Supplementary materials to RSC Advances

Effect of overexpression of endogenous and exogenous *Streptomyces* antibiotic regulatory proteins on tacrolimus (FK506) production in *Streptomyces* sp. KCCM 11116P

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Supplementary tables

Table S1 Strains and plasmids used in this study.

	Description	**Source and references**
Strains		
E. coli DH5α	Cloning host	Laboratory stock
E. coli ET12567	*dam dcm hsdS cat tet*	[1]
E. coli ET12567/ pWHM3	Strain to obtain non-methylated pWHM3	This study
E. coli ET12567/ pWHM3- *Sx5140*	Strain to obtain non-methylated pWHM3- *Sx5140*	This study
E. coli ET12567/ pWHM3- *bulZ*	Strain to obtain non-methylated pWHM3- *bulZ*	This study
E. coli ET12567/ pWHM3- *bulY*	Strain to obtain non-methylated pWHM3- *bulY*	This study
Streptomyces sp. KCCM 11116P	Wild-type FK506 producer	Laboratory stock
S. xinghaiensis S187^{T}	Source to obtain *Sx5140*	[2]
Streptomyces sp. CC01	*Streptomyces* sp. KCCM 11116P transformed with the empty plasmid pWHM3 which was used as control strain, Tsr^{r}	This study

Table S1 Cont.

	Description	**Source**
Streptomyces sp. CC02	*Streptomyces* sp. KCCM 11116P transformed with plasmid pWHM3-*bulZ* , Tsr^r	This study
Streptomyces sp. CC03	*Streptomyces* sp. KCCM 11116P transformed with plasmid pWHM3-*bulY*, Tsr^r	This study
Streptomyces sp. C5140	*Streptomyces* sp. KCCM 11116P transformed with plasmid pWHM3-*Sx5140*, Tsr^r	This study
Plasmids		
pMD-19T	Cloning vector, Amp^r	Takara
pMD-19T- *bulZ*	pMD-19T carrying *bulZ*, Amp^r	This study
pMD-19T- *bulY*	pMD-19T carrying *bulY*, Amp^r	This study
pMD-19T- *Sx5140*	pMD-19T carrying *Sx5140*, Amp^r	This study

Table S1 Cont.

	Description	**Source**
pWHM3	Cloning vector, colE1 replicon, pSG5 replicon, Tsr^r, Amp^r	[3]
pWHM3- *bulZ*	pWHM3 carrying *bulZ* in *Bam* HI-*Hin*d III sites, Amp^r, Tsr^r	This study
pWHM3- *bulY*	pWHM3 carrying *bulY* in *Bam* HI-*Hin*d III sites, Amp^r, Tsr^r	This study
pWHM3- *Sx5140*	pWHM3 carrying *Sx5140* in *Bam* HI-*Hin*d III sites, Amp^r, Tsr^r	This study

Table S2 Primers used in this study*.

Primers	5’ to 3’ primer sequences	Product size	Purpose
bulZ-FP[a]	GCTCTAGAATGAGAATTCAGGTTCTGGG	822 bp	To amplify
bulZ-RP[b]	AAAAAGCTTTCAGGCGGCGAAGAGGTC		*bulZ*
	GA		
bulY-FP[a]	GCTCTAGAATGGATATTGATGTGCTGGG	867 bp	To amplify
bulY-RP[b]	AAAAAGCTTTCACACCTGCGCCATGCGG		*bulY*
	C		
Sx5140-FP[c]	AAAGGATCCATGGACATCAAGGTATTG	795 bp	To amplify
	GGCCCG		*Sx5140*
Sx5140-RP[b]	AAAAAGCTTTCAGCCCGCGGCGGGCAC		
hrdB-FP	AGCCGTTTCCATCGTTCC	126 bp	Control for
hrdB-RP	GATCTGCCCATCAGCCTTTC		qRT-PCR
tsuR1-FP	TGCTTCTGTGTTCGACGAGTG	105 bp	qRT-PCR
tsuR1-RP	TTGGACGGGAAGTGAAAGTAGAG		*of tsuR*
tsuS1-FP	ATGTCGTCCTCGCCGAAA	95 bp	qRT-PCR
tsuS1-RP	GTAGTGGTCGCTGGTGTGCT		of *tsuS1*
tsuS2-FP	TGGAAGGCGACCACTTCA	98 bp	qRT-PCR
tsuS2-RP	CGTTTCTGCGGCAATCAG		of *tsuS2*
fkbN-FP	ATCACCTCACTGACGGAGTCG	80 bp	qRT-PCR
fkbN-RP	TGGTCGGCTATCTCCCTGTT		of *fkbN*
fkbG-FP	GGGTCGTTCGATGTCGTGT		

Table S2 Cont.

Primers	5’ to 3’ primer sequences	Product size	Purpose
fkbG-RP	GCTTCCGCAACAAGGTGA	256 bp	qRT-PCR of *fkbG*
fkbH-FP	CTGGGATCTGGACAACACCTT	143 bp	qRT-PCR
fkbH-RP	GGTCGTGGTCGTTCTTGCT		of *fkbH*
fkbI-FP	ATGACATCGCAGGGAATGG	85 bp	qRT-PCR
fkbI-RP	TGGTCAGCTCCTTCAGATAGGTC		of *fkbI*
fkbQ-FP	CACGCCGATCACCGTATGTA	121 bp	qRT-PCR
fkbQ-RP	GAAATGCCCACCCGAGAA		of *fkbQ*
tcsA-FP	GCTTCTTCTACTCCCACGGTCT	81 bp	qRT-PCR
tcsA-RP	GGGTCATGTCGTTCACCATC		of *tcsA*
tcsB-FP	CGGACTGTTACCCGACGACT	178 bp	qRT-PCR
tcsB-RP	CCCGTGCAGTTTGGAGAA		of *tcsB*
tcsD-FP	CAGGTGTCGTTCTCCGACTG	96 bp	qRT-PCR
tcsD-RP	GATTCTGCTGGCGTTGAGG		of *tcsD*

*FP and RP stand for forward primer and reverse primer in each primer pairs, respectively, the purpose of PCR using these primers was indicated in the table. Underlined: a. *Xba*I site TCTAGA; b. *Hind*III site AAGCTT; c. *Bam*HI site GGATCC.

Supplementary figures

Figure S1. Effect of precursor addition on FK506 production in the transformants. Disodium malonate[4] was selected as a precursor and was added at 4th day during fermentation of *Streptomyces* sp. CC01 and *Streptomyces* sp. C5140 at a final concentration of 5 mM. Samples were collected from the 5th day of fermentation, and the fermentation and detection procedures were described in Materials and methods in section 2.5 and 2.6.

Figure S2. Quantitative real-time RT-PCR analysis of *tsuS2* in *Streptomyces* sp. CC01, *Streptomyces* sp. CC02, *Streptomyces* sp. CC03 and *Streptomyces* sp. C5140. *tsuS2* expression levels at 24 h and 48 h were compared with that of the control strain *Streptomyces* sp. CC01, and was quantified by the fold change. The expression level of *tsuS2* at 72 h was undetectable and thus the result was not included in this manuscript.

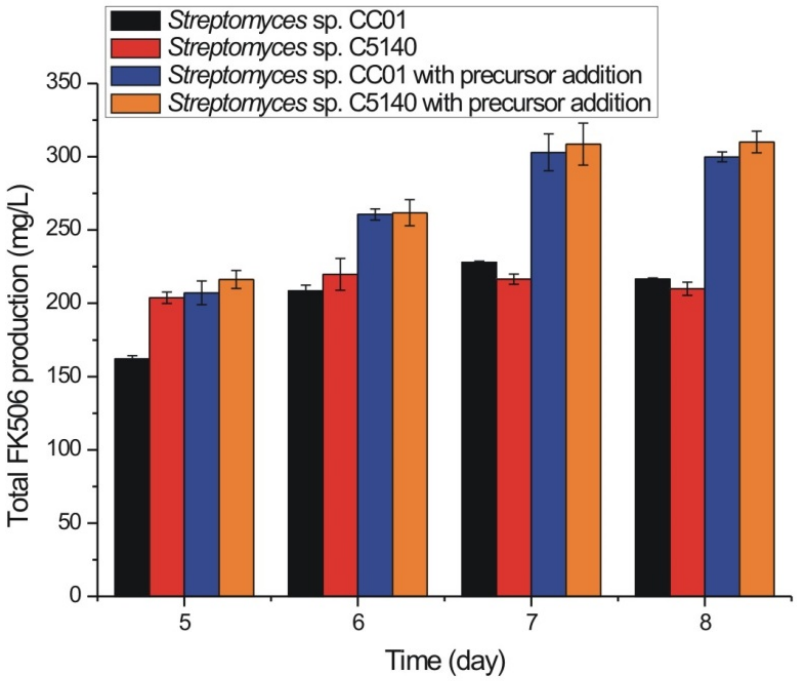
Streptomyces sp. CC01
Streptomyces sp. C5140
Streptomyces sp. CC01 with precursor addition
Streptomyces sp. C5140 with precursor addition
Total FK506 production (mg/L)
0
50
100
150
200
250
300
350
5
6
7
8
Time (day)

Figure S1

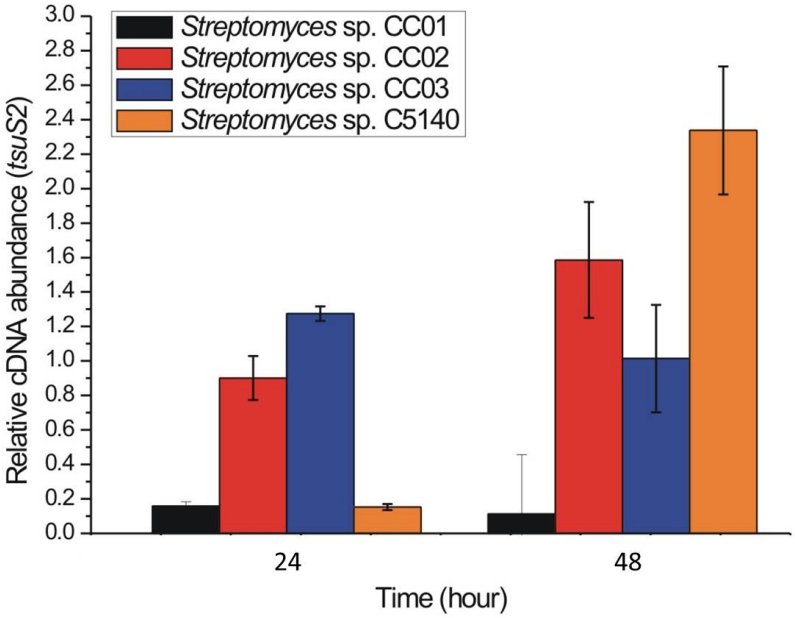
Streptomyces sp. CC01
Streptomyces sp. CC02
Streptomyces sp. CC03
Streptomyces sp. C5140
Relative cDNA abundance (tsuS2)
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
24
48
Time (hour)

Figure S2

References

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[3] J. Vara, M. Lewandowska-Skarbek, Y. G. Wang, S. Donadio and C. Hutchinson, *J. Bacteriol.*, 1989, **171**, 5872-5881.

[4] W. J. Du, D. Huang, M. L. Xia, J. P. Wen and M. Huang, *J. Ind. Microbiol. Biotechnol.*, 2014, **41**, 1131-1143.