

Table 1 Bacterial strains used in this study

Strains	Description	Source or reference
<i>Escherichia coli</i>		
DH5 α	General cloning host strain	Promega
DY330	Strain used for PCR-targeted mutagenesis	Yu <i>et al.</i> (2000)
S17.1	Strain used for conjugation from <i>E. coli</i> to <i>S. lividans</i>	Simon <i>et al.</i> (1983)
<i>Streptomyces coelicolor</i>		
M145	SCP1 ⁻ , SCP2 ⁻	Hopwood <i>et al.</i> (1983)
<i>Streptomyces lividans</i>		
TK24	SLP2 ⁻ , SLP3 ⁻ , <i>strR</i>	Hopwood <i>et al.</i> (1983)
Δppk (<i>SLI_4384</i>)	TK24 with <i>ppk::Ωhyg</i>	Chouayekh <i>et al.</i> (2002)
$\Delta phoP$ (<i>SLI_4466</i>)	TK24 with <i>phoP::Ωaac</i>	Ghorbel <i>et al.</i> (2006a)
ΔSLI_4382	TK24 with <i>SLI_4382::att3-aac</i>	This work
$\Delta pptA$ (<i>SLI_4383</i>)	TK24 with <i>SLI_4383::att3-aac</i>	This work
$\Delta pptA^{IFD}$	TK24 with <i>SLI_4383::att3</i>	This work
$\Delta pptA^{IFD}$ -C(-)	$\Delta pptA^{IFD}$ with pSET152	This work
$\Delta pptA^{IFD}$ -C(+)	$\Delta pptA^{IFD}$ with pMES18, complementation strain of $\Delta pptA^{IFD}$	This work

IFD stands for In Frame Deletion

Table 2 Plasmids used in this study

Plasmids/Cosmids	Description	Source or reference
Construction of mutant strains		
StD84	Cosmid from the <i>S. coelicolor</i> genome library containing <i>SLI_4383</i> and <i>SLI_4382</i>	Bentley <i>et al.</i> (2002)
pGEM-T Easy	<i>E. coli</i> vector for cloning of PCR DNA fragment, Amp ^R	Promega
pOSV236	<i>E.coli-Streptomyces</i> shuttle conjugative vector, Amp ^R , Tsr ^R , Pur ^R , containing <i>ptrc-xis-int</i> , used for apramycin cassette excision	Nguyen <i>et al.</i> (2010)
pOSV234	<i>E. coli-Streptomyces</i> shuttle vector, source of the <i>aac</i> cassette, Amp ^R , Apr ^R	Karray (2005)
pSET152	<i>E.coli-Streptomyces</i> shuttle conjugative vector, replicative in <i>E. coli</i> , Apr ^R , integrative in <i>Streptomyces</i> , used for genetic complementation in <i>Streptomyces</i>	Bierman <i>et al.</i> (1992)
pMES <i>pptA::aac</i>	<i>pptA::att3aac</i> in StD84, Amp ^R , Apr ^R	This work
pMES <i>SLI_4382::aac</i>	<i>SLI_4382::att3aac</i> in StD84, Amp ^R , Apr ^R	This work
pMES17	1.5-kpb fragment containing <i>pptA</i> and its promoter region in pGEM-T Easy, Amp ^R	This work
pMES18	1.5-kpb fragment containing <i>pptA</i> and its promoter region in pSET152, Apr ^R	This work

Amp^R; ampicillin resistant, Apr^R; apramycin resistant, Pur^R; puromycin resistant, Tsr^R; thiostrepton resistant

Table 3 Oligonucleotides used in this study

Purpose	Primer Pairs	5' → 3' sequence
Gene disruption		
<i>pptA</i> (<i>SLI_4383</i>)	NS1	CGCCCCGCACGCCGGCACCGAAGCGGGCACCGA CGCCCTGATCGCGCGCGCTTCGTTGCGGACGAA
	NS2	ACGCCTCCCCTCGTCTCTGCGGCCGTTCTGCGA CCGTTCCATCTGCCTCTTCGTCCCGAAGCAACT
	NS3	TCGCAGGAACGCCGCGCAGGACGACGGAGGCGT ACGCACCATCGCGCGCGCTTCGTTGCGGACGAA
		NS4
<hr/>		
Amplification of <i>pptA</i> (<i>SLI_4383</i>)	ED73 EC74	CTCGAGAACGGCATGTCCGA TGCGGCCGTTCTACGCCAG
<hr/>		
Southern Blot verification of the mutants	NS-F NS-R	TCTTGACGTCGACGGTCTTGATGC ACCGGACGTCCGGCGACTCCCCCA
<hr/>		
Primers for 5'-RACE PCR		
<i>SLI_4382</i>	<i>SLI_4382</i> -GSP1	TAGCCCGTCTCCTCCGCGAC
	<i>SLI_4382</i> -GSP2	CCTTCGGGTGCGACCAAGTCG
	<i>SLI_4382</i> -GSP3	GGACGCCACAGTACGCAGCC
<i>pptA</i> (<i>SLI_4383</i>)	<i>pptA</i> -GSP1	GCTCCAGACGGGACGCGTAC
	<i>pptA</i> -GSP2	GCGGCTGGAAGGTGTGCAGG
	<i>pptA</i> -GSP3	GTTCCCGGAGGGCACCGTTC
<hr/>		
Primers for qRT-PCR		
<i>Reference genes</i>		
<i>SLI_6088</i> (<i>hrdB</i>)	F	AAGGAAGACGGCGAGCTTCT
	R	GCACCGGGATACGGATGGTG
<i>SLI_2451</i> (<i>glk</i>)	F	CAGCGCTCCACGGTCTACTT
	R	GTGATGCAGATGACGTTGC
<i>SLI_4040</i> (<i>aspS</i>)	F	CTGCTGATGATCTCGGGCTT
	R	CGAGCTGGTAGAACTCGCC
<i>SLI_4129</i> (<i>gyrA</i>)	F	GGCGACTCCTCCATCTACGA
	R	GACCATCTCCATCGACAGC
<i>SLI_4131</i> (<i>gyrB</i>)	F	TCGAGACCACCGACTACTCCTT
	R	TCTTGACCTCGTGCTTCTCG
<i>SLI_4926</i> (<i>rpoB</i>)	F	CTTCGAGCCTCCCAAGAACA
	R	TTGGTCATGAGCGGGAAGT
<i>Target genes</i>		
<i>Ppk</i> (<i>SLI_4384</i>)	F (A)	AGTCCGCCAACATCAAGTGG
	R (B)	GCTTGCAGTGGGTCTTCAGG
<i>ppk-pptA</i> junction	F (C)	CTGCGCAACATCCAGGAGAT
	R (D)	GGGGTTCCGGTGGAAAGT
<i>pptA</i> (<i>SLI_4383</i>)	F (E)	GTACGCGTCCGCTCGGAG
	R (F)	GCCCACCGTGAGGTTGC
<i>pptA-SLI_4382</i> junction	F (G)	CAGCGCATGAGGTGGAG
	R (H)	TCGTCTGCGGGGCTCA
<i>SLI_4382</i>	F (I)	CCCGAAGTACGACGACTGGT
	R (J)	GAGGTAGCGCACGGTGGTC
<hr/>		
Primers for RT-PCR		
<i>pptA</i> (<i>SLI_4383</i>)	F (K)	TCTCCGGTTCGACGGCGTTC
	R (L)	TGGCCAGTAAGCGACCTTG