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Characterization of *Streptomyces* plasmid-phage pFP4 and its evolutionary implications

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ABSTRACT

Autonomous-replicating plasmid pFP4 of *Streptomyces* sp. FR1 isolated from a heavy metal-contaminated land was cloned and sequenced. Surprisingly, the 40,949-bp pFP4 contains a cluster of 20 genes, resembling these chromosome-integrated prophages of *Streptomyces* sp. SPB78 and *Streptomyces scabiei* 87.22. Plasmid pFP4 could transfer by conjugation and a replication locus, iteron/*repA*/*repB*, was identified. The filtered FR1 culture could infect both FR1 and FR1 cured of pFP4 to form plaques, and also six out of 13 strains from the same land, but failed to form plaques on other seven strains from same source and all ten *Streptomyces* species from different sources. pFP4 phage particles were observed by transmission electron microscopy. Major structural proteins (capsid, portal and tail, etc.) of pFP4 virions were encoded by twelve pFP4 genes. pFP4 phage DNA contained 3′ protruding cohesive ends of 9-nt. *Streptomyces* pFP4 represents a novel plasmid-phage.

1. Introduction

Streptomyces are Gram-positive, mycelial bacteria with high G + C content in their DNA, and are a major source of antibiotics and pharmacologically active metabolites (Bérdy, 2005). They usually harbor circular and/or linear plasmids in autonomous and/or chromosomally-integrated forms (Hopwood and Kieser, 1993). Some *Streptomyces* circular plasmids use rolling-circle-replication (RCR) (e.g., pIJ101, pJV1, pSG5 and pSN22, etc.: Hopwood and Kieser, 1993; Grohmann et al., 2003) and others use theta-replication (e.g., SCP2, pFP11 and pZL12, etc.: Haug et al., 2003; Zhang et al., 2008; Zhong et al., 2010). *Streptomyces* plasmids usually need a major *tra* gene, which encodes a DNA translocase containing a cell-division FtsK/SpoIIIE domain, for conjugal transfer (Pettis and Cohen, 1994; Hopwood, 2006).

Streptomyces temperate phages, including ΦC31 (Lomovskaya et al., 1972), SAt1 (Ogata et al., 1985), TG1 (Foor et al., 1985), FP43 (McHenney and Baltz, 1988), ΦSPK1 (Kuhn et al., 1987), ΦSC623 (Schneider et al., 1990), DAH2/DAH4/DAH5/DAH6 (Burke et al., 2001), mu1/6 (Farkasovská et al., 2007), ΦHAU3 (Zhou et al., 1994), VWB (Van Dessel et al., 2005), ΦBT1 (Gregory et al., 2003) and ΦSASD1 (Wang et al., 2010), etc. can infect and form plaques among *Streptomyces* species and many of these phage also integrate into host chromosomes as prophages. They range in size from 36 kb (Kuhn et al., 1987) to 121 kb (Burke et al., 2001), with 50–71.2% G + C content (Smith et al., 1999; Farkasovská et al., 2007; Van Dessel et al., 2005). ΦC31 is the most studied *Streptomyces* phage (Chater, 1986; Khaleel et al., 2011), and its derived vectors have widely been used in *Streptomyces* species (Chater and Bruton, 1983) and as valuable site-specific-integrating vectors in mammals (Thorpe and Smith, 1998; Calos, 2006).

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We report here cloning and sequencing of a 40,949-bp plasmid pFP4 of *Streptomyces* sp. FR1 from a heavy metal-contaminated land. Surprisingly, a cluster of genes on pFP4 resemble the chromosome-integrated prophages of *Streptomyces* species. We demonstrate that the phage segment on pFP4 is functional.

2. Materials and methods

2.1. Bacterial strains, plasmids, and general methods

Thirteen *Streptomyces* strains, including FR1 (Table 1), were isolated from heavy metal-contaminated land (9.1 mmol arsenic, 5.1 mmol copper, 1.3 mmol lead and 0.8 mmol zinc per kg soil) at a mine in Chenzhou suburb, Hunan province, China, and identified as *Streptomyces* species by PCR sequencing of the 16S rDNA (Zhang et al., 2006). Strains and plasmids used in this work are listed in Table 1. Plasmid isolation, transformation of *Escherichia coli* and Southern hybridization followed Sambrook et al. (1989). *E. coli* DH5α was used as a cloning host. *Streptomyces lividans* ZX7 (Zhou et al., 1988) was the host for propagating plasmids and pQC156 (Qin et al., 2003) was used as cloning vector. *Streptomyces* culture, plasmid isolation, preparation of protoplasts, transformation, conjugation, plaque formation, phage isolation and pulsed-field gel electrophoresis followed standard protocols of Kieser et al. (2000).

2.2. Cloning, sequencing and analysis of pFP4

pFP4 DNA was partially digested with BamHI and ligated to a Supercos1-derived cosmid pHAQ31 (Xia et al.,

Table 1
Strains and plasmids used in this study.

Strain and plasmid	Genotype or description	Source or reference
Strains		
Streptomyces		
S. lividans ZX7	*pro-2 str-6 rec-46 dnd* SLP2$^-$ SLP3$^-$	Zhou et al. (1988)
S. venezuelae ISP5230	A jadomycin B producer	Yang et al. (2001)
S. glaucescens GLA 4-26	A tetracenomycin C producer	Motamedi et al. (1986)
S. rochei 7434-AN4	A lankacidin producer	Hayakawa et al. (1979)
Streptomyces spp. 13 strains (FR1, F2, F3, F4, F6, F7, F8, F9, F11, F12, F13, P5, P7)	Isolated from a heavy metal-contaminated land in Chenzhou	Zhang et al. (2006)
Endophytic *Streptomyces* spp. 6 strains (9R-2, G53, Th7, Th8, Th91, Th92)	Isolated from Chinese medicinal herbs in the Shanghai Botanical Garden	Zhong et al. (2010)
Escherichia coli		
DH5α	*F- deoR recA1 endA1 hsdR17(rk- mk+) phoA supE44 λ- thi-1 gyrA96 relA1*	Life Technologies, Inc
DH10B	*F- mcrAΦ80dlacZΔM15 ΔlacX74 endA1 recA1 deoR Δ(ara,leu)7697 araD139 galU galK nupG rpsL λ-*	Life Technologies, Inc
Plasmids		
pIJ702	*melC tsr* pIJ101 origin	Katz et al. (1983)
pQC156	A 2.6-kb *Bcl*I-fragment of *melC/tsr* cloned in pSP72 (*Bgl*II)	Qin et al. (2003)
pQC578	A 6-kb *Mlu*I-fragment of pSLA2 containing the *rlrA/rorA/reps* cloned in pQC156	pQC578
pSET152	*Streptomyces* phage φC31-derived integration vector, *apr*^{r}	Bierman et al. (1992)
pHAQ31	*amp colEI-ori cos melC tsr*	Xia et al. (2009)
pCZH126	A 40-kb *Bam*HI-partially-digested fragment of pPF4 cloned in pHAQ31	This work
pCZH341	A 1.4-kb (30,743–32,145 bp) fragment (PCR) of pFP4 cloned in pQC156 (*Eco*RI and *Xba*I)	This work
pCZH344	A 3.8-kb (28,339–32,145 bp) fragment (PCR) of pFP4 cloned in pQC156 (*Eco*RI and *Xba*I)	This work
pCZH375	2.4-kb (28,339–30,760 bp) and 0.3-kb (31,830–32,145 bp) fragments (PCR) of pFP4 cloned in pQC156 (*Eco*RI and *Xba*I)	This work
pCZH377	A 3.5-kb (28,339–31,860 bp) fragment (PCR) of pFP4 cloned in pQC156 (*Eco*RI)	This work
pCZH379	A 3.4-kb (283,416–31,860 bp) fragment (PCR) of pFP4 cloned in pQC156 (*Eco*RI)	This work
pCZH421	A 3.1-kb (28,800–31,860 bp) fragment (PCR) of pFP4 cloned in pQC156 (*Eco*RI)	This work
pCZH422	A 6.1-kb (34,786–40,916 bp) fragment (PCR) of pFP4 cloned in pQC578 (*Hin*dIII)	This work