***Saccharopolyspora hirsuta* strain 367 releases JHJ-1, a bacteriophage capable of propagation on old mycelium**

JACQUES HAKET, JR, DANIELLE DESMARAIS, KHALIL MEHINDATE and CLAUDE V. DÉRY*

Département de Biologie, Faculté des Sciences, Université de Sherbrooke, Sherbrooke (Québec) J1K 2R1, Canada

(Received 4 September 1989; revised 14 November 1989; accepted 24 November 1989)

Bacteriophage JHJ-1 was isolated from *Saccharopolyspora hirsuta* strain 367 NRRL 12045 as an endogenous but virulent phage. The plaque size was not self-limiting, since a few p.f.u. could completely lyse a lawn. Electron microscopy showed that this phage belonged to group B of Bradley's morphological classification. The JHJ-1 genome is a linear DNA molecule of 41.1 kbp with cohesive ends and a G + C content of 68.8–70.0 mol %. The DNA cleavage map was established for 12 restriction endonucleases. The host range is apparently very narrow, being limited to two strains of *S. hirsuta* (NRRL 12045 and NRRL B-5792). However, JHJ-1 did not lytically infect *S. hirsuta* strain 367 UC 8106. Phage JHJ-1 was shown, by Southern blot analysis, to lysogenize both *S. hirsuta* NRRL 12045 and UC 8106. It thus appears to behave as a virulent mutant of a temperate phage on one, but not on the other, JHJ-1 lysogen.

Introduction

A large number of bacteriophages have been isolated from actinomycetes. These phages are known to decrease the yield of antibiotics and other metabolites during the fermentation process (Ogata, 1980; Chater, 1986). Actinomycete phages have also been used as models for the study of cellular regulatory mechanisms and for the development of cloning vectors (for reviews, see Chater, 1986; Lomovskaya *et al.*, 1980).

Saccharopolyspora hirsuta 367 is the producer of nodusmicin, a typical macrolide antibiotic (Whaley *et al.*, 1980). During the course of our experiments on *S. hirsuta* 367 NRRL 12045, a phage, JHJ-1, endogenous to this strain was isolated. Its morphology, host range, genome and intracellular state are described here. This phage exhibits two interesting features: the formation of plaques on some, but not all, strains carrying it as a prophage; and the ability of plaques to continue growth for many days.

Methods

Strains. *S. hirsuta* strains referred to as 12045, UC 8106 and IC-11 are all versions of the original isolate strain 367 (Whaley *et al.*, 1980): 12045 was obtained from NRRL; UC 8106 was obtained from the UpJohn Company; and IC-11 was a colony of 12045 that did not release phage (see Results). The independently isolated *S. hirsuta* NRRL B-5792 is the type strain. Other strains were obtained from NRRL, ATCC and the collection of the John Innes Institute (Norwich, UK).

Media, buffers and chemicals. The culture medium W, used for phage titration, amplification and host range experiments, contained 10 g yeast extract, 0.5 g K₂HPO₄ and 10 g D-glucose per litre, and was adjusted to pH 6.5 (Ostrowska-Krysiak *et al.*, 1971). Medium W was used as liquid medium or solidified with 0.8% (w/v) (soft agar) or 1.5% (w/v) (bottom agar) Bacto Agar (Difco). The sporulation medium ATCC no. 5 (SPM) contained 1.0 g yeast extract, 1.0 g beef extract, 2.0 g tryptose, 10 g glucose, 15 g agar and a trace of FeSO₄ per litre. The phage buffer contained 50 mM-Tris/HCl pH 7.5, 10 mM-Ca(NO₃)₂ and 10 mM-MgSO₄ (Smorawska *et al.*, 1988). Restriction enzymes, Bal-31 exonuclease, T4 DNA ligase and the Klenow fragment of the DNA polymerase I of *Escherichia coli* were obtained from Boehringer Mannheim Biochemicals or New England Biolabs and were used in accordance with the manufacturer's recommendations.

Virus titration, amplification and purification. JHJ-1 was titrated and amplified on plates with a lawn of IC-11, using the double-layer technique (Adams, 1959). For large-scale amplification, twenty 10 cm plates were routinely used: approximately 10³ p.f.u. of JHJ-1 were mixed with 0.3 ml of a dense liquid mycelial culture of IC-11 in a 4 ml soft agar supplemented with 10 mM-MgSO₄, and overlaid on the bottom agar. For strains UC 8106 and IC-11, liquid mycelial cultures were used since these strains did not sporulate sufficiently to provide a confluent lawn. The plates were incubated at 30 °C for 3 d, cooled at 4 °C for 1 h, and flooded with 5 ml of phage buffer containing 2–3 drops of chloroform. After 8 h at 4 °C, the phage lysate was harvested and centrifuged at 4 °C for 15 min at 4000 g. The supernatant was collected and adjusted to 0.5 M-NaCl and 10% (w/v) PEG 8000 (Yamamoto *et al.*, 1970). After at least 4 h at 4 °C, the suspension was centrifuged as described above. The pellet was then resuspended in phage buffer and layered over a CsCl step gradient. The gradient was made from three 2 ml solutions with densities of 1.360, 1.490 and 1.600 g cm⁻³ in phage buffer. The gradients were centrifuged at 30000 r.p.m. for 3 h at 16 °C in a SW 41 rotor (Smorawska *et al.*, 1988). The phage banded at the 1.490 and 1.600 interface and was harvested by puncturing the side of the tube. The phage suspension was maintained at 4 °C in this solution

and the phage yield averaged 10^{11} – 10^{12} p.f.u. Such phage suspensions were stable for at least 12 months.

Determination of the host range. For rapid determination of the host range of phage JHJ-1, 10 µl of a CsCl-purified phage suspension was spotted on a lawn of mycelial culture or heat shocked (10 min, 55 °C) spores (10^7 c.f.u.) of each strain (Grund & Hutchinson, 1987). The plates were incubated at 30 °C, except for *Faenia rectivirgula*, which was incubated at 43 °C (Smorawska *et al.*, 1988), and examined daily for up to 10 d for evidence of lysis. For a few strains, the virus stocks were diluted to allow visualization of isolated plaques.

Extraction and characterization of JHJ-1 DNA. CsCl-purified phage particles were dialysed against phage buffer. The suspension was then treated with DNAase and RNAase (Sigma), both at a final concentration of 50 µg ml⁻¹, for 1 h at 37 °C. Proteinase K (0.1 mg ml⁻¹) and SDS (final concentration, 0.5%) were then added. After incubation for 1 h at 37 °C, the viral DNA was purified by extraction with phenol, phenol/chloroform and chloroform/isoamyl alcohol (24:1, v/v). The DNA was recovered by ethanol precipitation, dried under vacuum and resuspended in TE buffer (10 mM-Tris/HCl, 1 mM-EDTA, pH 8.0).

Actinomycete DNA was purified as described by Hopwood *et al.* (1985).

Agarose gel electrophoresis was performed in Tris/borate/EDTA (TBE) buffer with agarose concentrations varying from 0.5 to 1.2% (w/v). The gels were stained with ethidium bromide (1 µg ml⁻¹, final concentration) and photographed (Maniatis *et al.*, 1982).

The mol% G+C content was determined by the ultraviolet spectroscopic method of Ulitzur (1972) and by the CsCl density gradient analysis of Mandel *et al.* (1968) as modified by Robin & Rodrigue (1980), except that the peaks of DNA in the gradient were localized by spotting 1 µl of each fraction on an agarose gel containing 1 µg ethidium bromide ml⁻¹ (Maniatis *et al.*, 1982). For both methods, *E. coli* K12 DNA (density 1.710 g ml⁻¹) was included as reference standard (Mandel *et al.*, 1968).

Genomic DNA blot analysis. JHJ-1 and *S. hirsuta* genomic DNA were digested with restriction enzymes and electrophoresed on 0.8% agarose gels. DNA was transferred to Hybond N membranes (Amersham) according to the method of Smith & Summers (1980). DNA probes were labelled with the Multiprime labeling system (Amersham). DNA was hybridized according to the method of Wahl *et al.* (1979), except that the hybridization and wash temperatures were 52 °C and 70 °C respectively, to take account of the mol% G+C content of JHJ-1 DNA.

Electron microscopy. Carbon-coated grids (300 mesh) were positively charged by treatment with 1% (w/v) Alcian Blue for 5 min. Dialysed phage particles (10^9 p.f.u. ml⁻¹) were adsorbed for 1 min and stained for 30 s with 2% (w/v) uranyl acetate or 2% (w/v) phosphotungstic acid, pH 7.1. The grids were examined in a Philips 201 electron microscope. The magnification was calibrated using a carbon grating replica.

Results

Strain 12045 spontaneously releases bacteriophage JHJ-1

We originally obtained a culture of *S. hirsuta* strain 367 from NRRL (NRRL 12045). After the second cycle of growth and sporulation, plaques appeared 2 d after plating a dense mycelial culture on SPM. In order to obtain an indicator strain for phage propagation, we isolated, by plating dilutions of a mycelial culture of

strain 12045 on medium W, a colony named IC-11 that showed no plaque formation after amplification in liquid medium W, replating at high density on SPM or medium W and incubation for 5 d. At this point, strain IC-11 was assumed not to carry extracellular phages nor to spontaneously release phages. However, mock-infected plates were always used for each experiment involving this strain (titration, amplification). The chromosomal DNA of IC-11 was identical to that of 12045 as judged by their restriction patterns (data not shown). Strain IC-11 was used to amplify one plaque to demonstrate the presence of a bacteriophage, which was isolated and named JHJ-1. Nine phage DNA preparations isolated from other plaques appearing on 12045 were identical to JHJ-1 DNA by restriction mapping.

Since strain 12045 released a bacteriophage, the frequency of the phenomenon was briefly investigated. Strain UC 8106 and 16 colonies resulting from spores of 12045 (see later) never showed spontaneous plaque formation when mycelial cultures were plated at high density and incubated for up to 2 weeks, whereas under the same conditions, IC-11 occasionally showed spontaneous clear plaque formation after 8–10 d. The phage DNA isolated from three of these plaques was identical to that of JHJ-1 as determined by restriction mapping. No phage were detected when culture supernatants of IC-11 or UC 8106 were plated on IC-11 or B-5792 as indicator strains. However, B-5792 is not a sensitive indicator strain since evidence of restriction was found in this strain (see later).

Plaque and phage morphology

When JHJ-1 was grown on a lawn of strain IC-11, the plaques were clear with a diameter of 3–4 mm after 2 d incubation. However, when such plates were incubated for longer periods, the diameter of the plaques increased. In fact, 10 plaques (diameter 3–4 mm after 2 d) completely lysed the lawn after incubation for 2–3 weeks. This effect was also observed on YEME (Hopwood *et al.*, 1985), Tryptic Soy Agar (Difco) and SPM. This contrasts with the self-limiting plaque size observed with most, if not all, bacteriophages described thus far.

Phage JHJ-1 has a binal symmetry, with an icosahedral head and a long flexible and striated tail (Fig. 1). The tail (170 nm) is more than three times longer than the head (50 nm). In some particles, a possible part of the channel mediating DNA injection into the host was observed (Fig. 1c). When the virions were stained with phosphotungstic acid, three structures resembling spikes at the end of the tails were observed (Fig. 1c). Such structures have been recorded in other actinophages (Berthiaume & Ackermann, 1977) and, accordingly, phage JHJ-1 is classified as a member of morphological

Fig. 1. Electron micrographs of JHJ-1 virions. CsCl-purified virions were adsorbed on the grids and stained with uranyl acetate (b) or phosphotungstic acid (a, c). The arrows show the striated tail (b) and the spikes at the end of the tail (c). The bars represent 0.5 μ m (a) and 0.2 μ m (b, c).

group B (Bradley, 1967), characterized by an isometric head, a hexagonal shape, and a very long non-contractile tail. In this group, the tail is often striated and may possess a terminal bulb.

Host range of phage JHJ-1

S. hirsuta strain 367 belongs to a genus which comprises only two species, *S. hirsuta* and *S. erythraea*; *S. hirsuta* NRRL B-5792 is the type strain of the species (Lacey & Goodfellow, 1975). In order to determine the host range of phage JHJ-1, particles were spotted on lawns of mycelium of UC 8106 and B-5792 and on lawns of heat-shocked spores of B-5792 and three *S. erythraea* strains

(NRRL 2360, ISP 5517 and NRRL 2359). Only *S. hirsuta* NRRL B-5792 exhibited lysis of the lawn, on preparations both from mycelium and from heat-shocked spores. No evidence of lysis was visible with UC 8106, even at 10^{10} p.f.u. per plate. Since strains NRRL 12045 and UC 8106 have the same DNA restriction patterns, UC 8106 would be expected to be sensitive to infection by JHJ-1. Strain UC 8106 may be a phage-resistant isolate of the culture sent to NRRL by the UpJohn Co. However, B-5792 exhibited a DNA restriction pattern completely different from both UC 8106 and IC-11 (data not shown), and *S. hirsuta* strain 367 may therefore represent a different *Saccharopolyspora* species. There was evidence of restriction in

Fig. 2. Restriction map of JHJ-1 DNA. ●, *HpaI*; ◆, *HindIII*; ■, *AseI*; ○, *EcoRV*; ◇, *BglII*; □, *ScaI*; ▲, *EcoRI*; ▽, *BstBI*; ▼, *SmaI*; ●, *StuI*; ☆, *BamHI*; ★, *PstI*.

B-5792, since there was a 10^4 -fold difference in the plating efficiency on B-5792 and IC-11 of JHJ-1 grown in IC-11, yet JHJ-1 grown on B-5792 plated equally well on both B-5792 and IC-11. No plaque or clearing effects were visible on the *S. erythraea* strains, even after 10 d incubation.

Seven *Streptomyces* strains, namely *Strep. lividans* TK24 (John Innes Institute collection), *Strep. labedae* NRRL B-5616 (formerly *Strep. erythraeus*) (Lacey, 1987), *Strep. avermitilis* NRRL 8165, *Strep. clavuligerus* ATCC 27064, *Strep. coelicolor* A(3)2 (John Innes Institute collection) and *Strep. albus* G wild-type and *SalI* restriction-defective mutant J1074 (John Innes Institute Collection), were also refractory to infection by JHJ-1, as were *Faenia rectivirgula* DSM 43747, *Amycolatopsis rugosa* NRRL 2295, *Saccharothrix aerocolonigenes* NRRL B-11267, *Micromonospora rosaria* NRRL 3718, *Actinoplanes caeruleus* NRRL 5325, *Streptoverticillium flavopersicum* NRRL B-2820 and *Nocardia argentinensis* ATCC 31306, a producer of nargenicin, an antibiotic similar to nodusmicin (Snyder & Rinehart, 1984). Plaque assays performed on *S. hirsuta* UC 8106, *Strep. lividans* TK24, *F. rectivirgula*, *Strep. labedae*, and *S. erythraea* NRRL 2360 and NRRL 2359 did not allow visualization of plaques.

Analysis of the DNA of phage JHJ-1

The G+C content of JHJ-1 DNA was 68.8 mol% as determined by the buoyant density method. The spectrophotometric method gave 70.0 mol%. The DNA of the

host IC-11 contained 73.8 mol% and 71.1 mol% G+C, as determined by the CsCl buoyant density and the spectrophotometric methods respectively. The genome length of the phage, calculated as the sum of restriction fragments, is 41.1 kb. The genome has cohesive ends, confirmed by three different techniques: labelling of the end with Klenow fragment of DNA polymerase I in the presence of [α - 32 P]dCTP then digestion with restriction endonuclease; ligation followed by digestion; heating restriction enzyme digests at 90 °C for 10 min and cooling on ice before loading on agarose gels (results not shown).

The phage DNA was digested with 31 restriction enzymes (Table 1) and 12 enzymes were chosen for the restriction map (Fig. 2). Maps were deduced from partial digestion, double digestion and Bal-31 exonuclease sequential digestion, and by comparing heated and non-heated restriction digests. In two cases, large fragments (*EcoRV* A and B; *StuI* A and B) could not be resolved even on 0.5% agarose gels. These fragments were arbitrarily placed on the map. Complete digestion by *EcoRI*, *BstBI* and *SmaI* could not be achieved, possibly due to the high G+C content of JHJ-1 DNA. It is known that some restriction sites are refractory to digestion by some enzymes (Brown & Smith, 1977; Gingeras & Brooks, 1983).

JHJ-1 DNA was not digested by *MluI*, *XbaI* or *XhoI*. In these cases, the phage DNA was co-digested with plasmids having at least one of each restriction site to demonstrate the absence of enzyme inhibitors (data not shown). Genomic DNA of IC-11, B-5792 and UC 8106

Table 1. Restriction enzymes used to digest JHJ-1 DNA and the numbers of fragments obtained

Enzyme	No. of fragments	Enzyme	No. of fragments	Enzyme	No. of fragments
<i>AseI</i>	2	<i>HindIII</i>	2	<i>SalI</i> *	>20
<i>ApaI</i>	>15	<i>HpaI</i>	2	<i>ScaI</i>	3
<i>BamHI</i> *	7	<i>KpnI</i> *	9	<i>SmaI</i>	5
<i>BclI</i> *	11	<i>MluI</i>	0	<i>SphI</i> *	>10
<i>BglII</i>	3	<i>NarI</i> *	>20	<i>SstI</i> *	>13
<i>BssHII</i>	>20	<i>NruI</i> *	>12	<i>SstII</i> *	>20
<i>BstBI</i>	4	<i>NsiI</i>	6	<i>StuI</i> *	5
<i>BstEII</i>	>13	<i>PstI</i> *	9	<i>XbaI</i>	0
<i>ClaI</i>	5	<i>PvuI</i> *	>20	<i>XhoI</i> *	0
<i>EcoRI</i>	4	<i>PvuII</i> *	>15	<i>XmnI</i>	6
<i>EcoRV</i>	3				

* Denotes that isoschizomers of those enzymes were found in actinomycetes (Roberts, 1984).

was resistant to *XbaI* and *XhoI* and was digested by *MluI*, suggesting that these strains may encode *XhoI* and *XbaI* methylase specificity.

Strains UC 8106 and IC-11 carry intracellular phage DNA

In order to demonstrate the presence of intracellular phage sequences, purified DNA from JHJ-1, UC 8106 and IC-11 was digested with *PstI* and heat-treated at 90 °C to denature the cohesive sequences. Equal amounts of the bacterial genomic DNA were electrophoresed with JHJ-1, blotted and hybridized with JHJ-1 genomic DNA or with a plasmid clone containing the fused cohesive *PstI* fragments D (2.5 kbp) and F (1.8 kbp) (Fig. 3). JHJ-1 DNA was present in strains IC-11 (and 12045) and UC 8106 but not in B-5792 (not shown). This DNA was present in an intracellular form, since the 1.8 and 2.5 kbp *PstI* fragments were fused to give a 4.3 kbp fragment (Fig. 3). However, the sensitivity of IC-11 (or 12045) to phage JHJ-1 could still be due to the presence of lysogenic phage in a small proportion of the bacterial population; this would explain the clear plaques caused by JHJ-1 infection. In order to rule out this possibility, we plated spores of 12045 from our initial stock of spores. Isolated colonies were amplified and analysed for JHJ-1 phage sensitivity and for the presence of intracellular phage DNA. All 16 clones were sensitive to phage JHJ-1, none released any phages and their hybridization patterns were identical to that of IC-11. Therefore JHJ-1 can infect a bacterial strain which contains a resident phage.

Fig. 3. Pattern of hybridization of IC-11 (A), UC 8106 (B) and JHJ-1 (C) DNA. The DNAs, digested with *PstI*, were hybridized with phage JHJ-1 DNA (A–C) or a cloned 4.3 kbp fragment containing the cohesive *PstI* fragments D and F (A'–C'). The 1.8 and 2.5 kbp fragments represent the two *PstI* cohesive fragments; the 4.3 kbp fragment represent the fused fragments.

Discussion

Phage JHJ-1 is different from any previously characterized actinophage as judged by its host range, mol% G+C content, the number of restriction enzyme cleavage sites, and from its restriction map. An intriguing feature of JHJ-1 is its potential to grow on both young and 'aged' cultures. The plaque size is not self-limiting and there was no difference in the titre when the phage was plated on heat-shocked spores or a dense mycelial culture of *S. hirsuta* B-5792. JHJ-1 may be able to exploit the characteristic stationary phase of ageing actinomycete cultures in which the cells, unlike those of dividing bacteria, are continuing to metabolize (Hopwood, 1988).

The host range of JHJ-1 is narrow, limited to only two strains, *S. hirsuta* NRRL B-5792 and NRRL 12045, among the 19 strains tested. It appears to be characteristic of phages isolated on *Saccharopolyspora* strains that they have a host range limited to this genus (Katz *et al.*, 1988; Brzezinski *et al.*, 1986; Grund & Hutchinson, 1987; Donadio *et al.*, 1986). The recent observations that phages P517, 121, SE-3 and SE-5 of *S. erythraea* can infect *Faenia rectivirgula* are exceptions (Kempf *et al.*, 1987; Smorawsinska *et al.*, 1988). However, *Faenia* and *Saccharopolyspora* are closely related wall type IV actinomycete genera (Embley *et al.*, 1988a, b).

Phage JHJ-1 DNA was resistant to cleavage by three of the 31 restriction enzymes tested. Of these three enzymes, *Xho*I isoschizomers are known to be produced by actinomycetes, whereas *Xba*I and *Mlu*I isoschizomers have not been reported thus far (Roberts, 1984). The fact that JHJ-1 DNA can be restricted by 13 restriction enzymes found in actinomycetes suggests, according to Cox & Baltz (1984), that the phage has not evolved recently from a broad-host-range actinophage.

JHJ-1 DNA was shown to be present in an intracellular form in strains 12045 and UC 8106, as the cohesive *Pst*I fragments were fused in their genomes. Whether the phage DNA is present as a plasmid or integrated in the bacterial chromosome remains to be determined.

The most surprising result was the difference in JHJ-1 sensitivity between strains 12045 and UC 8106. Only 12045 was infected lytically by phage JHJ-1. However, both strains should be representatives of the same species [in fact, they should be genetically identical, since Whaley *et al.* (1980) cited them as the same strain and their restriction patterns were identical] and they are lysogenized by the same phage. Moreover, all the 16 clones isolated from our first spore stock of 12045 were sensitive to JHJ-1 infection and contained intracellular phage DNA. The most obvious explanation would be that JHJ-1 represents a virulent mutant of a lysogenic phage, thus allowing phage JHJ-1 to avoid repression in strain 12045; in this case we believe that the resistance of UC 8106 would be caused by a block during the adsorption of the phage particle or during the early phase of the viral cycle. Alternatively, it is possible that, for unknown reasons, a wild-type JHJ-1 phage could bypass the regulatory circuit of repression of a mutant 12045. Thus UC 8106 would represent a normal lysogenic strain immune to super-infection by the same phage. However, a phenomenon similar to the Pgl phenotype, a genetically unstable character of *Streptomyces coelicolor*, might be involved. The Pgl⁺ phenotype causes phage ϕ C31 to be unable to undergo more than one round of lysis in *Strep. coelicolor* and hence prevents plaque formation, while Pgl⁻ mutants give plaques upon phage infection (Chater, 1986). Thus, UC 8106 would be Pgl⁺ and contain a prophage, and 12045 (and IC-11) would be Pgl⁻ and therefore be sensitive to any virulent mutation that spontaneously occurred during culture maintenance. If UC 8106 was a Pgl⁺ strain, this character would have to be very stable since JHJ-1 never yielded plaques on this strain.

Evidence that JHJ-1 is a virulent mutant of a prophage has been obtained. Following treatment of strains UC 8106 and IC-11 with mitomycin C, culture supernatants yielded turbid plaques of self-limiting size on B-5792 as indicator strain; the induced phages did not yield any plaques on IC-11 (L. R. Gaudreau & C. V.

Déry, unpublished results). This induced temperate phage is presently being characterized and its properties will be compared with JHJ-1.

We wish to thank the UpJohn Company and Dr A. J. Lyons of NRRL for providing bacterial strains; Ryszard Brzezinski, Brian G. Talbot and François Denis for discussions and critical reading of the manuscript; Dominique Boucher for providing *E. coli* DNA; Gilles Grondin for the electron microscopy analysis and Anne Rousseau for secretarial assistance. Danielle Desmarais is the recipient of a fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERCC). This research was supported by a grant from the NSERCC to C. V. D.

References

- ADAMS, M. H. (1959). Bacteriophages. New York, Interscience.
- BERTHIAUME, L. & ACKERMANN, H.-W. (1977). La classification des actinophages. *Pathologie Biologie* **25**, 195-201.
- BRADLEY, D. E. (1967). Ultrastructure of bacteriophages and bacteriophages. *Bacteriological Reviews* **31**, 230-314.
- BROWN, N. L. & SMITH, M. (1977). Cleavage specificity of the restriction endonuclease isolated from *Haemophilus gallinarum* (Hga I). *Proceedings of the National Academy of Sciences of the United States of America* **74**, 3213-3216.
- BRZEZINSKI, R., SURMACZ, E., KUTNER, M. & PIEKAROWICZ, A. (1986). Restriction mapping and close relationship of the DNA of *Streptomyces erythraeus* phages 121 and SE-5. *Journal of General Microbiology* **132**, 2937-2943.
- CHATER, K. F. (1986). *Streptomyces* phages and their applications to *Streptomyces* genetics. In *The Bacteria*, vol. IX, pp. 119-158. Edited by S. W. Queener & L. E. Day. Orlando: Academic Press.
- COX, K. L. & BALTZ, R. H. (1984). Restriction of bacteriophage plaque formation in *Streptomyces* spp. *Journal of Bacteriology* **159**, 499-504.
- DONADIO, S., PALADINO, R., COSTANZI, I., SPARAPANI, P., SCHREIL, W. & IACCARINO, M. (1986). Characterization of bacteriophages infecting *Streptomyces erythraeus* and properties of phage-resistant mutants. *Journal of Bacteriology* **166**, 1055-1060.
- EMBLEY, T. M., O'DONNELL, A. G., ROSTON, J. & GOODFELLOW, M. (1988a). Chemotaxonomy of wall type IV actinomycetes which lack mycolic acids. *Journal of General Microbiology* **134**, 953-960.
- EMBLEY, T. M., SMIDA, J. & STACHEBRANDT, E. (1988b). Reverse transcriptase sequencing of 16S ribosomal RNA from *Faenia rectivirgula*, *Pseudonocardia thermophila* and *Saccharopolyspora hirsuta*, three wall type IV actinomycetes which lack mycolic acids. *Journal of General Microbiology* **134**, 961-966.
- GINGERAS, T. R. & BROOKS, J. E. (1983). Cloned restriction/modification system from *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 402-406.
- GRUND, A. D. & HUTCHINSON, C. R. (1987). Bacteriophages of *Saccharopolyspora erythraea*. *Journal of Bacteriology* **169**, 3013-3022.
- HOPWOOD, D. A. (1988). Towards an understanding of gene switching in *Streptomyces*, the basis of sporulation and antibiotic production. *Proceedings of the Royal Society B* **235**, 121-138.
- HOPWOOD, D. A., BIBB, M. J., CHATER, K. F., KIESER, T., BURTON, C. J., KIESER, H. M., LYDIAE, D. J., SMITH, C. P., WARD, J. M. & SCHREMPF, H. (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich: The John Innes Foundation.
- KATZ, L., CHIANG, S.-J. D., TUAN, J. S. & ZAPLEN, L. B. (1988). Characterization of bacteriophage ϕ C69 of *Saccharopolyspora erythraea* and demonstration of actinophage propagation by transfection of *Streptomyces* and *Saccharopolyspora*. *Journal of General Microbiology* **134**, 1765-1771.
- KEMPF, A., GREINER-MAI, E., SCHNEIDER, J., KORN-WENDISCH, F. & KUTZNER, H. J. (1987). A group of actinophages of *Faenia rectivirgula*. *Current Microbiology* **15**, 283-285.

- LACEY, J. (1987). Nomenclature of *Saccharopolyspora erythraea* Labeda 1987 and *Streptomyces erythraeus* (Waksman, 1923) Waksman and Henrichi 1948, and proposals for the alternative epithet *Streptomyces labedae* sp. nov. *International Journal of Systematic Bacteriology* **37**, 458.
- LACEY, J. & GOODFELLOW, M. (1975). A novel actinomycete from sugar-cane bagasse: *Saccharopolyspora hirsuta* gen. et sp. nov. *Journal of General Microbiology* **88**, 75–85.
- LOMOVSKAYA, N. D., CHATER, K. F. & MKRTUMIAN, N. H. (1980). Genetics and molecular biology of *Streptomyces* bacteriophages. *Microbiological Reviews* **44**, 206–229.
- MANDEL, M., SCHILDKRAUT, C. L. & MARMUR, J. (1968). Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **12B**, 184–195.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- OGATA, S. (1980). Bacteriophage contamination in industrial processes. *Biotechnology and Bioengineering* **22**, 177–193.
- OSTROWSKA-KRYSIAK, B., GURKAU, M., SOKOŁOWSKA, B., SIKORA, D. & BLONSKA, A. (1971). New variants of actinophages acting upon *Streptomyces erythraeus*. *Acta Microbiologia Polonica* **B3**, 195–201.
- ROBERTS, R. J. (1984). Restriction and modification enzymes and their recognition sequences. *Nucleic Acids Research* **12** (supplement), r167–r204.
- ROBIN, J. & RODRIGUE, A. (1980). Isolation and preliminary characterization of herpes Channel Catfish virus DNA. *Canadian Journal of Microbiology* **26**, 130–134.
- SMITH, G. E. & SUMMERS, M. D. (1980). The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzoyloxy methyl paper. *Analytical Biochemistry* **109**, 123–129.
- SMORAWINSKA, M., DENIS F., DÉRY, C. V., MAGNY, P. & BRZEZINSKI, R. (1988). Characterization of SE-3, a virulent bacteriophage of *Saccharopolyspora erythraea*. *Journal of General Microbiology* **134**, 1773–1778.
- SNYDER, W. C. & RINEHART, K. L. (1984). Biosynthesis of nargenicin and nodusmicin. *Journal of the American Chemical Society* **106**, 787–789.
- ULITZUR, S. (1972). Rapid determination of DNA base composition by ultraviolet spectroscopy. *Biochimica et Biophysica Acta* **272**, 1–11.
- WAHL, G. M., STERN, M. & STARK, G. R. (1979). Efficient transfer of large DNA fragments from agarose gels to diazobenzoyloxymethyl paper and rapid hybridization by using dextran sulfate. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 3683–3687.
- WHALEY, H. A., CHIDESTER, C. G., MISZAK, S. A. & WNUK, R. J. (1980). Nodusmicin: the structure of a new antibiotic. *Tetrahedron Letters* **21**, 3659–3662.
- YAMAMOTO, K. R., ALBERTS, B. M., BENZINGER, R., LAWHORNE, L. & TREIBER, G. (1970). Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. *Virology* **40**, 734–744.