width was 10 ± 3 nm. Intact actinophage ϕSPK1 also had a buoyant density in CsCl of 1.5461 g cm^{-3}. The shape and size of these phage particles indicated that they belong to the group B type phages described by Bradley (1).

Phage genome. The DNA from actinophage ϕSPK1 was purified and analyzed for its size, its buoyant density, and its digestion by several restriction endonucleases. The DNA of ϕSPK1 was double stranded, and it had a buoyant density in CsCl of 1.7240 g cm^{-3}, from which the G+C content was calculated as 69 mol%. The DNA of streptomycetes has a G+C content of greater than 70 mol% (14). Lomovskaya et al. (14) showed that the G+C content of several actinophages ranged from 59 to 73 mol%. Thus, at least a few phages contained DNA with a significantly lower G+C content than their hosts. The DNA purified from ϕSPK1 was digested by only 5 of 17 restriction endonucleases tested. Digestion with the restriction endonuclease *Eco*RI yielded four high-molecular-mass bands, but apparently, only partial digestions were obtained irrespective of the length of incubation with the restriction endonuclease (Fig. 3). Typically, the DNA of streptomycete phages are cleaved at one to seven sites by restriction endonuclease *Eco*RI (14). Digestion of ϕSPK1 DNA with *Sal*GI, *Apa*I, and *Alu*I yielded more than 20 small fragments and digestion with *Pvu*II yielded 37 discernible bands (Fig. 4). No apparent restriction sites were present in ϕSPK1 DNA for the restriction endonucleases *Bam*HI, *Bcl*I, *Bgl*II, *Cla*I, *Hin*dIII, *Mlu*I, *Pst*I, *Sma*I, *Sph*I, *Sst*I, *Xba*I, or *Xho*I. The lack of digestion of ϕSPK1 DNA with *Pst*I, *Sma*I, *Sph*I, *Sst*I, enzymes that recognize sequences containing high G+C contents, was a curious result, especially because of the high G+C content of the phage DNA. According to Chater (2), a 40-kilobase phage with a G+C content of 66 mol% should have 60

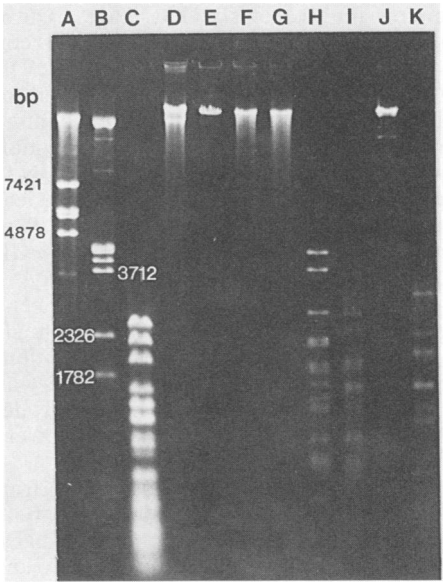

FIG. 3. Agarose gel (0.6%) electrophoresis of fragments generated from restriction digests of DNA purified from actinophage ϕSPK1. Lanes A, B, C, and D, lambda phage DNA digested with *Eco*RI, *Pvu*II, *Alu*I, and *Xba*I, respectively; lanes E through K; undigested actinophage ϕSPK1 DNA and actinophage ϕSPK1 DNA digested with *Xba*I, *Bcl*I, *Pvu*II, *Alu*I, *Eco*RI, and *Sal*I, respectively. Size standards are given in base pairs (bp).

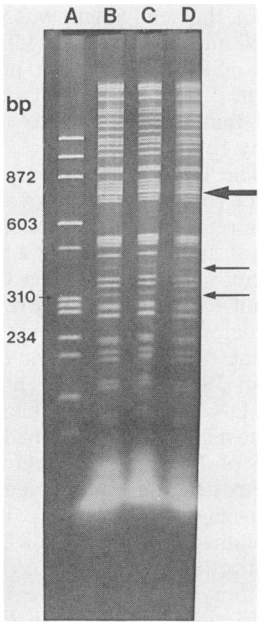

FIG. 4. Polyacrylamide gel (0.5%) electrophoresis of fragments generated from digestion of actinophage ϕSPK1 DNA with *Pvu*II restriction endonuclease. Lanes: A, ϕX174 digested with *Hae*III (size standards are shown in base pairs [bp]); B, ϕSPK1 digested with *Pvu*II; C, ϕSPK1 digested with *Pvu*II followed by incubation at 80°C for 10 min; D, ϕSPK1 preligated with T4 DNA ligase and then digested with *Pvu*II. The large arrow points out annealed fragments joined at their ends, and the small arrows show unannealed terminal fragments which should contain cohesive ends.

cleavage sites for restriction endonucleases in which six of six bases are G+C, 15 sites for four of six G+C bases, and 4 cleavage sites for two of six G+C bases. Out of the 10 restriction endonucleases that we used that recognized at least four of six G+C bases, 7 did not digest the DNA. Only *Pvu*II, *Sal*GI, and *Apa*I from that group cleaved the ϕSPK1 DNA, and all of those resulted in >20 fragments, denoting many cleavage sites. Cleavage sites for the endonucleases *Bam*HI and *Pst*I, however, are rare in the DNA of most actinophages (14). Of the restriction endonucleases from streptomycetes (or their isoschizomers), i.e., *Pst*I (*Sal*PI), *Sal*GI, *Sph*I, *Sst*I, *Xho*I (*Sex*I) (2), only *Sal*GI cleaved ϕSPK1 DNA. *Sal*GI also cleaves the DNA of other streptomycete phages at a frequency much higher than that of other restriction endonucleases isolated from streptomycetes (2). Interestingly, ϕSPK1 infected *S. albus* G, the organism from which *Sal*GI is obtained, but this was done by using the overlay assay, and we have not determined whether the frequency of infection was much lower than that with other nonrestrictive strains.

The approximate apparent length of the DNA from ϕSPK1 was calculated to be about 35,800 ± 500 base pairs, based on the addition of the linear DNA fragments after digestion with *Pvu*II (Fig. 3 and 4). This would be equivalent to an apparent approximate molecular weight of ca. 24,000, which is similar to the size of the DNA from the temperate actinophage SAt1 of *S. azureus* (16), and slightly smaller than the genomes of most other actinophages (14).

We attempted to determine if the genome of phage ϕSPK1 was linear or circular. After digestion of the phage DNA with *Pvu*II, a band was observed with an approximate apparent size of 780 base pairs which stained more intensely with ethidium bromide (Fig. 4). Two minor bands also were detected with apparent sizes of approximately 380 and 320 base pairs (Fig. 4). Although the addition of the two smaller bands did not equal the larger band, they were within the error in these types of measurements. When the DNA from ϕSPK1 was subjected to treatment with heat (80°C, 15 min) or formamide (50%, 15 min) to dissociate the presumptive joined ends, the intensity of the two smaller fragments increased only slightly (Fig. 4). Milder treatments completely failed to separate the terminal fragments, and harsher treatments resulted in degradation of the DNA. On the other hand, when the DNA was preligated prior to digestion with *Pvu*II, the 780-base-pair band was increased in intensity (Fig. 4). Thus, it appears that ϕSPK1 has overlapping (cohesive) ends which are difficult to separate in vitro. This would suggest that the ϕSPK1 actinophage genome is circular; however, further experiments should be carried out to confirm this. The genomes of several other actinophages have been shown to be circular with cohesive ends (2, 7, 14, 16).

Although the morphology and size of ϕSPK1, as well as its DNA size (molecular mass), buoyant density, and G+C molar ratios, are similar to those of other isolated actinophages, the *S. galilaeus* temperate bacteriophage ϕSPK1 is unique due to its host range and the restriction endonucleases digestion patterns of its DNA. Unless restriction enzymes are found which would cleave the ϕSPK1 DNA at only a few sites, it is unlikely that this phage would make a good candidate as a potential cloning vector.

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LITERATURE CITED

1. **Bradley, D. E.** 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. **31**:230–314.
2. **Chater, K. F.** 1987. *Streptomyces* phages and their applications to *Streptomyces* genetics, p. 119–158. *In* S. W. Queener and L. E. Day (ed.), The bacteria. A treatise on structure and function, vol. 9. Antibiotic-producing streptomcytes. Academic Press, Inc., Orlando.
3. **Chater, K. F., D. A. Hopwood, T. Kieser, and C. J. Thompson.** 1982. Gene cloning in *Streptomyces*, p. 69–95. *In* P. H. Hofschneider and W. Goebbel (ed.), Gene cloning in organisms other than *E. coli*. Springer-Verlag, Berlin.
4. **Chung, S. T.** 1982. Isolation and characterization of *Streptomyces fradiae* plasmids which are prophages of the actinophage ϕSF1. Gene **17**:239–246.
5. **Dean, D. H., I. C. Orrego, K. W. Hutchison, and H. O. Halvorson.** 1976. New temperate bacteriophage of *Bacillus subtilis*, ρ11. J. Virol. **20**:509–519.
6. **Dekleva, M. L., J. A. Titus, and W. R. Strohl.** 1985. Nutrient effects on anthracycline production by *Streptomyces peucetius* in a defined medium. Can. J. Microbiol. **31**:287–294.
7. **Donadio, S., R. Paladino, I. Costanzi, P. Sparapani, W. Schreil, and M. Iaccarino.** 1986. Characterization of bacteriophages infecting *Streptomyces erythreus* and properties of phage-resistant mutants. J. Bacteriol. **166**:1055–1060.
8. **Dowding, I. E., and D. A. Hopwood.** 1973. Temperate bacteriophages for *Streptomyces coelicolor* A3(2) isolated from soil. J. Gen. Microbiol. **78**:349–359.
9. **Eckhardt, T.** 1978. A rapid method for identification of plasmid desoxyribonucleic acid in bacteria. Plasmid **1**:584–588.
10. **Holms, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
11. **Ikeda, H., H. Tanaka, and S. Omura.** 1981. Isolation and

characterization of covalently closed circular DNA associated with chromosomal and membrane fraction of *Streptomyces ambofaciens*. J. Antibiot. **35**:497–506.

12. **Klaus, S., H. Kriegel, F. Suss, M. Neigenfind, I. Zimmerman, and U. Traubeneck.** 1981. Properties of the temperate actinophage SH10. J. Gen. Microbiol. **123**:269–279.
13. **Lampel, J. S., and W. R. Strohl.** 1986. Transformation and transfection of anthracycline-producing streptomycetes. Appl. Environ. Microbiol. **51**:126–131.
14. **Lomovskaya, N. D., K. F. Chater, and N. M. Mkrtumian.** 1980. Genetics and molecular biology of *Streptomyces* bacteriophages. Microbiol. Rev. **44**:206–229.
15. **Luftig, R. B.** 1968. Further studies on the dimensions of viral and protein structure using the catalase crystal internal marker technique. J. Ultrastruct. Res. **23**:178–181.
16. **Ogata, S., H. Suenaga, and S. Hayashida.** 1985. A temperate phage of *Streptomyces azureus*. Appl. Environ. Microbiol. **49**: 201–204.
17. **Okanishi, M., and T. Manome.** 1980. Isolation and characterization of plasmid DNA's in actinomycetes. J. Antibiot. **33**: 88–91.
18. **Okanishi, M., K. Suzuki, and H. Umezawa.** 1968. Factors affecting infection of protoplasts with deoxyribonucleic acid of actinophage PK-66. J. Virol. **2**:686–691.
19. **Xue, Y., Z. Zhuang, Y. Zhu, Y. Yu, and K. Dong.** 1981. Plasmid deoxyribonucleic acid replication in *Streptomyces griseus*. J. Bacteriol. **146**:412–414.
20. **Yugisawa, M. T., S. R. Hwang, and J. E. Davies.** 1978. Possible involvement of plasmids in biosynthesis of neomycin. J. Antibiot. **31**:809–813.