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Actinophages and restriction enzymes from *Micromonospora* species (Actinomycetales)

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SUMMARY

To develop a screening procedure for the detection of restriction endonucleases in micromonosporae and catellatosporae based on efficiency of plating, eight different actinophages were isolated from soils enriched with *Micromonospora* species and one from *Catellatospora*-enriched soil. The lytic actinophages all contained double-stranded DNA and the majority appeared, when examined by electron microscopy, to belong to Ackermann's type B1 since they had isometric heads and noncontractile tails. One actinophage was classified as type C1 because of its isometric head and very short noncontractile tail. The host ranges of the actinophages were determined on strains of *Micromonospora* and selected species from other actinomycete genera of cell wall chemotype II. Type II restriction enzymes were isolated from *M. echinospora* ssp. *echinospora* (ATCC 15837), *M. purpurea* (ATCC 15835) and *M. zionensis* (LL-100-125) and were designated *Mec*I, *Mpu*I and *Mzi*I, respectively. Restriction enzymes *Mec*I and *Mpu*I are isoschizomers of *Xho*I, while *Mzi*I is an isoschizomer of *Pvu*II.

INTRODUCTION

The occurrence of host-controlled restriction-modification (R-M) systems is widespread in the prokaryotic kingdom. R-M activity was first inferred from bacteriophage restriction data [5,21] because of the decreased efficiency of plating (EOP) when a bacteriophage, capable of infecting two hosts, was propagated by passage through one host and plated on the other host. EOP studies have been used as indicators of R-M in *Streptomyces antibioticus* [26] and *Streptomyces* species [11]. Biological screening has also provided evidence for two R-M systems in *Halobacterium cutirubrum* [23].

The basis of the present study was a search for restriction endonucleases among members of the important antibiotic-producing genus *Micromonospora*. R-M systems impose a barrier to interspecies gene transfer [4,11], therefore knowledge about the R-M systems present in *Micromonospora* species could foster the development of transformation or transduction systems for the manipulation of anti-

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Table 1

Five groups of *Micromonospora* species created by actinophage infection patterns

+ = host infected by actinophages as revealed by plaque formation; − = host not infected by actinophages.

Species	Strain No.[b]	Actinophages								
		MBφ A15	MCφ A02	MCφ A16	MEφ VO1	Mlφ A08	Mlφ A15	MPφE RCPM	MZφ 11	CSφ A09
M. halophytica ssp. *halophytica*[a]	ATCC 27596	−	−	−	−	−	−	−	−	−
'M. lilacina'	NRRL B16080	−	−	−	−	−	−	−	−	−
'M. parva'	NRRL B16093	−	−	−	−	−	−	−	−	−
'M. viridifaciens'	NRRL B16087	−	−	−	−	−	−	−	−	−
M. carbonacea	ATCC 27114	+	−	−	−	−	−	−	+	−
'M. sagamiensis spp. *sagamiensis'*	ATCC 21826	+	−	−	−	−	−	−	+	−
M. aurantiaca	NRRL B16091	+	−	−	−	−	−	−	+	−
'M. lacustris'	NRRL B11050	−	−	−	−	−	+	−	+	−
M. scalabitana spp. *sporogenes*	NRRL B16086	−	−	−	−	−	−	+	+	−
M. coerulea[a]	NRRL B16092	−	+	+	−	+	−	+	+	−
M. purpureochromogenes[a]	IMRU 3343	−	−	−	+	−	−	−	+	−
M. rosaria	NRRL 3718	−	−	−	+	−	−	−	+	−
'M. saitamica'	NRRL B16084	−	−	−	+	−	−	−	+	−
'M. globosa'	NRRL 11299	+	−	−	+	−	−	−	+	−
M. chalcea	ATCC 12452	+	−	−	+	−	+	−	+	−
'M. peucetica'	NRRL B16082	+	−	−	+	+	+	−	+	−
'M. inyoensis'	ATCC 27600	+	+	−	+	+	+	−	+	−
M. olivoasterospora	ATCC 21819	+	+	−	+	−	−	−	+	−
M. echinospora ssp. *echinospora*[a]	ATCC 15837	−	−	−	+	−	−	+	+	−
'M. zionensis'[a]	LL-100-125	−	−	−	+	−	−	+	+	−
M. purpurea[a]	ATCC 15835	−	−	−	+	+	−	+	+	−
M. chalcea spp. *izumensis*	ATCC 21561	+	−	−	+	−	−	+	+	+
M. brunnea[a]	NRRL B16079	+	−	−	+	+	−	+	+	−
M. carbonacea spp. *aurantiaca*	NRRL 2997	+	−	−	+	+	−	+	+	−
'M. scalabitana spp. *rubra'*	NRRL B16085	+	−	−	+	+	+	+	+	−
M. purpureochromogenes ssp. *halotolerans*[a]	LL-RV-79-9-101	+	−	−	+	+	+	+	+	−
M. rhodorangea	NRRL 5326	+	+	−	+	+	+	+	+	+
M. halophytica spp. *nigra*	NRRL 3097	−	+	−	+	−	+	+	+	−
M. inositola[a]	NRRL B16095	−	−	−	−	+	+	+	−	+
'M. grisea'	NRRL 3800	−	−	−	−	+	+	−	−	+

[a] Screened for R-M activity using efficiency of plating studies.

[b] NRRL = Northern Regional Research Laboratory; ATCC = American Type Culture Collection; IMRU = Institute of Microbiology, Rutgers University; LL = Lechevalier and Lechevalier.

biotic biosynthesis genes [3,14,15]. As observed by the frequent appearance of actinomycetes in the list of restriction enzyme-producing microorganisms [25], these bacteria constitute a valuable source for the isolation of new restriction enzymes.

The host ranges of the actinophages were determined for *Micromonospora* strains and representative species from nine actinomycete genera. The patterns of host susceptibility to actinophage infection can be used to divide actinomycetes into taxonomic groups [6,12,20,24]. In *Micromonospora* species, phage infection patterns have not been exploited as a standard taxonomic criterion.

MATERIALS AND METHODS

Growth of microorganisms

Table 1 lists the *Micromonospora* species included in the actinophage host-range studies and identifies those strains screened for restriction endonucleases. Table 2 lists other cell wall chemotype II actinomycetes investigated for actinophage host range.

The inocula for actinophage propagation, titering and EOP studies were grown overnight to a cell density of approximately 500 Klett units (green filter No. 55), in 10 ml TY broth contained in a glass test tube. The glass tube contained a freely-moving glass rod to disperse the growth. Agitation was at 400 rpm on a 1 in stroke rotary shaker at 30°C. TY medium consisted of 5 g tryptone (Difco) and 5 g yeast extract (Difco) in a final volume of 900 ml distilled water; pH was adjusted to 7.2 with NaOH. Added after sterilization were 50 ml each of 40% glucose and 0.5 M morpholinepropanesulfonic acid (Mops), pH 7.2. For the production of inocula, $MgSO_4$ and $Ca(NO_3)_2$ were each added to the medium post-sterilization to a 5 mM final concentration. Broth for soil enrichment and actinophage propagation in flasks, as well as top agar (0.7%) and basal agar (1.5%) prepared for titering and EOP studies, were supplemented to 20 mM $Ca(NO)_3)_2$; the $MgSO_4$ level remained at 5 mM. The inocula for host-range studies and cell mass for the isolation of restriction enzymes were grown in STY broth. STY medium contained 25 g Maltrin M040 (Grain Processing Corp., Muscatine, IA) in TY broth. Other type II cell wall actinomycetes were grown under the same conditions as *Micromonospora* species.

Actinophage soil enrichment

The soil samples were stored in plastic bags after collection. In the case of wet samples, the bags were left open to air-dry before sealing. The environment of the soil collection area was noted and soil pH was determined. The soil samples used throughout this study were collected in Australia.

The micromonosporae selected for actinophage soil enrichment were grown for 24 h as described above. Enrichment for *M. echinospora*-specific actinophages required the addition of 5–15 mM NaCl to the TY broth rather than 20 mM $Ca(NO_3)_2$. The glass rods were removed from each tube, 1 g of soil was added and the tubes returned to the shaker machine. After 7 days, 1 ml samples were collected from each tube. The samples were centrifuged at 12000 × *g* for 5 min and the supernatants were filtered through Durapore HVLP 0.45 μm Swinnex filter units (Millipore Corp.). Ten microliters of the filtered material were streaked onto TY soft-agar overlays [2] containing cells of the putative host. Streaked plates were incubated overnight at 30°C and stored at room temperature for 5 days of observation. Clear and turbid spots were soaked in 0.5 ml phage extraction buffer (0.1 M NaCl, 20 mM $Ca(NO_3)_2$, 5 mM $MgSO_4$ and 25 mM Mops, pH 7.2) for 3 h at 28°C, filtered and restreaked. Single plaque purification was repeated three times. Actinophage MPφE-RCPM was a clear plaque-forming mutant of the temperate phage MPφWR-1. The latter phage was isolated by one of us (B.C.T.) from a soil moistened with TY medium and incubated for 3 days at 28°C.

The names of the actinophages referred to the enrichment host and the soil sample from which they were isolated and were determined by the following method: first letter of the host genus and species names (capitalized), followed by the Greek letter phi (φ) and the soil isolate designation.

Table 2

Host-ranges of actinophages on other type II actinomycetes not belonging to the genus *Micromonospora*.

+ = host infected by actinophages; − = host not infected by actinophages.

Species	Strain No.[b]	Actinophages								
		MBφ A15	MCφ A02	MCφ A16	MEφ VO1	Mlφ A08	Mlφ A15	MPφE RCPM	MZφ 11	CSφ A09
Actinoplanes italicus	NRRL 27366	−	−	−	−	−	−	−	−	−
Actinoplanes philippinensis	NRRL 5462	−	−	−	−	−	−	−	−	−
Actinoplanes utahensis	ATCC 14539	−	−	−	−	−	−	−	−	−
Amorphosporangium auranticolor	ATCC 15330	−	−	−	−	−	+	−	−	−
Ampullariella lobata	ATCC 15350	+	−	−	+	−	+	+	+	−
Ampullariella sp.	LL-37Q-46	−	−	−	−	−	+	−	+	−
Catellatospora sp.[a]	LL-2926	−	−	−	−	+	−	+	−	+
Catellatospora sp.	LL-Y13-9	−	−	−	−	−	−	−	−	−
Catellatospora sp.	LL-10-25	−	−	−	−	−	−	−	−	−
Catenuloplanes sp.	LL-Pall No. 2	−	−	−	−	−	−	−	−	−
Catenuloplanes japonicus	ATCC 31637	−	−	−	−	−	−	−	−	−
Dactylosporangium aurantiacum	LL-D748	−	−	−	−	−	−	−	−	−
Glycomyces rutgersensis	NRRL B16106	−	−	−	−	−	−	−	−	−
Glycomyces harbinensis	NRRL 15337	−	−	−	−	−	−	−	−	−

[a] Host checked for presence of R-M activity using efficiency of plating studies.

[b] NRRL = Northern Regional Research Laboratory; ATCC = American Type Culture Collection; LL = Lechevalier and Lechevalier.

Propagation and concentration of actinophages

Actinophages were propagated in 50 ml TY broth using a 4% (v/v) overnight host inoculum and the addition of a single plaque. Agitiation was at 300 rpm on a 2 in stroke orbital shaker in a 250 ml Erlenmeyer flask at 28°C. The overnight lysate was centrifuged at 10000 × *g* for 20 min at 10°C and filtered through Durapore HVLP 0.45 μm Swinnex filter units.

The lysate was incubated at 37°C for 1 h with 10 μg/ml RNase A (Cooper Biomedical) and 10 μg/ml DNase I (Sigma). Actinophages were concentrated by adding 0.6 g NaCl and 1.1 g PEG 6000 (Fluka) per 10 ml lysate to yield an overnight precipitation at 4°C [22]. The sample was centrifuged at 15000 × *g* for 30 min at 4°C. The pellets were resuspended in CsCl dissolved in phage extraction buffer to a density equal to 1.481 g/cm^3. The sample was centrifuged at 35000 rpm for 44 h at 4°C, the phage band collected and then dialyzed for 24 h at 4°C against three changes of phage extraction buffer.

Host-range studies

Micromonosporae and the other type II cell wall actinomycetes used for host-range studies were grown for 24 hours in STY broth as described. Actinophage stocks were diluted in phage extraction buffer, and 10 μl of each dilution were both spotted and streaked onto TY soft-agar overlays of the test host. The plates were incubated at 30°C overnight, then stored at room temperature for 2 weeks. Clear and turbid spots were soaked in phage extraction buffer and dilutions plated to discriminate between phage infection and nonspecific clearing caused by any inhibitory substance in the lysate. A phage infection was confirmed by the presence of plaques.

Transmission electron microscopy

A drop of the dialyzed actinophage suspension was pipetted onto a Formvar-coated, carbon-stabilized 400-mesh copper grid, fixed with 1% glutaraldehyde and negatively stained with 2% uranyl acetate. The grid was blotted with filter paper and observed with a JEOL transmission electron microscope (JEM-100CX) operated at 100 KV.

Actinophage DNA isolation and restriction digests

The dialyzed actinophage stock was mixed with EDTA to a 100 mM final concentration, extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1 v/v) and twice with chloroform [22]. The DNA was precipitated in the presence of 0.3 M sodium acetate and 2 vol. of cold isopropanol, then reprecipitated in absolute ethanol and washed in 95% ethanol. The DNA was dissolved in 10 mM Tris, pH 7.5 and 1 mM EDTA.

Actinophage DNA was digested according to the manufacturer's directions with the restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, *Pvu*II, *Xba*I and *Xho*I (BRL and New England Biolabs). If the DNA was refractory to cleavage by these six enzymes, further digests were conducted with the enzymes *Nar*I, *Pst*I, *Bgl*II, *Sau*3AI and *Kpn*I. Restriction patterns generated among the actinophages were used to confirm the uniqueness of each isolate [13] and to approximate the size of the genome [7].

Efficiency of plating studies

Actinophage stocks (10^{12} PFU/ml) propagated for one growth cycle on the enrichment host were diluted from 10^{-1} to 10^{-10} in 100 μl phage extraction buffer. Added to the dilution tubes were 0.3 ml of host inoculum and 3 ml soft-agar which were spread over TY basal agar. The plates were incubated overnight at 30°C and stored at room temperature for 5 days of observation. A comparison of titers on the enrichment host and on multiple secondary hosts in the first round of propagation suggested which secondary hosts possessed R-M activity. The system was then reversed for the second round of propagation by soaking an isolated plaque from the lawn of the secondary host (obtained from the first round of propagation) and plating it on the enrichment and secondary hosts to determine whether the enrichment host possessed possible R-M activity. The third and final cycle of propagation soaks an isolated plaque from the lawn of the enrichment host (obtained from the second round of propagation) to repeat and confirm the results from the first round of propagation.

When used for in vivo detection of R-M activity,

EOP results must demonstrate alternance (reversibility) in titer dependence on the last host strain used for propagation. To compensate for the experimental variability of counting plaques on an irregular host lawn, a 100-fold difference was selected as the indicator of putative R-M activity.

Isolation of endonuclease activity

Micromonospora species were grown as described in STY broth for 24 h. The cells were washed once in 1 M NaCl, 20 mM Tris buffer, pH 7.5 and twice in 20 mM Tris buffer. Five grams of cells were resuspended and brought up to a 10 ml volume with buffer A (20 mM Tris buffer, pH 7.5, 1 mM EDTA, pH 8.0 and 10% glycerol) and stored at −70°C. The cells were ruptured by one passage at 1300 psi through a chilled French pressure cell (American Instrument Company), the lysate collected in a tube containing 10 mM β-mercaptoethanol (final concentration) and 20 μg/ml phenylmethylsulfonyl fluoride and kept at 4°C for subsequent manipulations. To remove the nucleic acids, 20% streptomycin sulfate in 100 mM Tris buffer pH 8.0 (actual pH = 6.8) was added to the viscous crude extract to give a final concentration of 2% [28]. The mixture was stirred for 20 min, then centrifuged at 15000 × *g* for 25 min. The crude extract was filtered through a Millex GVWP 0.22 μm syringe filter unit.

To assay for type II restriction enzyme activity, dilutions of crude extract were incubated for 1 h at 37°C in 50 mM NaCl restriction digest buffer (10 mM Tris buffer, pH 7.5, 10 mM $MgSO_4$, 50 mM NaCl, 10 mM β-mercaptoethanol) with the following DNA types: adenovirus 2, Simian virus 40, pBR322, *Micromonospora* actinophages, bacteriophages lambda, T7 and T4 [27]. The reactions were terminated by the addition of 1/10 vol. of stop dye (0.25% bromophenol blue, 15% Ficoll type 400 and 50 mM EDTA) and by heating the reaction to 65°C for 10 min. The fragments were separated by electrophoresis at 20 V for several hours in a 0.8% agarose gel containing 1 μg/ml ethidium bromide.

Restriction enzymes were separated from interfering material using the Pharmacia Fast Protein Liquid Chromatography (FPLC) system (Piscataway, NJ). Partially purified enzyme was eluted from the FPLC Mono Q anion exchange column using a 0–1 M KCl-buffer A linear gradient.

RESULTS

The enrichment protocol led to the isolation of actinophages for *M. brunnea, M. coerulea, M. echinospora* spp. *echinospora, M. inositola* and *Catellatospora* sp. Actinophages enriched on *M. purpurea* and *M. zionensis* were isolated previously using a different method. The host-ranges of the actinophages on the *Micromonospora* species are listed in

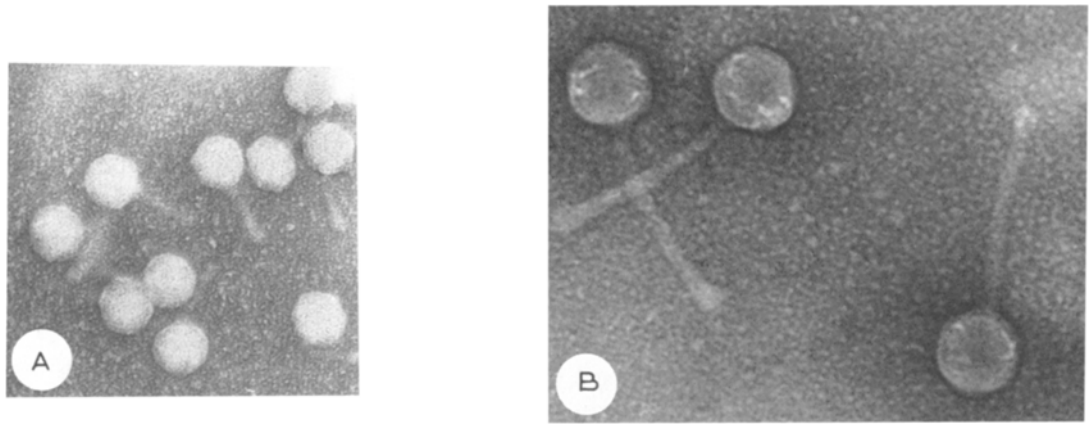

Fig. 1. Transmission electron micrographs of actinophage isolates negatively stained with 2% uranyl acetate. (A) Actinophage MCφA02; morphotype C1; 160000 × magnification. (B) Actinophage MIφA15; morphotype B1; 160000 × magnification.

Table 3

Characteristics of the actinophage isolates

Actinophage (Enrichment host)	Head/tail (nm)	Genome size (kb)	Number of cleavage fragments from the restriction enzymes[a]					
			*Xba*I	*Eco*RI	*Hin*dIII	*Xho*I	*Pvu*II	*Bam*HI
MBφA15 (*M. brunnea*)	46/234	51–53	3	4	5	>22	> 9	8
MCφA02 (*M. coerulea*)	40/46	39–40	3	5	3	> 8	> 5	8
MCφA16 (*M. coerulea*)	60/200	55–57	0	0	4	0	4	3
MEφV01 (*M. echinospora*)	56/236	42–46	3	5	5	>20	>16	>15
MIφA08[b] (*M. inositola*)	40/106	41–42	0	0	0	0	0	0
MIφA15[b] (*M. inositola*)	66/170	60–62	0	0	0	0	0	0
MPφE-RCPM (*M. purpurea*)	81/228	53–55	0	3	0	0	2	0
MZφ11 (*M. zionensis*)	60/290	41–43	3	4	6	> 9	9	>10
CSφA09 (*Catellatospora* sp.)	40/110	53–54	0	6	0	0	0	0

[a] Type II restriction endonucleases used for DNA cleavage.

[b] MIφA08 and MIφA15 were uncut with restriction enzymes *Nar*I, *Pst*I and *Bgl*II, and yielded 15 and >10 bands respectively, when digested with *Kpn*I.

Table 1. The actinophage infection patterns on selected species of other type II cell wall actinomycetes [19] are listed in Table 2. Transmission electron micrographs of two of the actinophages are shown in Fig. 1.

A summary of the characteristics of the actinophage isolates are listed in Table 3. Comparative restriction digests conclusively proved each actinophage isolate to be unique. The sizes, in kilobases, of the actinophage genomes were calculated by summing the fragment sizes from the restriction digests. The fragment sizes were determined by comparision with lambda *Hin*dIII digest mobility plots and represent the mean of three measurements.

Based on the results from the EOP studies, the presence of R-M activity was suspected in *M. brunnea, M. coerulea, M. inositola* and *Catellatospora* sp., but no restriction endonucleases were isolated from these species (results not shown). In addition, no type II restriction endonuclease was isolated from *M. halophytica* spp. *halophytica*, a species uninfected by all actinophages included in the EOP studies (results not shown). Table 4 gives examples of data from the EOP studies from those strains in which restriction enzymes were isolated. Type II restriction endonucleases were isolated from *M. echinospora* ssp. *echinospora, M. purpurea* and *M. zionensis*, and are designated *Mec*I, *Mpu*I and *Mzi*I, respectively. Fig. 2 shows the digestion patterns of these three enzymes on adenovirus 2 DNA. The partially purified enzymes eluted from the Mono Q

Table 4

Efficiency of plating (EOP) data for those hosts which yielded type II restriction enzymes

(A) EOP data for actinophage MPφE-RCPM propagated on *M. purpurea* and *M. inositola*. (B) EOP data for actinophage MZφ11 propagated on *M. zionensis* and *M. echinospora* ssp. *echinospora*. (C) EOP data for actinophage MZφ11 propagated on *M. zionensis* and *M. brunnea*. The EOP of the actinophages on the enrichment host is defined as unity, under the standard conditions established to yield the highest actinophage infectivity. Plaque titers on the secondary host are fractions of unity, and must be at least 100-fold less than those observed on the enrichment host to be considered a valid indicator of R-M activity. EOP data must be reversible and completely dependent on the last host for propagation.

(A) Previous Hosts in Sequence	EOP on *M. purpurea*	EOP on *M. inositola*
M. purpurea	1.0	4×10^{-3}
M. purpurea, M. inositola	3×10^{-3}	1.0
M. purpurea, M. inositola, M. purpurea	1.0	2×10^{-2}
(B) Previous Hosts in Sequence	EOP on *M. zionensis*	EOP on *M. echinospora*
M. zionensis	1.0	5×10^{-3}
M. zionensis, M. echinospora	83.0	1.0
M. zionensis, M. echinospora, M. zionensis	1.0	5×10^{-2}
(C) Previous Hosts in Sequence	EOP on *M. zionensis*	EOP on *M. brunnea*
M. zionensis	1.0	1.0
M. zionensis, M. brunnea	3×10^{-2}	1.0
M. zionensis, M. brunnea, M. zionensis	1.0	1.0

anion exchange column between 0.15 and 0.30 M KCl in buffer A. *Mec*I, *Mpu*I and *Mzi*I were most active in 50 mM, 150 mM and 100 mM NaCl digestion buffer, respectively.

CONCLUSIONS

The enrichment procedures used in this study permitted isolation of *Micromonospora*- and *Catel-*

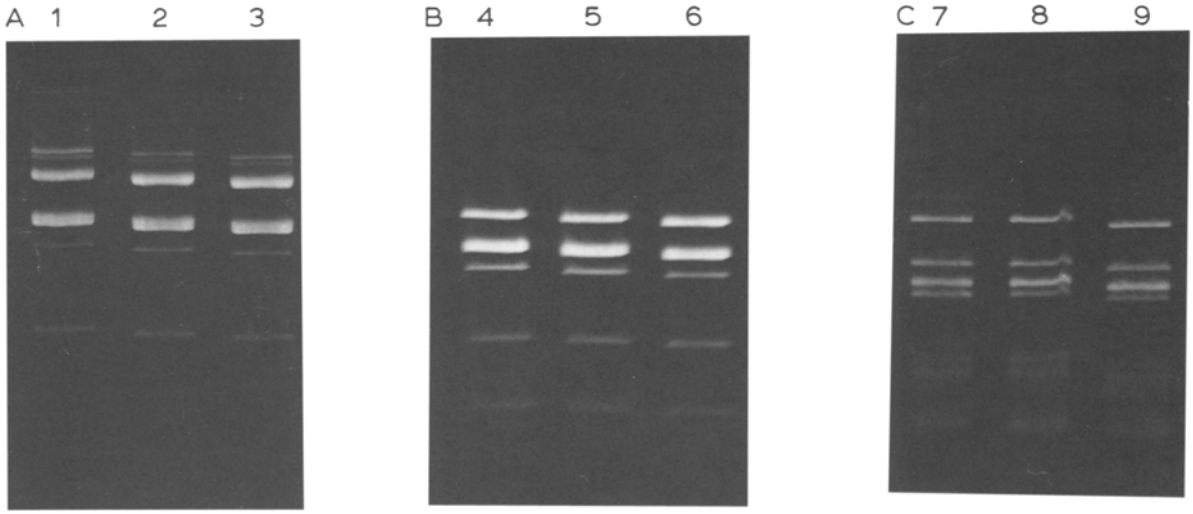

Fig. 2. Digests of adenovirus 2 DNA using the type II restriction enzymes *Mec*I, *Mpu*I and *Mzi*I compared to digests obtained with the corresponding isoschizomers *Pae*R71, *Xho*I and *Pvu*II, respectively. (A) Lane 1, *Mec*I; lane 2, *Pae*R71; lane 3, *Mec*I and *Pae* R71. (B) Lane 4, *Mpu*I; lane 5, *Xho*I; lane 6, *Mpu*I and *Xho*I. (C) Lane 7, *Mzi*I; lane 8, *Pvu*II; lane 9, *Mzi*I and *Pvu*II.

latospora-specific actinophages. Soils yielding actinophages were moist when collected, ranged in pH from 5.7 to 7.5 and had been regularly amended with fertilizer in their natural environment. Actinophages were discovered in a soil from a cattle-grazing field, the soils of three vineyards, two garden soils and a compost pile.

The majority of the new actinophages isolated in this study fall into a common morphological group described by Ackermann and coworkers as type B1 [1]. Type B1 is characterized by an isometric head, hexagonal in shape, and a very long noncontractile tail. In addition, the tails are often striated and may possess a terminal bulb. Actinophage MCφA02 had the C1 morphotype; it is characterized by a very short noncontractile tail and an isometric head. Like all actinophages described to date, the new isolates obtained in this study contained double-stranded DNA [9]. The new type B1 actinophages share the same morphology and general size of previously isolated *Micromonospora* phages φUW21 and φUW51 [17,18] and phages Mm1, Mm4, Mm5, φM2 and φM3 [8].

The host-range of each actinophage was determined on micromonosporae and representative species from nine genera of actinomycetes. The species infected included members of the genera *Amorphosporangium, Ampullariella* and *Catellatospora*. Uninfected strains belonged to the genera *Actinoplanes, Catenuloplanes, Dactylosporangium* and *Glycomyces*. The actinophages were unable to infect *Streptomyces* or *Streptoverticillium* species, both classified with a cell wall of chemotype I. This observation was in agreement with the results of Prauser [24] who demonstrated that actinophages were restricted in their host-range to organisms belonging to the same cell wall chemotype.

Actinophage infection patterns were used to sort the *Micromonospora* species into five groups. The divisions were based on the total number of infections produced by each phage and were as follows: (1) hosts uninfected by any of the actinophages, (2) hosts infected by MZφ11, (3) hosts infected by MZφ11 and MEφV01, (4) hosts infected by MZφ11, MEφV01 and MPφE-RCPM, and (5) hosts uninfected by MZφ11 and MEφ01. Chater and Carter [10] suggested that the host-range observed for *Streptomyces* phage R4 correlated with the absence of detectable type II restriction enzymes rather than with conventional taxonomic divisions. Cox and Baltz [11] supported this conclusion by stating that restriction was very important in determining host-range in *Streptomyces* phages. The host-range data for the micromonosporae may also correlate with host-controlled R-M.

Type II restriction enzymes were not isolated from every strain which revealed a positive EOP result. Table 4 contains some examples of the EOP results obtained. The data in section A demonstrate putative R-M activity in both hosts. That of *M. purpurea* is not related to *Mpu*I since the indicator phage, MPφE-RCPM, has no *Xho*I sites. This implies that an additional R-M system is present which was not identified. The results shown in section B of the table demonstrate restriction by *M. echinospora* but without accompanying modification, since DNA from MZφ11 grown on *M. echinospora* is still cut by *Xho*I (data not shown). The lack of *Xho*I modification activity in this system is confirmed by the demonstration of digestibility by *Xho*I of DNA from the phage MEφV01 grown on *M. echinospora* (Table 3). The data in section C of Table 4 indicate the presence of at least one putative R-M system in *M. zionensis*. *M. brunnea* may have R-M activity but this was not demonstrable with MZφ11. The presence of a type I restriction endonuclease in *M. brunnea, M. coerulea, M. inositola* and *Catellatospora* sp. could have produced positive EOP results and the DNA smears observed on agarose gels. DNA smearing was observed when crude lysate was incubated with DNA in the presence or absence of adenosyltriphospate (ATP) and *S*-adenosylmethionine (SAM). No attempt was made to separate any nonspecific nuclease activity away from possible type I restriction endonuclease activity. Also, a portion or all of the nuclease activity may be due to the presence of a type II restriction enzyme recognizing a four base-pair sequence, which statistically would produce many cuts in the DNA molecule, thus creating a smear.

The *M. echinospora* spp. *echinospora* restriction enzyme, designated *Mec*I, was an isoschizomer of

*Xho*I, with cleavage properties like *Pae*R7 1. The *M. purpurea* restriction enzyme, *Mpu*I, was also an isoschizomer of *Xho*I. The *M. zionensis* enzyme, designated *Mzi*I, was an isoschizomer of *Pvu*II. From an evolutionary point of view, it was interesting to note that, of the four type II restriction endonucleases isolated from *Micromonospora* species, three are isoschizomers of *Xho*I. The first *Xho*I isoschizomer was isolated from *M. carbonacea* and was designated *Mca*I [16].

A number of restriction systems remain to be identified in *Micromonospora* species. The availability of wide host-range actinophages makes available new vehicles for studying the actinomycetes. With most of the effort in this field having been previously concentrated on species of *Streptomyces*, the present study is an attempt at widening such knowledge to the micromonosporae.

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