

# Additional file 1

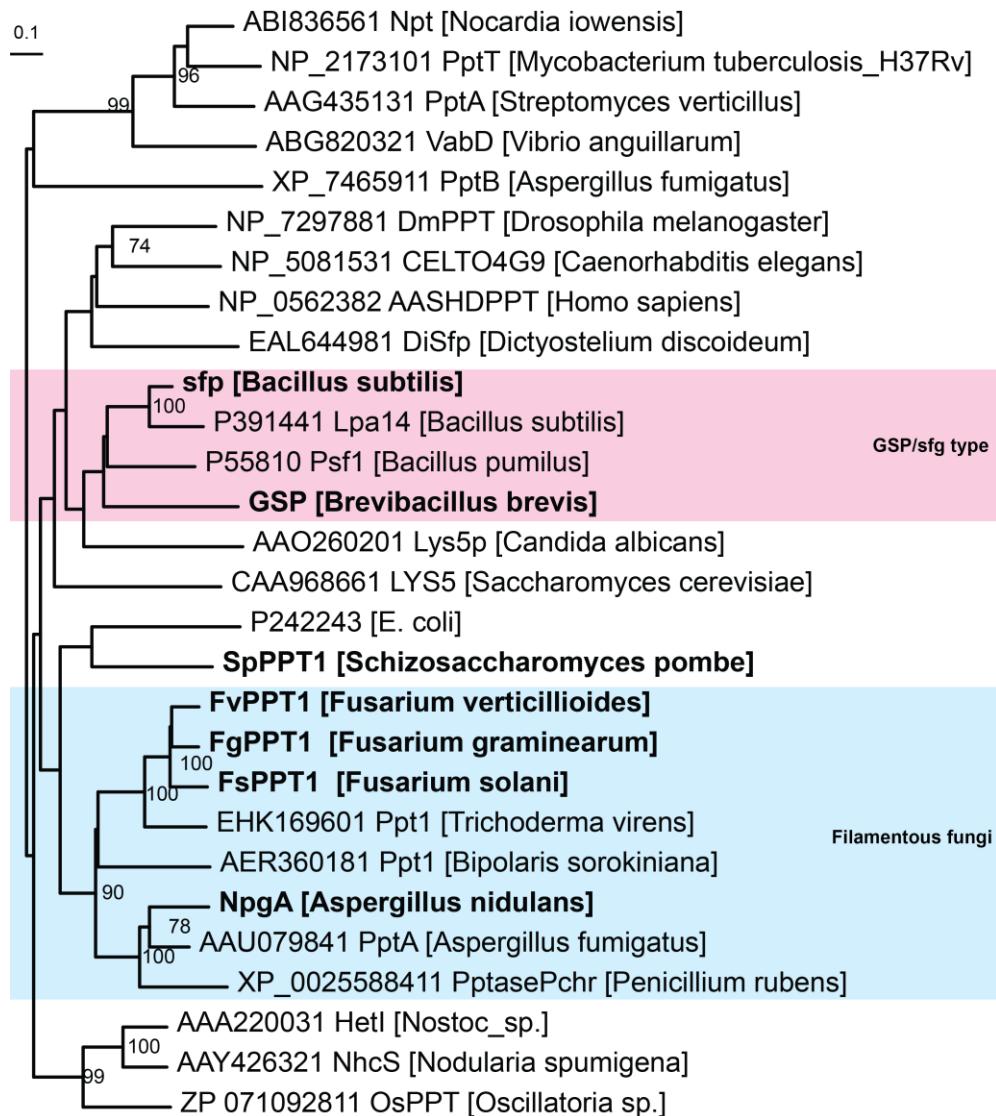
**Table S1.** This table contains the primer sequences of both the primers used for gene-amplification and the primer used for initial sanger-sequencing in fragments of around 700 bp, containing at least 50 bp overlap between each fragment.

Gene	Fragments	Product size (bp)	Name	TAR-region and annealing sequence for PCR
fsr1	2	3274	Fsr1.1-fw	<b>AAA ATT CGA ATT CAA CCC TCA CTA AAG GGC</b> ATG ACA GAC AAC TTA AAA TTA TAC TTA TTC G
			Fsr1.1-rv	CCT TCA AAG CTG CAC ACA AA
		3291	Fsr1.2-fw	TTC CAT ACG CAT TCC ATT CA
			Fsr1.2-rv	<b>ACA ACC TTG ATT GGA GAC TTG ACC AAA CCT</b> TCA AAC TCT TGG ACC CCA CA
fsr2	2	563	Fsr2.1-fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG CAC AAG ACT GAA AGA GAC G
			Fsr2.1-rv	<b>GGT GGC GGT AGA ACC GCT GCT TCC ACC AAC</b> ATC AAC GAC CTT GGC CTC T
		618	Fsr2.2-fw	<b>AAG GCT CTG GGA GAG GCC AAG GTC GTT GAT G</b> TTG GTG GAA GCA GCG GT
			Fsr2.2-rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> CTA AGC ATG CCC ATT CAG ACC
fsr3	1	1599	Fsr3-fw	<b>AAA ATT CGA ATT CAA CCC TCA CTA AAG GGC</b> ATG CAA ATC AAC GAC CAA AC
			Fsr3-rv	<b>GCC GAC AAC CTT GAT TGG AGA CTT GAC CAA</b> CTA TGC CCA GTC ACC GTC TT
bik1	1	6111	Bik1.Fw	<b>AAA ATT CGA ATT CAA CCC TCA CTA AAG GGC GGC C</b> ATG GCC TCC TCC GCA GAT GT
			Bik1.Rv	<b>TCT GGC GAA GAA TTG TTA ATT AAG AGC TCA</b> TCA GTT GAC ACC CAT TGC TT
bik2	1	1470	Bik2.Fw	<b>AAA ATT CGA ATT CAA CCC TCA CTA AAG GGC GGC C</b> ATG GCT GAA CCA AAC CAA CA
			Bik2.Rv	<b>TCT GGC GAA GAA TTG TTA ATT AAG AGC TCA</b> TTA AGA ACC AAC TTC AAC AAC ACC
bik3	1	1362	Bik3.Fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG GTT TCT AAC GGT ATC TCA
			Bik3.Rv	<b>GCG GAT CTT AGC TAG CGG CGG TAC CAA GCT</b> TTA ACC TAA AAC AAC ATC AAT AAC TGA C
npgA	1	1035	npgA.Fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG GTT CAA GAT ACT TCT TCA GCT T
			npgA.Rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> TTA AGA TAA ACA ATT ACA AAC ACC TGT AGC
gsp	1	729	gsp.Fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG GGT GGT CAA AAG ATG AT
			gsp.Rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> TTA AAA ATT ATT ATT TTC TGA AAA AGT AGA
sfp	1	672	sfp.Fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG AAA ATC TAC GGT ATC TAC ATG GA
			sfp.Rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> TAA CAA TTC TTC GTA CGA AAC CAT AG
FgPPT	1	930	FgPPT.Fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG TCT CAA ACT CAA TCT TCA CCA
			FgPPT.Rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> TTA TGA AGA TGG CAA TCT TTC ACC
FsPPT	1	948	FsPPT.Fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG GGT GAA TCT ACT CCA ACA G
			FsPPT.Rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> TTA TAA AGC ATC TGT TGC ATC TTC
q10474	1	780	q10474.Fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG AAG CAA AAG GTT TAC AGA TTG T
			q10474.Rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> TTA CAA ATC ATT CAA TGT TTC CCA
FvPPT	1	879	FvPPT-fw	<b>ATA CTT TAA CGT CAA GGA GAA AAA ACC CCG</b> ATG TCC TCA GCA CAA TCA TCA
			FvPPT-rv	<b>GAT CTT AGC TAG CGG CGG TAC CAA GCT TAC</b> TTA TGA TTT AGG AGC CTT TTC ACC

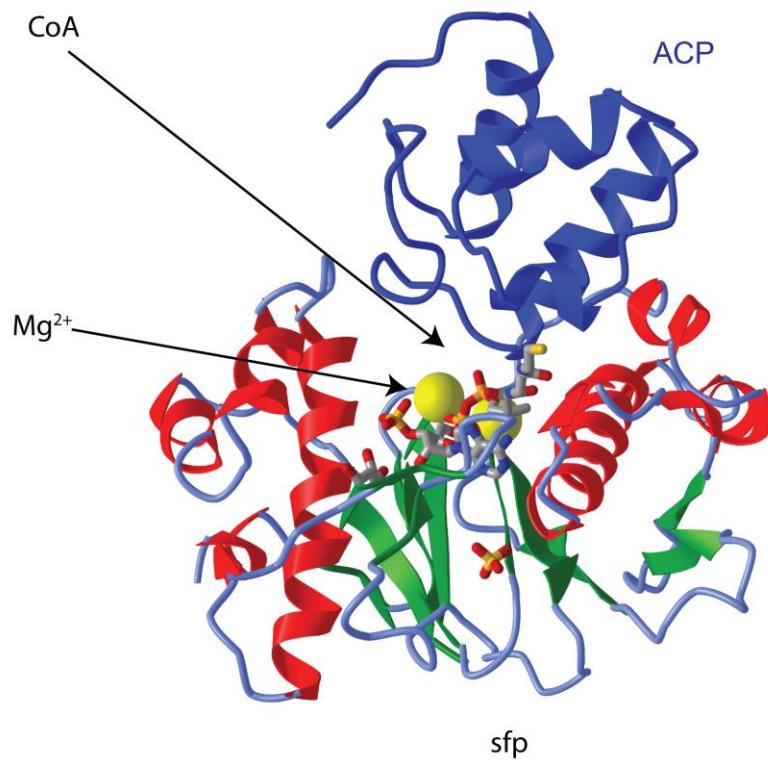
**Table S2.** This table contains the different plasmids utilized in the project, both the native plasmids used as expression vectors, but also plasmids purchased containing the synthetically derived codon optimized genes.

Plasmid	Gene inserted	Purchased	Constructed	Restriction enzymes for linearization	Resulting plasmid
pESC-URA	<i>empty</i>	•			
pESC-LEU	<i>empty</i>	•			
pUC57	<i>fsr1</i>	•			
pUC57	<i>fsr3</i>	•			
pUC57	<i>bik1</i>	•			
pUC57	<i>bik2</i>	•			
pUC57	<i>bik3</i>	•			
pUC57	<i>npgA</i>	•			
pUC57	<i>gsp</i>	•			
pUC57	<i>sfp</i>	•			
pUC57	<i>FgPPT</i>	•			
pUC57	<i>FsPPT</i>	•			
pJET1.2	<i>Q10474</i>	•			
pUC57	<i>FvPPT</i>	•			
pESC-LEU	<i>fsr1</i>		•	NotI/BglII	pESC-LEU:: <i>fsr1</i>
pESC-LEU+ <i>fsr1</i>	<i>npgA</i>		•	BamHI/XhoI	pESC-LEU:: <i>npgA+fsr1</i>
pESC-LEU+ <i>fsr1</i>	<i>gsp</i>		•	BamHI/XhoI	pESC-LEU:: <i>gsp+fsr1</i>
pESC-LEU+ <i>fsr1</i>	<i>sfp</i>		•	BamHI/XhoI	pESC-LEU:: <i>sfp+fsr1</i>
pESC-LEU+ <i>fsr1</i>	<i>FgPpt</i>		•	BamHI/XhoI	pESC-LEU:: <i>FgPpt+fsr1</i>
pESC-LEU+ <i>fsr1</i>	<i>FsPpt</i>		•	BamHI/XhoI	pESC-LEU:: <i>FsPpt+fsr1</i>
pESC-LEU+ <i>fsr1</i>	<i>Q10474</i>		•	BamHI/XhoI	pESC-LEU:: <i>q10474+fsr1</i>
pESC-LEU+ <i>fsr1</i>	<i>FvPpt</i>		•	BamHI/XhoI	pESC-LEU:: <i>FvPPT+fsr1</i>
pESC-URA	<i>fsr3</i>		•	NotI/BglII	pESC-URA:: <i>fsr3</i>
pESC-URA+ <i>fsr3</i>	<i>fsr2</i>		•	BamHI/XhoI	pESC-URA:: <i>fsr2+3</i>
pESC-LEU	<i>bik1</i>		•	NotI/BglII	pESC-LEU:: <i>bik1</i>
pESC-LEU+ <i>bik1</i>	<i>npgA</i>		•	BamHI/XhoI	pESC-LEU:: <i>npgA+bik1</i>
pESC-LEU+ <i>bik1</i>	<i>gsp</i>		•	BamHI/XhoI	pESC-LEU:: <i>gsp+bik1</i>
pESC-LEU+ <i>bik1</i>	<i>sfp</i>		•	BamHI/XhoI	pESC-LEU:: <i>sfp+bik1</i>
pESC-LEU+ <i>bik1</i>	<i>FgPpt</i>		•	BamHI/XhoI	pESC-LEU:: <i>FgPpt+bik1</i>
pESC-LEU+ <i>bik1</i>	<i>FsPpt</i>		•	BamHI/XhoI	pESC-LEU:: <i>FsPpt+bik1</i>
pESC-LEU+ <i>bik1</i>	<i>Q10474</i>		•	BamHI/XhoI	pESC-LEU:: <i>q10474+bik1</i>
pESC-LEU+ <i>bik1</i>	<i>FvPpt</i>		•	BamHI/XhoI	pESC-LEU:: <i>FvPPT+bik1</i>
pESC-URA	<i>bik2</i>		•	NotI/BglII	pESC-URA:: <i>bik2</i>
pESC-URA+ <i>bik3</i>	<i>bik3</i>		•	BamHI/XhoI	pESC-URA:: <i>bik2+3</i>

**Figure S1.** Phylogenetic tree of the PPTases used in the present study (**bold**) together with 22 additional published PPTases. Bootstrap values (>70%) from 1000 replications are indicated at the respective nodes.



**Figure S2.** Predicted structure of sfp/ACP interaction with the CoA and Mg<sup>2+</sup> ion highlighted by arrows.



**Figure S3.** Production levels of bikaverin and bostrycidin in the individual strains (relative to OD at 48 hours) in the supernatant and pellets. The mean of the supernatant from BY4743::*FvPPT1* was set to 100 for both compounds.

