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Short Communication

MPphiWR-2, a cryptic phage associated with *Micromonospora purpurea* ATCC 15835

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SUMMARY

A previously undescribed cryptic phage was found associated with *Micromonospora purpurea* ATCC 15835.

INTRODUCTION

Micromonospora purpurea ATCC 15835 produces the gentamicin comples of antibiotics and derivatives of this actinomycete have been used for commercial antibiotic production [2]. A temperate phage (MPphiWR-1) was isolated using this microorganism [4], and during this study, a novel cryptic phage was found to be present in ATCC 15835. A partial description of this phage is presented.

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MATERIALS AND METHODS

Actinomycetes used were *M. purpurea* ATCC 15835, *M. purpurea* 6-5000-8 (Schering Corporation) and *M. purpurea* AA3 (the source of MPphiWR-1) [4]. The phages studied included MPphiWR-1, a temperature actinophage [4], MPphiE-RCPM, a lytic mutant of MPphiWR-1 [4], and MPphiWR-2 (this study).

Media, buffers, culture storage, growth conditions and other procedures were as described elsewhere [4].

RESULTS/DISCUSSION

In a CsCl gradient purification of the temperate actinophage MPphiWR-1, the predominant band was less dense than expected [4]. The gradient mate-

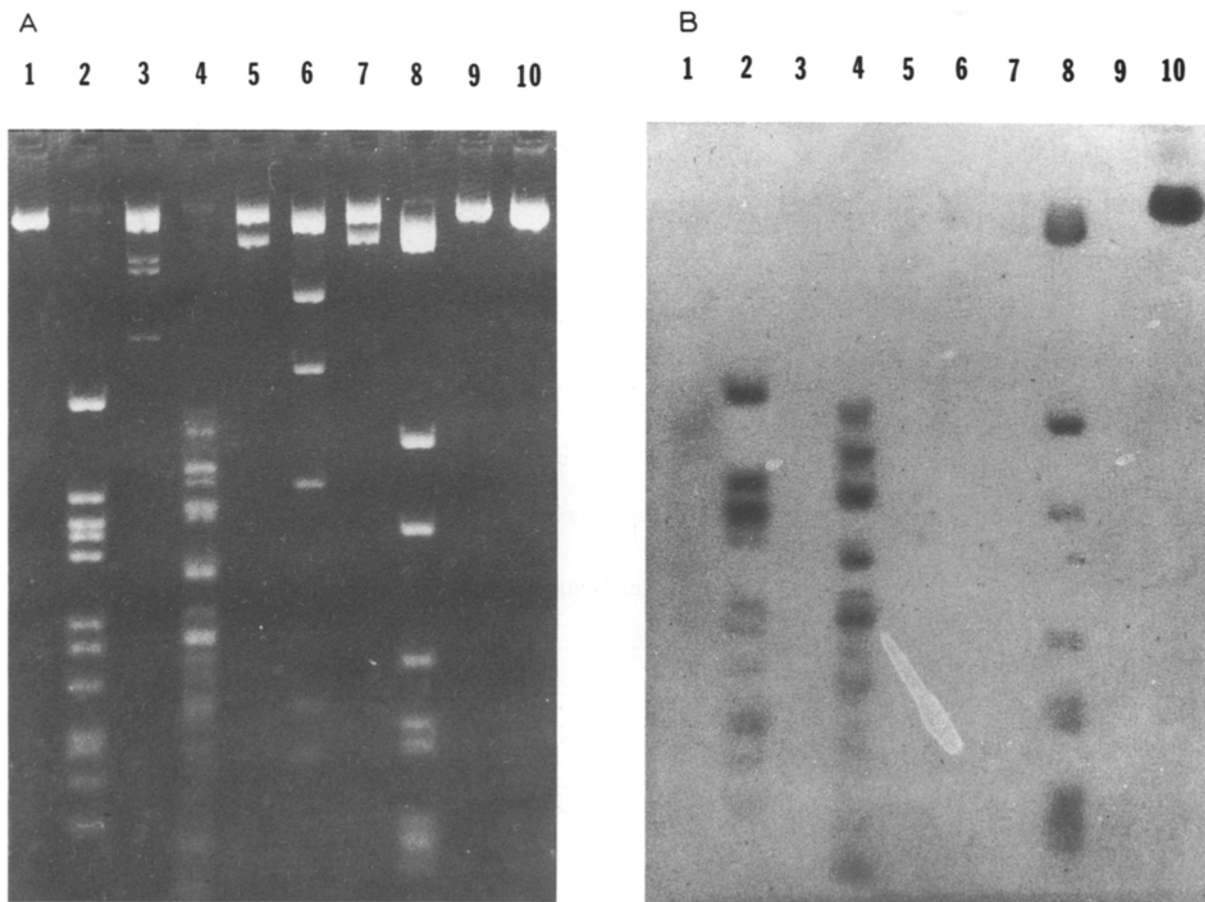

Fig. 1. Gel comparison and Southern blot of restriction enzyme digestions of MPphiWR-2 and MPphiE-RCPM DNA. A. Gel, B. Blot. Lanes are from left to right, origin is at the top. Probe DNA was MPphiWR-2. Lane 1, MPphiE-RCPM DNA treated with *Bam*HI, fragment is 57.9 kb; Lane 2, MPphiWR-2 DNA cut with *Bam*HI; Lane 3, MPphiE-RCPM DNA cut with *Sac*II, fragments are 24.8, 12.9, 12.1 and 8.1 kb; Lane 4, MPphiWR-2 DNA cut with *Sac*II; Lane 5, Lambda DNA cut with *SalI*, fragments are 32.7 and 15.3 kb; Lane 6, Lambda DNA cut with *Hind*III, fragments are 23.1, 9.4, 6.6, 4.4, 2.3 and 2.0 kb; Lane 7, MPphiE-RCPM DNA cut with *Pvu*II, fragments are 41.1 and 16.8 kb; Lane 8, MPphiWR-2 DNA cut with *Pvu*II, Lane 9, uncut MPphiE-RCPM DNA, fragment is 57.9 kb; Lane 10, uncut MPphiWR-2 DNA.

rials had been obtained by eluting lawns of *M. purpurea* ATCC 15835 having confluent turbid plaques of MPphiWR-1 [4]. This unexpected low-density band was collected and DNA isolated. Restriction enzyme digestion patterns of this DNA (Figure 1, Table 1) were different than those from MPphiE-RCPM DNA. It should be noted that MPphiWR-2, like MPphiE-RCPM, was not cut by *Xho*1 (Table 1). This was to be expected, since an isoschizomer of that enzyme, *Mpu*1, has been isolated from *M. purpurea* ATCC 15835 [3].

The characteristic phage CsCl band, differences in DNA restriction patterns and DNA sizes, suggested that a new phage, different from MPphiWR-1, was present in ATCC 15835. The putative new phage was named MPphiWR-2 (M = *Micromonospora*, P = *purpurea*, phi = phage, W = Waksman Institute, R = Rutgers University, 2 =

Table 1

MPphiWR-2 and MPphiE-RCPM restriction fragments

Enzyme	Phage DNA	
	MPphiWR-2	MPphiE-RCPM
*Bam*HI	>11	1
*Eco*RI	3	3
*Hind*III	2	1
*Kpn*I	> 4	7
*Pst*I	> 5	1
*Pvu*II	7	2
*Sal*I	>20	>15
*Sac*II	>15	4
*Xba*I	3	1
*Xho*I	1	1

second new phage). The genome size for MPphiWR-2 was estimated to be 45 kb by totaling the fragment sizes observed in the gels.

As MPphiWR-2 had only been isolated from cells infected with MPphiWR-1, the origin of the phage was unknown. To determine the relationship of MPphiWR-2 to MPphiWR-1 and its host, several strains of *M. purpurea* were tested for the presence of MPphiWR-2. Lawns of our laboratory stock and an independent strain (D. Labeda) of *M. purpurea* ATCC 15835, an improved gentamicin producing mutant, *M. purpurea* 6-5000-8 (Schering Corp.), and *M. purpurea* ATCC 15835 with confluent MPphiWR-1 plaques were eluted and this material centrifuged to equilibrium in CsCl (starting density 1.5 g/cc).

Every gradient had a low-density band present and the MPphiWR-1-infected material had an additional higher-density minor band. All bands were collected and the DNA isolated but only the bands from our laboratory stock culture uninfected and MPphiWR-1-infected, were titered against *M. purpurea* ATCC 15835 and densities measured. The density of the gradient band prepared from our laboratory stock *M. purpurea* ATCC 15835 was 1.49 g/cc and produced no plaques when titered. The major band from the MPphiWR-1-infected culture had a density of 1.44 g/cc with a titer of 6.25×10^5 pfu/ml phenotypically like MPphiWR-1 [4] and the

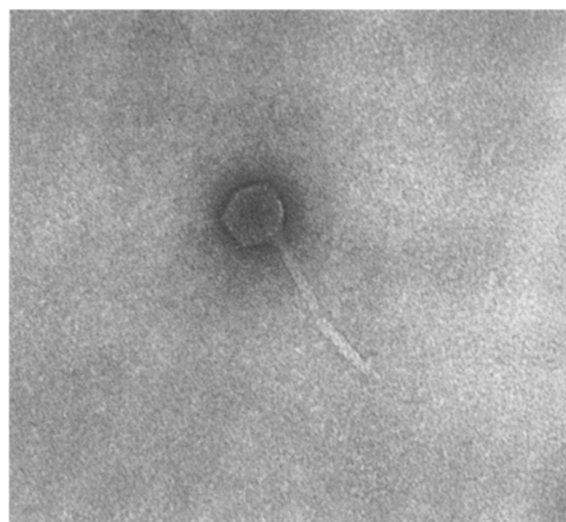

Fig. 2. Electron micrograph of MPphiWR-2 from uninfected *M. purpurea* ATCC 15835 (153 000 × magnification).

density of the minor band was 1.54 g/cc with a titer of 4.3×10^8 pfu/ml of the same phenotype. The 1.54 g/cc density corresponded to that expected for MPphiRCPM (MPphiWR-1) [4].

Restriction analysis of the isolated DNA with *Bam*HI and *Pvu*II indicated that all the low-density band materials were identical but different from MPphiE-RCPM (data not shown, see Fig. 1 for example). The DNA from the high-density band was found to be identical to MPphiE-RCPM by restriction analysis [4].

An additional demonstration of difference between MPphiE-RCPM (MPphiWR-1) and MPphiWR-2 was achieved by probing a Southern blot of the DNA of these two phages with labeled MPphiWR-2. The results (Fig. 1) show no homology between the two phages. This was expected since no homology between the total DNA of *M. purpurea* ATCC 15835, and the DNA of MPphiE-RCPM had been observed [4].

Proof that the low-density opaque band seen in the CsCl gradients was composed of phage particles was obtained by electron microscopy of dialyzed material from the MPphiWR-1-uninfected *M. purpurea* ATCC 15835 preparation. Fig. 2 shows a typical phage particle from the band. MPphiWR-2,

like MPphiWR-1, has a B1 morphotype [1] with a symmetrical head having an approximate diameter of 71 nm and a tail approximately 172 nm long.

The restriction patterns of MPphiWR-2 DNA were nearly identical to those of a plasmid found in a gentamicin production strain of *M. purpurea* superior to 6-5000-8 (E. Barron and S. Goldberg, Schering Corporation, personal communication). Southern blot analysis of genomic DNA from this production strain with MPphiWR-2 (data not shown) confirmed the presence of homologous material. The plasmid may represent a defective form of the phage, a replicative intermediate or a normal lysogenic state.

CONCLUSIONS

The identification of a novel, cryptic, possibly temperate, phage associated with *Micromonospora purpurea* ATCC 15835 may open additional avenues for research into the molecular biology of the host. This phage, MPphiWR-2, may serve as a means to transfer genetic material, either by natural transduction or development into a cloning vector. The persistance of genetic material from this phage in the different improved gentamicin-producing mutants suggests a possible involvement of MPphiWR-2 with production of this antibiotic.

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