

# New thermal inducible *Streptomyces* phages isolated from tropical soils

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## ABSTRACT

Two new *Streptomyces* phages, øBP1 and øBP2, were isolated from tropical soil samples. These phages presented a large host range and developed both lytic and lysogenic responses in different *Streptomyces* species tested. Variations in the incubation temperature showed to be important in the development of the replication cycle. Increasing incubation temperature from 30°C to 42°C induced the lytic response of øBP2 and lysogenic of øBP1 in the host strain *Streptomyces* sp. WL6. øBP1 and øBP2 have icosahedral heads with long tails and were characterized in relation to morphology, G + C content, genome size and adsorption curve.

## INTRODUCTION

*Streptomyces* phages have been isolated mainly from temperate soil samples and investigated for a variety of reasons, including: interest in controlling the problems they cause in fermentation processes, use as taxonomic tools for typing strains in screening programs (Anné *et al.*, 1984; Kurtboke *et al.*, 1992; Long *et al.*, 1993), and studies of fundamental biological processes (Lomovskaya *et al.*, 1980; Long and Amphlett, 1996). Actinophages are also of interest in *Streptomyces* genetics because of their potential use in the construction of cloning vectors (Kuhstoss *et al.*, 1991). Genetic studies of *Streptomyces* phages were initiated with phages R4, øC31 and S10 (Lomovskaya *et al.*, 1980). øC31 is the best characterized actinophage, and several cloning vectors have been derived from it (Suarez and Chater, 1980; Kobler *et al.*, 1991). Ecological studies of tropical phages are important for obtaining information about interactions between hosts and parasites, and the control exerted by phages on several chemical transformations. The aim of the present study was to

screen tropical soil samples in search of new actinophages which could develop lytic and lysogenic cycles on *Streptomyces* species. Interestingly, many of these phages were different from *Streptomyces* phages isolated from temperate environments, mainly in their lytic response, that was thermoinducible.

## MATERIAL AND METHODS

Phages were isolated from tropical soil samples from different regions: Colombian Amazon basin and Minas Gerais and São Paulo States, Brazil. The soil pH was between 4.6 to 6.5 and soil temperature from 18°C to 23°C. *Streptomyces* sp. WL6 and *S. lydicus* were chosen as hosts because they grow easily at high temperatures. The *Streptomyces* strains used in this work are listed in Table I.

*Streptomyces* strains and phages were manipulated as described by Chater and Carter (1979), but using modified R5 medium (R5M = R2YE medium without saccharose and with 3 g/l Tris instead of TES) (Hopwood *et al.*, 1985). In order to detect phages, 2 g of soil samples were diluted in 4 ml of R5M medium, and

enriched by incubating with 50  $\mu$ l ( $10^7$  c.f.u.) of *Streptomyces* sp. WL6 spores for 60 h at 30°C (adapted from Dowding, 1973). Phages were detected from supernatant solutions by their ability to form plaques on a bacterial lawn after overnight incubation, and distinguished by variations in morphology, size and turbidity of the plaques. High titer phage suspensions were obtained according to Anné *et al.*, 1984, and phage DNA was extracted from pellets as described by Chater and Carter (1979). Growth step and adsorption experiments were carried out with phage suspensions added to mycelial fragments after 8-h incubation at 30°C, according to Lomovskaya *et al.* (1980), Haket *et al.* (1990), and Long and Amphlett (1996). Electron microscopy observations of negative stained phage samples were done as described by Suarez and Chater (1980). Temperate phages could be distinguished by the appearance of immune colonies at the center of the plaques that were resistant to superinfection by the same phage. Host range was determined by using 100- $\mu$ l phage suspension ( $10^{10}$  p.f.u. ml<sup>-1</sup>) on lawns of different strains. If plaques appeared, the strain was considered to be sensitive to the specific phage.

In order to study the influence of the temperature in the morphology of the lysis plaques and infection efficiency, spore suspensions ( $10^7$  c.f.u ml<sup>-1</sup>) were mixed with 50  $\mu$ l ( $10^{10}$  p.f.u. ml<sup>-1</sup>) of phage suspensions and incubated at 30°C, 37°C or 42°C for 20 h. The stability of phage particles at high temperatures was tested by incubation for 10 min at 65°C before plating at 30°C. The G + C content of phage DNAs was determined by high-performance liquid chromatography (Ko *et al.*, 1977). Restriction enzyme analysis of phage DNA was carried out with 23 different restriction enzymes, using assay conditions described by the manufacturers.

## RESULTS AND DISCUSSION

Nine different phages, named  $\phi$ BP1 to  $\phi$ BP9, were isolated from tropical soils and characterized according to morphology, genome size and adsorption curve. The incubation time during phage isolation was

Table I - Host range of the phages  $\phi$ BP1 and  $\phi$ BP2.

Streptomyces strains	Phages					
	$\phi$ BP1			$\phi$ BP2		
	p.f.u.*	Plaque type	Size (mm)	p.f.u.	Plaque type	Size (mm)
<i>S. albocyanus</i> 5139 <sup>1</sup>	+	turbid	1	+	clear	2
<i>S. alni</i> M6615 <sup>2</sup>	++	clear	2	+	turbid	1
<i>S. aureofaciens</i> M3031 <sup>2</sup>	+	turbid	2	+	turbid	1
<i>S. bobili</i> 3310 <sup>3</sup>	++	turbid	2	-	-	-
<i>S. capoamus</i> M3123 <sup>2</sup>	+++	clear	3	+++	clear	3
<i>S. clavuligerus</i> 27064 <sup>3</sup>	-	-	-	-	-	-
<i>S. coelicolor</i> A3(2) <sup>4</sup>	++	turbid	2	-	-	-
<i>S. coelicolor</i> 1501 <sup>4</sup>	++	turbid	1	-	-	-
<i>S. felleus</i> M3079 <sup>2</sup>	+++	clear	3	+++	clear	1
<i>S. felleus</i> M3182 <sup>2</sup>	+++	turbid	1	++	turbid	1
<i>S. griseus</i> M3059 <sup>2</sup>	-	-	-	-	-	-
<i>S. lavendulae</i> M3003 <sup>2</sup>	-	-	-	-	-	-
<i>S. lydicus</i> 5461 <sup>1</sup>	+++	turbid	1	+++	turbid	2
<i>S. lividans</i> TK24 <sup>4</sup>	+	clear	3	++	turbid	1
<i>S. lividans</i> TK54 <sup>4</sup>	+	turbid	1	+	turbid	2
<i>S. melanochromogenes</i> 3182 <sup>3</sup>	-	-	-	-	-	-
<i>S. nigellus</i> 5490 <sup>1</sup>	-	-	-	-	-	-
<i>S. novaecarsariae</i> 3074 <sup>3</sup>	+	clear	3	+++	clear	4
<i>S. olindensis</i> M5622 <sup>2</sup>	+++	clear	3	+++	clear	3
<i>S. parvulus</i> M3042 <sup>2</sup>	+++	turbid	3	+++	turbid	4
<i>S. plicatus</i> M3166 <sup>2</sup>	-	-	-	-	-	-
<i>S. recifenci</i> M3064 <sup>2</sup>	+	clear	2	-	-	-
<i>S. rimosus</i> M3198 <sup>2</sup>	+	clear	1	+	turbid	1
<i>Streptomyces</i> sp. WL6 <sup>5</sup>	+++	clear	1	+++	turbid	2

\*p.f.u./ml: + less than 30; ++ between 30 to 70; +++ more than 70; - none; growth temperature 30°C.

(1) ISP - International *Streptomyces* Project.

(2) DAUFPE - Department of Antibiotics, University of Pernambuco, Brazil.

(3) ATCC - American Type Culture Collection.

(4) JI - John Innes Institute, Norwich, England.

(5) CDBB - Biotechnology Department, University of Mexico.

relatively short (in average 20 h) to avoid problems such as readsorption, death and the presence of older phages. Previous reports have demonstrated the isolation of actinophages after 1 week of incubation such as phage SA<sub>t</sub>1, phages of *Micromonospora purpurea* and *Brevibacterium lactofermentum* (Klaus *et al.*, 1981). Due to their ability to undergo lytic and lysogenic growth in *Streptomyces* sp. WL6, phages  $\phi$ BP1 and  $\phi$ BP2 were selected for more detailed characterization.  $\phi$ BP1 infected 18 out of 24 *Streptomyces* strains tested, developing a lysogenic response in 9 strains (Table I). Lysogeny was demonstrated by immunity of putative lysogens to super infection and by release of phage particles from the lysogenic host strain. The phages could be adsorbed but yielded no progeny.  $\phi$ BP2 infected 14 *Streptomyces*

strains and evoked the lysogenic response in 9 strains. Both phages did not infect *S. clavuligerus*, *S. griseus*, *S. lavendulae*, *S. melanochromogenes*, *S. nigellus* and *S. plicatus*, probably because these species have strong restriction systems (Baltz and Cox, 1984 and Diaz *et al.*, 1989). The host range of both phages is wider than related actinophages, such as  $\phi$ C31, R4 and  $\phi$ A7 (Lomovskaya *et al.*, 1980; Baltz and Cox, 1984; Diaz *et al.*, 1991). This feature could be useful for taxonomic applications and also for the elimination of *Streptomyces* strains most frequently found in soil samples, in the screening of novel organisms (Kurtboke *et al.*, 1992; Long *et al.*, 1993). The optimum temperature for plaque formation was 30°C in *S. felleus*, *S. parvulus*, *S. olidensis*, *S. capoamus*, *S. lydicus* and *Streptomyces* sp. WL6. Plaque formation required the presence of 10 mM Ca<sup>2+</sup>. Electron microscopy of purified  $\phi$ BP1 and  $\phi$ BP2 virions revealed that both phages have icosahedral heads of 70 nm and 60 nm in diameter, and tails of 300 nm and 290 nm in length, respectively (Figure 1). The phages belong to Type B according to Bradley's classification system (Bradley, 1967).

Growth step and adsorption experiments showed that  $\phi$ BP1 replicated more efficiently in both host strains than  $\phi$ BP2, inducing total bacterial lysis and liberating more particles to the culture medium in a shorter period of time. The most efficient adsorption rates were obtained for mycelium germinated from spores after 8-h incubation. The latent period (biosynthesis and maturation) was 60 min for  $\phi$ BP1 in both hosts;  $\phi$ BP2 took 60 min in *S. lydicus* and 120 min in *Streptomyces* sp. WL6 (Figure 2), whereas the reference phage  $\phi$ C31 took only 40 min in *S. coelicolor* (Lomovskaya *et al.*, 1980). The long rise period of both phages indicated that phage release from single infected cells was asynchronous.

In order to study the influence of the temperature on the replication of phages  $\phi$ BP1 and  $\phi$ BP2, the host strains were incubated at 30°C, 37°C and 42°C (Table II). When the incubation temperature increased from 30°C to 37°C, phage  $\phi$ BP1 developed lysogenic and lytic responses, respectively. In *Streptomyces* sp. WL6, the plaque and burst sizes were greater at 37°C. At 30°C,  $\phi$ BP2 replicated by lysogenic growth in both hosts (Table II); temperature shift to 37°C and 42°C induced lytic response and also

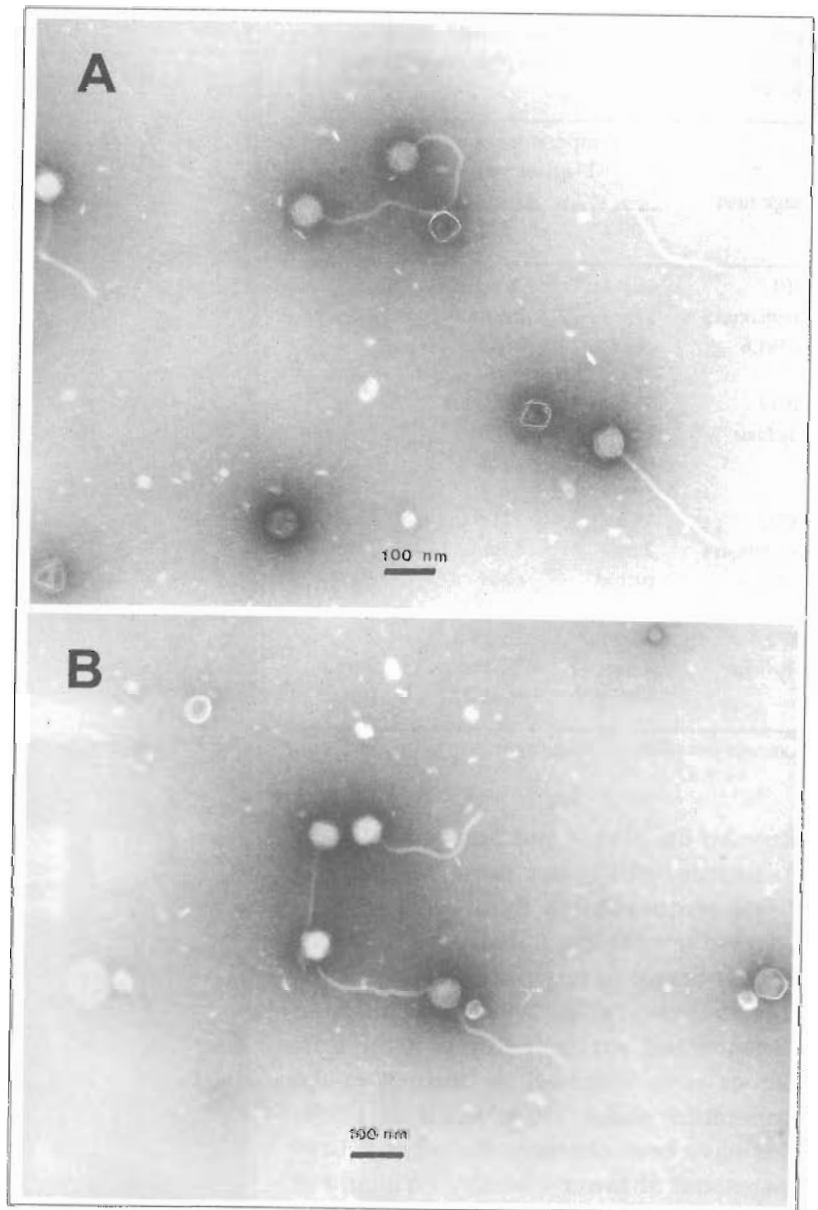


Figure 1 - Electron micrographs of phages  $\phi$ BP1 (A) and  $\phi$ BP2 (B) negatively stained with 2% uranyl acetate. Magnification: 45000X. Dimensions are indicated in nanometers.

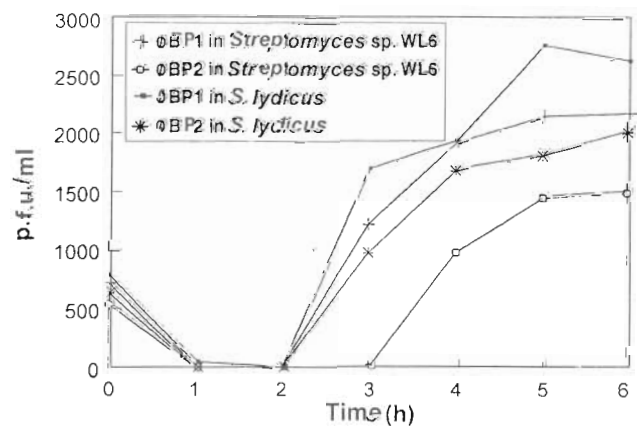


Figure 2 - One-step growth curve of phages  $\phi$ BP1 and  $\phi$ BP2 in the hosts *Streptomyces* sp. WL6 and *S. lydicus*. Growing mycelial cultures (8 h) were inoculated with phage suspensions and incubated at 30°C for 16 h.

Table II - Influence of the temperature on the development of the life cycles of phages  $\phi$ BP1 and  $\phi$ BP2 after 20 h of incubation.

Phage host	Temperature of incubation		
	30°C	37°C	42°C
$\phi$ BP1/ <i>Streptomyces</i> sp. WL6	46 p.f.u.*	36 p.f.u.	-
	1 mm	2-3 mm	-
	clear	turbid	-
$\phi$ BP1/ <i>S. lydicus</i>	81 p.f.u.	206 p.f.u.	-
	1 mm	2 mm	-
	turbid	clear	-
$\phi$ BP2/ <i>Streptomyces</i> sp. WL6	45 p.f.u.	124 p.f.u.	23 p.f.u.
	2 mm	4 mm	7 mm
	turbid	clear	clear
$\phi$ BP2/ <i>S. lydicus</i>	25 p.f.u.	28 p.f.u.	27 p.f.u.
	2 mm	2-3 mm	2 mm
	turbid	turbid	turbid

\*Average per plate; - = no growth.

increased the plaque and burst sizes (Table II). Because  $\phi$ BP2 was more resistant to higher temperatures than  $\phi$ BP1, it was subjected to heat-shock treatment (65°C for 15 min) before normal incubation at 30°C with *Streptomyces* sp. WL6. Low infection efficiency and irregular forms of the lytic plaques were observed as product of the temperature shifts. These kinds of alterations have been observed for other actinophages, but at lower intensity (Williams *et al.*, 1987). Tolerance to high temperatures (max. 45°C) has been described only for phages of thermophilic microorganisms such as *Bacillus*, *Micromonospora* and *Thermoactinomyces* (Haket *et al.*, 1990).

The G + C content of the phages (69.6% for  $\phi$ BP1 and 67.2% for  $\phi$ BP2) was in agreement with the G + C range of *Streptomyces* spp., which is about 60 to 76 mol%. The genome size of  $\phi$ BP1 (43.5 kb) and  $\phi$ BP2 (35.5 kb) was determined by restriction analysis. The most informative restriction profiles were obtained using *Cla*I (7 fragments) and *Eco*RI (4 fragments) for  $\phi$ BP1, and *Cla*I (10 fragments), *Eco*RV (2 fragments) and by *Bam*HI (3 fragments) for  $\phi$ BP2 (Figure 3A). Restriction profiles of phage genomes digested with restriction enzymes that cut preferentially G/G-rich sites are shown in Figure 3B. The remaining enzymes had either multiple sites or did not cut the phage

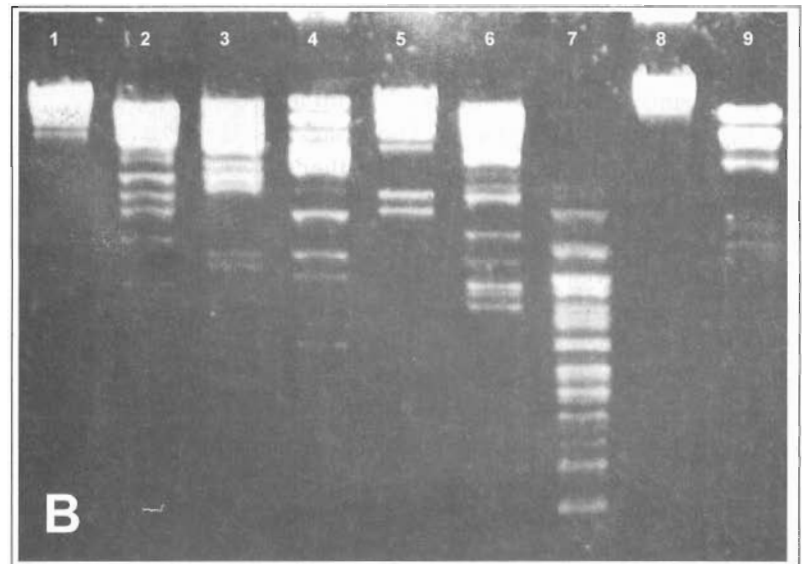
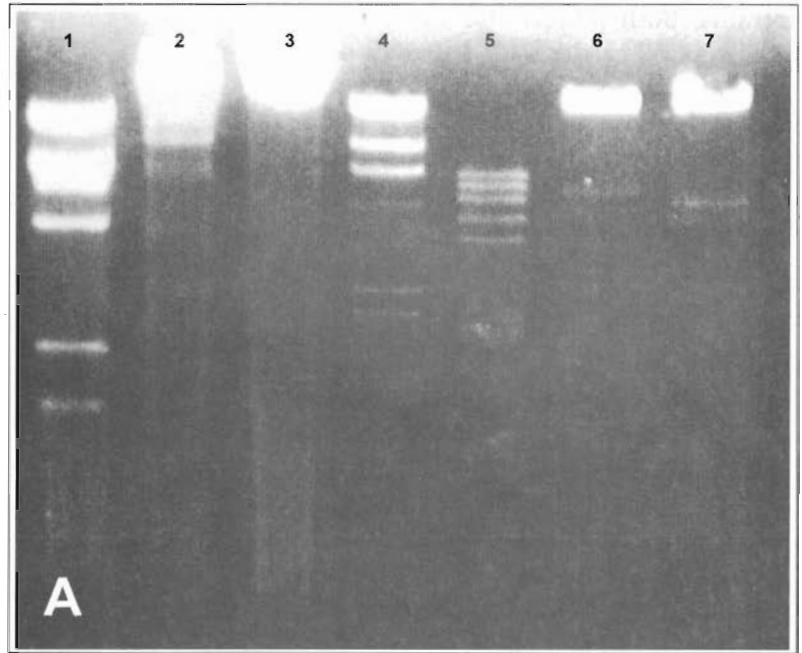


Figure 3 - Restriction analysis of the phages  $\phi$ BP1 and  $\phi$ BP2. (A): 1,  $\phi$ BP1/*Cla*I; 2,  $\phi$ BP1/*Eco*RI; 3,  $\phi$ BP1/*Bam*HI; 4,  $\lambda$ HindIII (23.1, 9.4, 6.5, 4.3, 2.3, 2.0, 0.5 kb); 5,  $\phi$ BP2/*Cla*I; 6,  $\phi$ BP2/*Eco*RV; 7,  $\phi$ BP2/*Bam*HI. (B): 1,  $\phi$ BP1/*Hae*III; 2,  $\phi$ BP1/*Ava*I; 3,  $\phi$ BP1/*Apa*I; 4,  $\phi$ BP1/*Xho*I; 5,  $\lambda$ HindIII (23.1, 9.4, 6.5, 4.3, 2.3, 2.0, 0.5 kb); 6,  $\phi$ BP2/*Hae*III; 7,  $\phi$ BP2/*Ava*I; 8,  $\phi$ BP2/*Apa*I; 9,  $\phi$ BP2/*Xho*I.

genomes (Table III). The comparison of  $\phi$ BP1 and  $\phi$ BP2 restriction profiles with those of other actinophages did not show any obvious similarities between them (Lomovskaya *et al.*, 1980; Anné *et al.*, 1984; Diaz *et al.*, 1989). These results lead to the conclusion that  $\phi$ BP1 and  $\phi$ BP2 are two novel *Streptomyces* phage isolated from tropical soils. They present interesting characteristics, particularly a broad host range and replication cycle inducible by high temperatures, that could be used as molecular tools.

**Table III** - Physical properties of DNA of phages  $\phi$ BP1 and  $\phi$ BP2.

Phage	G + C content (mol%)	DNA size (Kb)	Restriction endonucleases cleavage sites	Enzymes with no cleavage sites
$\phi$ BP1	69.6	43.5	<i>Apa</i> I (> 15); <i>Ava</i> I (> 11); <i>Bal</i> I (> 9); <i>Bgl</i> II (> 13) <i>Cla</i> I (7); <i>Eco</i> RI (4); <i>Hae</i> III (> 6); <i>Kpn</i> I (> 13); <i>Mlu</i> I (> 11); <i>Nco</i> I (> 11); <i>Sca</i> I (> 5); <i>Xho</i> I (> 12)	<i>Acc</i> I; <i>Alu</i> I; <i>Bcl</i> I; <i>Bam</i> HI; <i>Bgl</i> I; <i>Dra</i> I; <i>Eco</i> RV; <i>Hinc</i> II; <i>Hpa</i> I; <i>Pst</i> I; <i>Pvu</i> I
$\phi$ BP2	67.2	35.5	<i>Ava</i> I (> 20); <i>Apa</i> I (> 4); <i>Bal</i> I (> 8); <i>Bam</i> HI (3); <i>Bgl</i> II (> 15); <i>Cla</i> I (10); <i>Eco</i> RV (2); <i>Hae</i> III (> 14) <i>Mlu</i> I (> 15); <i>Nco</i> I (> 11); <i>Sca</i> I (> 12); <i>Xho</i> I (> 8)	<i>Acc</i> I; <i>Alu</i> I; <i>Bcl</i> I; <i>Bgl</i> III; <i>Dra</i> I; <i>Eco</i> RI; <i>Hinc</i> II; <i>Hpa</i> I; <i>Kpn</i> I; <i>Pst</i> I; <i>Pvu</i> I

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## RESUMO

Dois novos fagos de *Streptomyces*, denominados  $\phi$ BP1 e  $\phi$ BP2, foram isolados de amostras de solos tropicais. Estes fagos apresentaram uma grande faixa de hospedeiros e foram capazes de desenvolver os ciclos lítico e lisogênico em diferentes espécies de *Streptomyces* testadas. A temperatura de incubação mostrou-se importante para o desenvolvimento do ciclo de replicação. O aumento desta temperatura de 30°C para 37°C ou 42°C induziu o ciclo lítico de  $\phi$ BP2 e lisogênico de  $\phi$ BP1 na linhagem hospedeira *Streptomyces* sp. WL6. Ambos os fagos apresentaram cabeça icosaédrica com talos longos e foram caracterizados em relação à morfologia, tamanho do genoma, conteúdo de G + C e curva de adsorção.

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