SUPPLEMENTARY FILE

Characterization of Two Toxin-Antitoxin Systems in Deep-Sea *Streptomyces* sp. SCSIO 02999

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Table S1. Sequence analysis results of the three putative TA pairs.

Query name	Top hit in Pfam annotation					Top hit in GenBank non-redundant database				
	Pfam family	alignment start	alignment end	Description	E-value	Accession No.	Description	Query cover	E-value	Identity
Orf5461	PF06769	5	84	YoeB_toxin	4.2e-41	WP_031030578	Txe/YoeB family	100%	8e-55	100.00%
Orf5462	PF02604	3	72	Phd/YefM_antitoxin	2.6e-18	WP_031030581	type II toxin-antitoxin system *[Streptomyces*]	100%	5e-56	100.00%
Orf2766	PF02604	9	57	Phd/YefM_antitoxin	4.1e-12	WP_031033384	Phd/YefM family antitoxin [*Streptomyces olivaceus*]	100%	2e-54	98.88%
Orf2767	PF05016	4	60	ParE_toxin	3.2e-10	WP_031033386	RelE/ParE family toxin [*Streptomyces olivaceus*]	98%	8e-36	95.31%
Orf2769	None					EMF54554	hypothetical protein [*Streptomyces bottropensis* ATCC 25435]	55%	1e-12	74.00%
Orf2770	None					WP_018845686	hypothetical protein [*Streptomyces*]	100%	8e-51	98.80%

Table S2. Mass spectroscopy results of the co-purified protein with NHis-YefM (refer to Fig. 2B, lane 3). Peptide fragments identified by mass spectrometry analysis are highlighted in different colors, and their loci in YoeB protein are also shown.

Peptide seq	Observed	Mr (expt)	Mr (calc)	ppm	M
MRVTFTSHGWEDYVHWAESDR	2609.2732	2608.2659	2608.1554	42.4	1
KVTKR	631.4585	630.4512	630.4177	53.2	2
INRLIDDITRDPFK	1716.0256	1715.0183	1714.9366	47.6	2
GIGKPEPLKGDLSGYWSR	1960.1277	1959.1204	1959.0214	50.6	1
RIDDTHR	912.5231	911.5158	911.4573	64.1	1
LVYKPADGVLVIVQARYHY	2204.3244	2203.3171	2203.2153	46.2	1

Protein YoeB [*Streptomyces* sp. SCSIO 02999]
YoeB Protein: MRVTFTSHGWEDYVHWAESDRKVTKRINRLIDDITRDPFKGIGKPEPLKGDL
Identified: MRVTFTSHGWEDYVHWAESDRKVTKRINRLIDDITRDPFKGIGKPEPLKGDL
YoeB Protein: SGYWSRRIDDTHRLVYKPADGVLVIVQARYHY
Identified: SGYWSRRIDDTHRLVYKPADGVLVIVQARYHY

Table S3. YoeB can not cross-activate *E. coli* TA systems. Fold changes of 14 TA transcripts and 1 RNase gene (*rbn*) in *E. coli* K-12 BW25113 WT or Δ*lon* cells overexpressing YoeB via pCA24N-*yoeB* as compared to empty vector pCA24N were quantified by qRT-PCR. The *yoeB* was used as positive control. Lower Ct (cycle threshold) values indicate higher expression levels. Mean and standard deviations are from three independent cultures.

Gene	WT/pCA24N	WT/pCA24N-*yoeB*		Δ*lon*/pCA24N	Δ*lon*/pCA24N-*yoeB*	
	Ct	Ct	Fold change (log_2)	Ct	Ct	Fold change (log_2)
***yoeB*$_{E.coli}$**	27.93±1.5	27.02±1.33	0.38 ±1.8	26.54±0.1	29.44±0.95	-2.7±0.24
***yefM*$_{E.coli}$**	26.24 ±0.46	25.31 ±0.5	1.17 ±0.52	26.4 ±0.62	27.9 ±0.99	-0.63 ±1.61
relE	26.93 ±0.18	26.79 ±0.49	0.94 ±0.38	27.25 ±0.83	27.54 ±0.73	0.48 ±1.72
relB	28.28 ±0.43	26.93 ±0.38	0.7 ±0.52	27.5 ±0.92	27.38 ±0.78	0.67 ±0.95
mazF	27.11±0.2	26.08±0.3	1.51±0.11	26.83±0.24	26.94±0.45	-0.2±0.57
mqsR	26.97 ±0.69	26.61 ±0.59	0.83 ±0.68	27.34 ±0.4	28.75 ±0.79	-1.49 ±1.03
higB	28.01 ±1.02	28.03 ±1.47	0.45 ±1.92	28.56 ±0.99	28.26 ±0.88	0.22 ±1.84
hicA	27.93 ±0.74	26.98 ±0.65	0.33 ±0.76	26.97 ±1.17	28.23 ±0.88	-0.22 ±1.67
yafO	29.42 ±1.12	28.2 ±1.44	0.86 ±1.66	28.12 ±0.73	30.87 ±0.65	-1.42 ±1.01
yhaV	29.15 ±1.09	28.62 ±1.33	0.76 ±1.46	27.33 ±0.79	29.04 ±1.1	-0.32 ±1.94
chpB	27.73 ±0.97	27.08 ±0.74	1.42 ±0.95	26.86 ±0.71	27.05 ±1.15	0.29 ±1.98
rbn	31.93 ±1.84	31.58 ±0.88	-0.21 ±1.29	29.3 ±0.17	32.38 ±0.79	-2.07 ±0.94
ralR	27.04 ±1.15	29.4 ±1.21	-2.55 ±1.64	27.36 ±0.24	30.55 ±1.68	-1.57 ±1.28
ghoT	27.46 ±0.97	29.09 ±0.21	-2.15 ±0.91	27.91 ±1.23	28.47 ±0.08	-0.21 ±1.61
yoeB	30.72 ±0.91	17.35 ±0.93	12.1 ±0.69	31.94 ±0.57	18.05 ±0.35	13.72 ±0.92
rrsG	10.95 ±0.51	10.89 ±0.31	-	11.21 ±0.48	11.67 ±0.13	-

Table S4. Orf2769 can not cross-activate *E. coli* TA systems. Fold changes of 14 TA transcripts and 1 RNase gene (*rbn*) in *E. coli* K-12 BW25113 WT or Δ*lon* cells overexpressing Orf2769 via pCA24N-*2769* as compared to empty vector pCA24N were quantified by qRT-PCR. The *orf2769* was used as positive control. Lower Ct (cycle threshold) values indicate higher expression levels. Mean and standard deviations are from three independent cultures.

Gene	WT/pCA24N	WT/pCA24N-*2769*		Δ*lon*/pCA24N	Δ*lon*/pCA24N-*2769*	
	Ct	Ct	Fold change (log_2)	Ct	Ct	Fold change (log_2)
***yoeB*$_{E.coli}$**	27.81±0.41	27.47±1.2	0.94 ±1.35	26.23±0.25	27.06±0.66	-0.69 ±0.83
***yefM*$_{E.coli}$**	26.08±0.26	26.39±0.88	0.3 ±0.99	25.56±0.68	25.99±0.36	-0.29 ±0.71
relE	27.56±0.14	27.56±0.75	0 ±0	27.33±0.18	27.38±0.63	0 ±0
relB	26.74±0.42	27.18±0.66	0.17 ±0.96	27.16±0.48	26.82±0.53	0.48 ±0.74
mazF	25.47±0.35	25.57±0.58	0.5±0.52	24.5±0.2	25.65±0.79	-1.01±0.16
mqsR	27.1±0.46	27.29±0.74	0.42 ±1	27.13±0.24	27.15±1.04	0.11 ±0.43
higB	28.28±1.01	27.46±0.65	1.43 ±1.44	27.22±0.25	27.46±1.26	-0.1 ±0.66
hicA	27.98±0.09	27.18±1.12	1.41 ±1.02	26.58±0.5	26.13±0.5	0.59 ±1.14
yafO	29.04±0.27	27.63±1.24	2.01 ±1.27	28.1±0.75	26.55±1.01	1.69 ±0.68
yhaV	28.58±0.36	26.95±1.15	2.23 ±1.09	26.47±0.66	26.16±0.93	0.45 ±0.51
chpB	27.64±0.15	27.06±1.68	1.64 ±0.52	26.98±0.62	27.15±0.76	1.18 ±0.71
rbn	29.84±0.84	28.21±1.3	1.21 ±0.28	28.57±0.4	28.76±1.32	0.86 ±0.39
ralR	29.61±0.69	27.9±1.52	0.56±0.56	28.8±0.23	28.21±1.14	0.61±0.18
ghoT	27.48±1.3	29.26±0.38	-2.38 ±1.52	27.64±0.61	26.73±0.89	1.05 ±0.77
orf2769	35.39±1.59	17.93±1.3	17.98 ±1.2	31.8±0.3	16.39±0.62	15.94 ±1.5
rrsG	10.27±0.19	10.87±0.6	-	10.87±0.36	11.01±0.49	-

Table S5. Oligonucleotides used for plasmid construction site-directed mutagenesis and DNA sequencing. If an enzymatic restriction site is included in the sequence, the enzyme restriction site is underlined. f indicates forward primer and r indicates reverse primer. P indicates promoter.

Purpose/Name	Sequence (5'-3')
Plasmid construction	
pCA24N-*yoeB*-f	CACCATCACCATACGGATCCGGCCCTGGTGCACCACCACCACCACCACAGGGTCACTTTCACGTC
pCA24N-*yoeB*-r	TAGCGGCCGCATAGGCCTCAGTAGTGGTAGCGGGCCTG
pCA24N-*yefM*-f	CACCATCACCATACGGATCCGGCCCTGATGCACCACCACCACCACCACCCCATCACCGCCAGCGA
pCA24N-*yefM*-r	TAGCGGCCGCATAGGCCTCACGCCCGCTCCGCGTCCGG
pCA24N-*yoeB-yefM*$_{E.coli}$-f	CACCATCACCATACGGATCCGGCCCTGATGCGTACAATTAGCTACAGCGAAGCGCGTCA
pCA24N-*yoeB-yefM*$_{E.coli}$-r	TAGCGGCCGCATAGGCCTCAGTAGTGGTAGCGGGCCTG
pCA24N-*yoeB*$_{E.coli}$-*yefM*-f	CACCATCACCATACGGATCCGGCCCTGATGCCCATCACCGCCAGCGAAGCCCGTCAGAA
pCA24N-*yoeB*$_{E.coli}$-*yefM*-r	TAGCGGCCGCATAGGCCTCAATAATGATAACGACATGC
pCA24N-*2769*-f	CACCATCACCATACGGATCCGGCCCTGATGCACCACCACCACCACCACGATCGGCTTCAGTGCCG
pCA24N-*2769*-r	TAGCGGCCGCATAGGCCTCATGGTGCCGATCCTACCGG
pCA24N-*2770*-f	CACCATCACCATACGGATCCGGCCCTGATGCACCACCACCACCACCACAGCGGTAGTAGGAAGTA
pCA24N-*2770*-r	TAGCGGCCGCATAGGCCTCAGGCGGCAGCCCCGCCGGC
pHGR01-P-*yoeB-yefM*-f	AGTCAATAAACCGGTGAATTCAGGTGACGAGGGCCTGCCAGT
pHGR01-P-*yoeB-yefM*-r	ACGACGGCCAGTGCCAAGCTTTCAGTAGTGGTAGCGGGCCTG
pHGR01-P-*2769-2770*-f	AGTCAATAAACCGGTGAATTCAGGCCGGCCCGCTCCACCTCG
pHGR01-P-*2769-2770*-r	ACGACGGCCAGTGCCAAGCTTTCAGGCGGCAGCCCCGCCGGC
pET28b-NHis-*yefM-yoeB*-f	TTAAGAAGGAGATATACCATGCACCACCACCACCACCACCCCATCACCGCCAGCGA
pET28b-*yefM-yoeB*-r	CGAGTGCGGCCGCAAGCTTAGTAGTGGTAGCGGGCCTG
pET28b-*yefM-yoeB*-f	TTAAGAAGGAGATATACCATGCCCATCACCGCCAGCGA
pET28b-*2769-2770*-f	TTAAGAAGGAGATATACCATGGATCGGCTTCAGTGCCG

pET28b-*2769-2770*-CHis-r	CGAGTGCGGCCGCAAGCTTAGTGGTGGTGGTGGTGGTGGGCGGCAGCCCCGCCGGCCT
pUT18C-*2770*-f	ACTCTAGAGGATCCCCGGGTACCGAGCGGTAGTAGGAAGTATTC
pUT18C-*2770*-r	ATTACTTAGTTATATCGATGAATTTCAGGCGGCAGCCCCGCCGG
pKT25-*2769*-f	CTAGAGGATCCCCGGGTACCTGATCGGCTTCAGTGCCGCCC
pKT25-*2769*-r	GAATTCTTAGTTACTTAGTCATGGTGCCGATCCTACCG
PCR and DNA sequencing	
pCA24N-f	GATAACAATTTCACACAGAATT
pCA24N-r	GTCAGAGGTTTTCACCGTCATCA
pET28b-f	TAATACGACTCACTATAGGG
pET28b-r	TATGCTAGTTATTGCTCAG
pHGR01-f	TTCTCCAGCCCCTCGGCGCGCATGA
pHGR01-r	CATAACCTCGCCTCCCAGGCAATGTTGGTG
pUT18C-f	GCGAGGGCTATGTCTTCTACG
pUT18C-r	GGGCTGGCTTAACTATGCGG
pKT25-f	CGCATCTGTCCAACTTCCGC
pKT25-r	CGCCAGGGTTTTCCCAGTCA
RT-PCR	
pET28b-*yefM-yoeB*-f	TTAAGAAGGAGATATACCATGCCCATCACCGCCAGCGA
pET28b-*yefM-yoeB*-r	CGAGTGCGGCCGCAAGCTTAGTAGTGGTAGCGGGCCTG
pET28b-*2769-2770*-f	TTAAGAAGGAGATATACCATGGATCGGCTTCAGTGCCG
pET28b-*2769-2770*-r	CGAGTGCGGCCGCAAGCTTAGGCGGCAGCCCCGCCGGCCT
QRT-PCR	
$yoeB_{E.coli}$-f	AGAACGCCATTTGAAGGTAAGG
$yoeB_{E.coli}$-r	TGAGCAGTGAATCGTCGGTAAC
$yefM_{E.coli}$-f	TGGAGAGGCTTGTGTTCTGATG

yefM$_{E.coli}$-r	TTTCCGTTCCTTTGCCTGAT
relE-f	CACTAAAGGAATGGCGAAAGCT
relE-r	CCAACAGAAATCACGAAAACGA
relB-f	GGTAGCATTAACCTGCGTATTG
relB-r	AGCCGTTCTTTCACTATCTCCAC
mazF-f	TATGGGCGATCTGATTTGGG
mazF-r	TTTCTTCGTTGCTCCTCTTGC
mqsR-f	CACATACACGTTTGAGTCAGGTTAA
mqsR-r	ATCAGAGTAGGTGGTCATGCTTTT
higB-f	AACATAAAACGGAGTTGGTGGC
higB-r	ACGATGAACAGCGGTAAAGAAA
hicA-f	AATCTCAGGGCGTCGATGTAG
hicA-r	CGAGTTGTTTCAGGATTGCTTTA
yafO-f	TTTCCTATAAGCGTGACGGTGTT
yafO-r	GAGGTTCAGGTTTCAGAATGGC
yhaV-f	ATCACGGTCAATCCATCATCAC
yhaV-r	GCTGAATACGGTATAGGCATCTGT
chpB-f	GTTCAAGCCTTTAATCAACTGGG
chpB-r	TAATAACGCCTCTTCCACCACC
rbn-f	AGTCGCGGCCATAGCTCTAC
rbn-r	GAAATCATTCGCCAGTTCAGTC
ralR-f	CATCAGTAACGGTGAAAGCCA
ralR-r	CCAGTGGTTCGTTTATTCCA
ghoT-f	CCTTTGTCATTATCTGGTTTATCTCAC
ghoT-r	AAAGAGAGAAAAAAGTAATGCCACAG

yoeB-f	GTGACCAAGCGGATCAACAGA
yoeB-r	CGTGACCAGTAGCCCGACA
2769-f	ATGGATCGGCTTCAGTGCC
2769-r	AATGACTGCCCCGAGGTTC
rrsG-f	TATTGCACAATGGGCGCAAG
rrsG-r	ACTTAACAAACCGCCTGCGT

Figure S1. Gene and protein sequences of *yoeB-yefM* operon in *Streptomyces* sp. SCSIO 02999. The sequences encoding *yoeB* and *yefM* is shown as indicated, and the protein sequences of YoeB and YefM were also shown together with length and size. The 500 bp of *yefM* 5' UTR region was also shown, the palindrome was highlighted, the green indicated the palindrome. The ribosome binding site (RBS) is highlighted box. The start and stop codons for *yoeB* and *yefM* are highlighted in red and blue, respectively. The overlapped four bases are underlined.

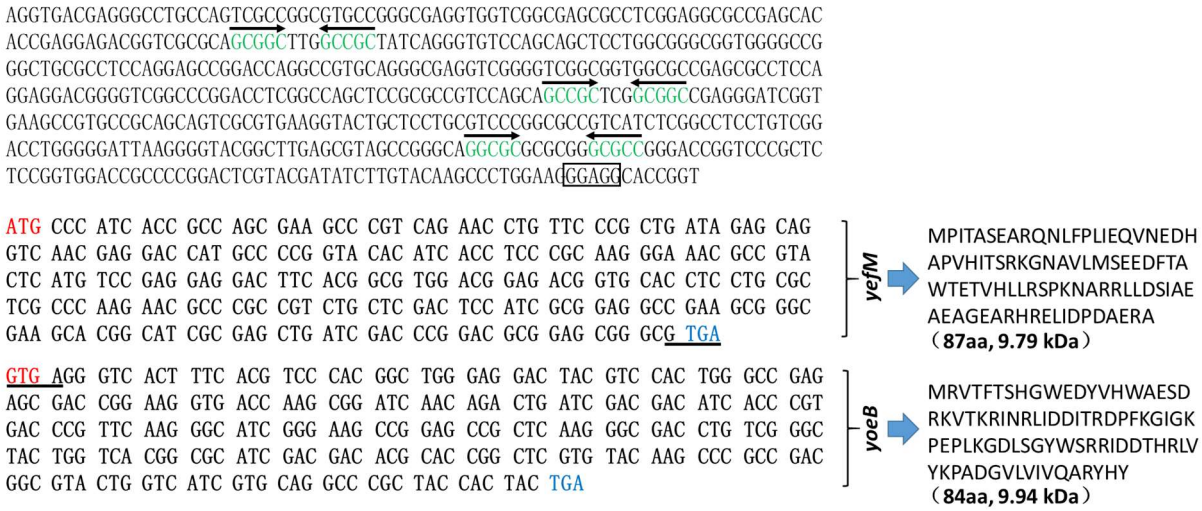

Figure S2. Comparison of amino acid sequences. (**A**) Comparison of the amino acid sequences of YoeB in *E. coli* K12 and in *Streptomyces* sp. SCSIO 02999. (**B**) Comparison of the amino acid sequences of YefM in *E. coli* K12 and in SCSIO 02999. (**C**) Comparison of the amino acid sequences of PhD in conjugative plasmid RK2 and Orf2766 in SCSIO 02999. (**D**) Comparison of the amino acid sequences of ParE in bacteriophage P1 and Orf2767 in SCSIO 02999.

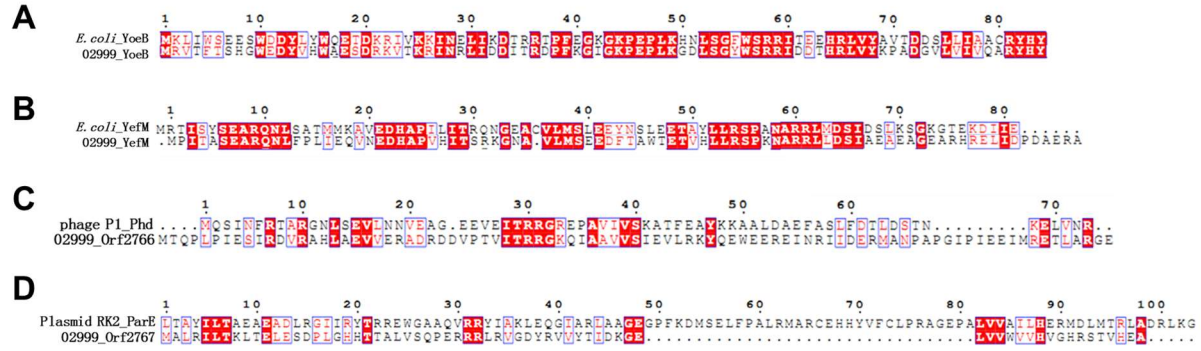

Figure S3. Growth and CFU of the three TA pairs. (**A**) Growth and CFU of *yoeB-yefM.* (**B**) Growth and CFU of *orf2769-orf2770*. (**C**) Growth and CFU of *orf2767-orf2766*. Monitor the growth of the *E. coli* strains harboring the pCA24N-based plasmids with IPTG (1 mM) at OD_{600}~0.1 by absorbance at 600 nm and cell viability (CFUs/ml) was determined on LB agar plates containing chloramphenicol (30 μg/ml) at the time points indicated.

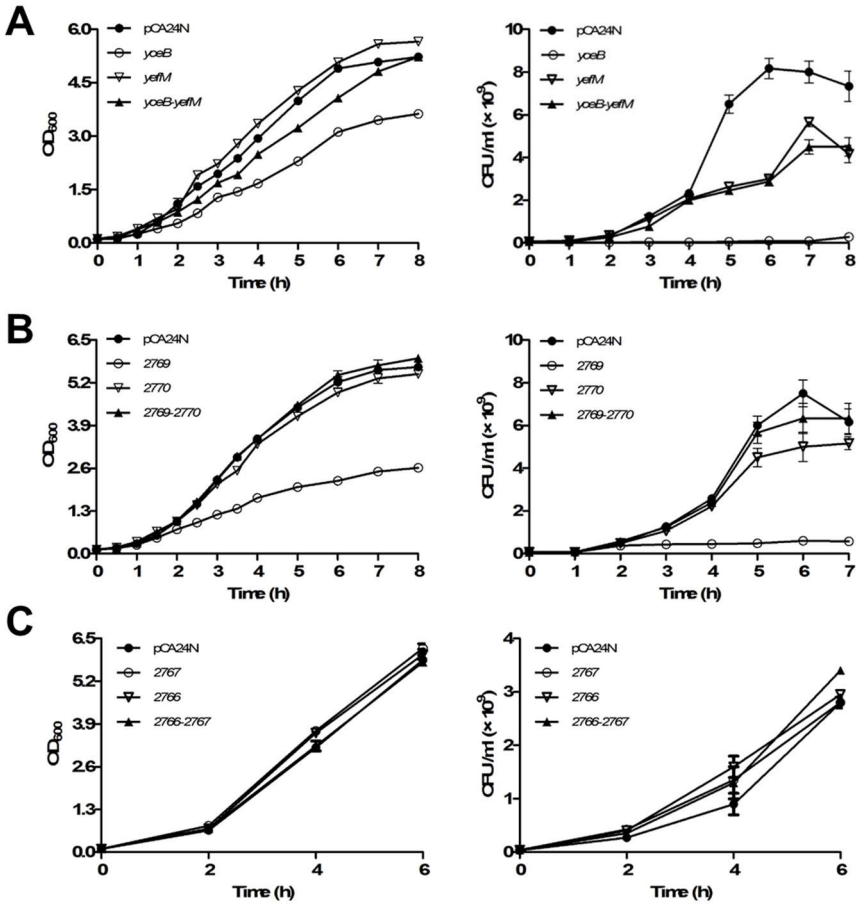

Figure S4. Gene and protein sequences of *orf2769-orf2770* operon in *Streptomyces* sp. SCSIO 02999. The sequence encoding *orf2769* and *orf2770* is shown as indicated, and the protein sequences of Orf2769 and Orf2770 were also shown together with length and size. The 500 bp of *orf2769* 5' UTR region was also shown, the palindrome was highlighted, the green indicated the palindrome. The start and stop codons for *orf2769* and *orf2770* are highlighted in red and blue, respectively. The overlapped four bases are underlined.

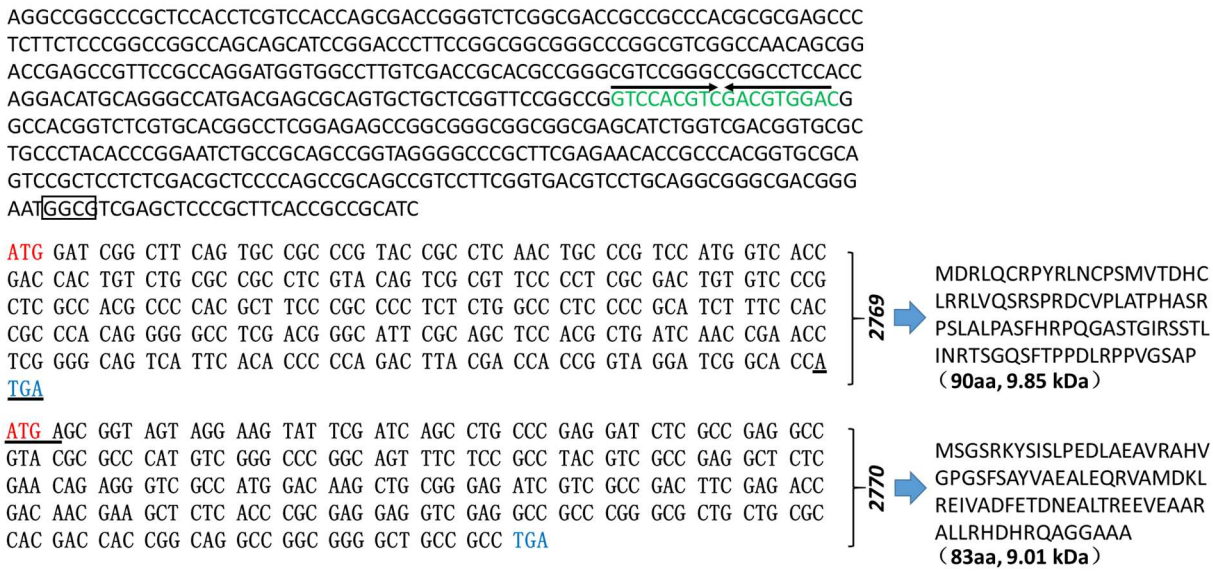

Figure S5. Gene and protein sequences of *orf2767-orf2766* operon in *Streptomyces* sp. SCSIO 02999. The sequence encoding *orf2766* and *orf2767* is shown as indicated, and the protein sequences of Orf2766 and Orf2767 were also shown together with length and size. The 500 bp of *orf2766* 5' UTR region was also shown, the palindrome was highlighted, the green indicated the palindrome. The start and stop codons for *orf2766* and *orf2767* are highlighted in red and blue, respectively.

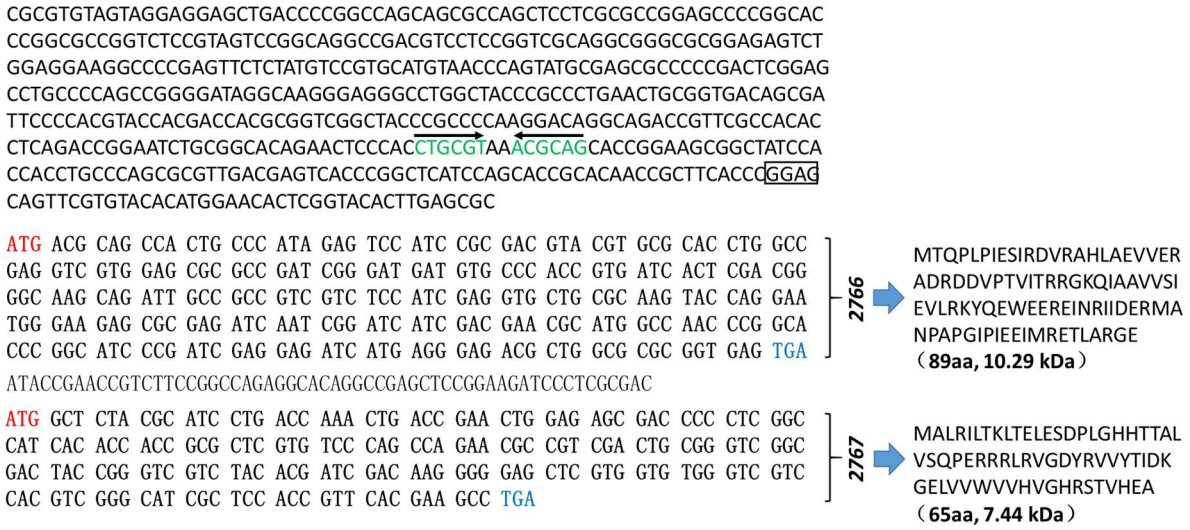

Figure S6. ClpXP proteases could not degrade the antitoxin YefM in *E. coli*. The mutant strains Δ*clpP* and Δ*clpX* harboring the plasmid pCA24N-NHis-*yefM* were induced with 0.5 mM IPTG for 30 min, and added 1% spectinomycin (100 μg/ml) into the strains to activate a stress response. Collected the equivalent amount cells at 0 min, 30 min, 60 min and 120 min and then ran the Tricine-SDS-PAGE for western blot assay. The Tricine-SDS-PAGE (upper panel) and western blot (lower panel) shown that the antitoxin YefM was degraded in the mutant strains Δ*clpP* (**A**) and Δ*clpX* (**B**).

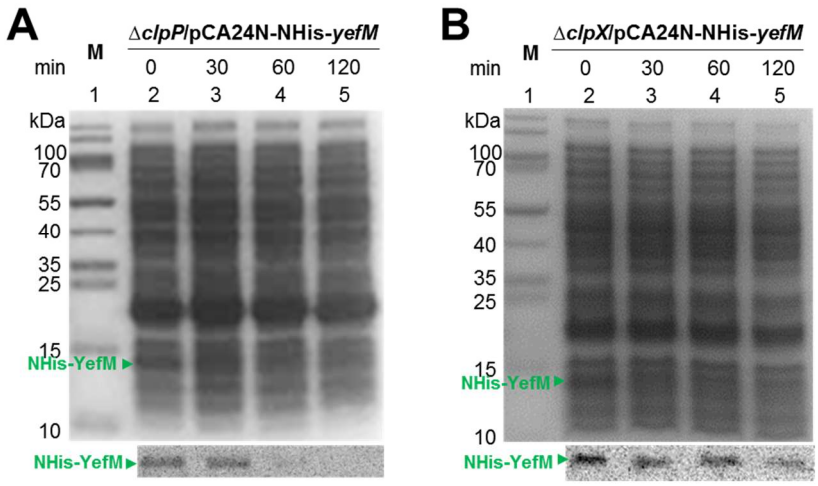

Figure S7. Lon protease degrades the antitoxin Orf2770 in *E. coli*. The strains *E. coli* K-12 BW25113 WT, Δ*lon*, Δ*clpP* and Δ*clpX* harboring the plasmid pCA24N-NHis-*2770* were induced with 0.5 mM IPTG for 30 min, and added 1% spectinomycin (100 µg/ml) into the strains to activate a stress response. Collected the equivalent amount cells at 0 min, 30 min, 60 min and 120 min and then ran the Tricine-SDS-PAGE for western blot assay. The Tricine-SDS-PAGE (upper panel) and western blot (lower panel) shown that the antitoxin Orf2770 was degraded in the WT (**A**) Δ*clpP* (**C**) and Δ*clpX* (**D**) but was not degraded in Δ*lon* strain (**B**).