

Supporting information

A single-domain small protein Med-ORF10 able to regulate the production of antitumor agent medermycin in *Streptomyces*

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Table S1. Primers used in this study.

Primer name	Sequence (with the restriction enzyme sites underlined and start/stop codon in bold)	Enzyme sites
med10-A-Red	GCGGACCGTCCGCGCGGCCTGACGAAGGGAGA ACC ATG ATTCCGGGGATCCGTCGACC CCACGGAAGGGAGCGGGGGGACGGGGGGCGGG	
med10-B-Red	GCC GG TCAT GTAGGCTGGAGCTGCTTC	
med10-A-PIJ	GGAATTCC ATATG TCCACCCACCGAGTCC	<i>NdeI</i>
med10-B-PIJ	CGGGATCCGG TCAC GATTCCGGCAGGTG	<i>BamHI</i>
med10-A-WHM	CGGGATCCCGGGAGAACC ATG TCCACC-3'	<i>BamHI</i>
med10-B-WHM	CGGAATTCCGG TCAC GATTCCGGCAG	<i>EcoRI</i>
med10-A-PET	GGAATTCC ATATG TCCACCCACCGAGTC	<i>NdeI</i>
med10-B-PET	CCCAAGCTTCGATTCCGGCAGGTGGTC GGAATTCC ATATG AGCGGAACCGGCCGGCCCAC	<i>HindIII</i>
med12-A-PET	CG TGGCG	<i>NdeI</i>
med12B-PET	CCCAAGCTTCGACGCGCTCCCGGGCTCGGCTTC	<i>HindIII</i>
Pmed12-A	CGGGATCCTGACTGACCTCGGAC	
Pmed12-B	GGGTACCCGTTTTCTTCTCCCG	
med12A-RT	CCACCAGAGGAACGATGT	
med12B-RT	ATGGGCGAGCGGATGAA	
23SrRNA-A-RT	GGAGTAGTGGCGAGCGAAA	
23SrRNA-B-RT	CGGCAGACCGTAAAAGAGAGA	
med29-A-pET	GGAATTCC ATATG CTGTCCGTGGTGATG	<i>NdeI</i>
med29-B-pET	CCCAAGCTTCGGTCCCGTTCGT	<i>HindIII</i>
00131-F	ATGTCGACCGCTTCCAGAC	
00131-R	AGGAGGAGTTCGTTGGTGG	
00208-F	GTGTTGATGAAGCCGAACG	
00208-R	CCTCTACGGCTACGTCTTC	
0682-F	AACGGCCTGTTCTCTAC	
0682-R	GACCTCCAGTTCGCTCTC	
2954-F	TCGTCGAGACGTACTTCAAG	
2954-R	CAGAGCCAGCTTCATCGAAC	
4359-F	ACGGGCTGAAGAACCTGGTC	
4359-R	AGTGCCTCCTCTTCGACCTC	
4360-F	CCTCTACGGCTACGTCTTC	
4360-R	GTGTTGATGAAGCCGAACG	
4361-F	ACCGACATCGCCCTAGTTC	
4361-R	CAGGTAGGAGTACGCGACG	
4412-F	TTCTGCTCGTCTCCGAACTC	
4412-R	TCCTGAGTCTCCTCGGTAC	
0760-F	AGTCGTTTCGACCTGGTGC	
0760-R	CGGGTACTTCCAGGCAG	
0774-F	TCTTCTCCACCTCCGTCATC	
0774-R	AGGTTCTGACCGAGGCACTG	
1174-F	AACTGCTACCACGCATACCC	
1174-R	TTGGGGCTGTAGGAACAAAG	
1124-F	CCAAGGACAAGGTCACCG	
1124-R	AGAACGGGCAGGAGATCAG	
1125-F	GTTTTCCCGAACGCACAC	
1125-R	GACCCTGGTGAGGAACTC	
1454-F	CATGTCGATGACGCTCAC	
1454-R	CAGGTGTAGGAGAGGCAG	

1866-F	GGGAAGACGTACACATC
1866-R	CAGTGGAAGTCCTCCTTG
1901-F	CTGATCGAACCGCTCTCC
1901-R	GAGCCGTAGATGAGGACG
2113-F	CAAGCTCGCGAAGTACAC
2113-R	AACATCTCGGTACGCTC
3073-F	GGATGCTGCAGAACAACCTC
3073-R	ATCGTCTCGTCCTGCTTGAG
3285-F	TGTTCAACACCCTCACCAAG
3285-R	CGTCGAGTTCGTACGTCTTG
3479-F	GGTAACAGCAACGGCTCAC
3479-R	GTCCTTCCACTCCCAGATG
5223-F	ACGACGACAACCTGGAGTTC
5223-R	CCTGTTCGAAGCGGTACTTC
6171-F	TCAACCTCACCGTCAAC
6171-R	CTCGCAGGCGTTCTTG
7470-F	TGGTCCGTATGACCGTG
7470-R	TCGGCCAGCAGGAAAAG

Table S2. The downregulated genes in CH999/pIK340- Δ med10 ($\log_2(\text{fold change}) > 1$ or < -1 and P-value < 0.05) using transcriptomics.

Name	Putative gene product	Fold-change
SCO3470	transposase	-
SCO3471	extracellular agarase	-
SCO3472	transposase remnant	-
SCO3473	aldolase	-
SCO3474	sugar kinase	-
SCO3475	galactonate dehydratase	-
SCO3476	short-chain dehydrogenase	-
SCO3477	dehydrogenase	-
SCO3478	dehydrogenase	-
SCO3479	beta-galactosidase	-
SCO3480	racemase	-
SCO3481	hypothetical protein	-
SCO3482	sugar-permease	-
SCO3483	integral membrane transport protein	-
SCO3484	sugar-binding protein	-
SCO3485	LacI family transcriptional regulator	-
SCO3486	aldehyde dehydrogenase	-
SCO3487	hydrolase	-
SCO3488	-//-	-
SCO1174	aldehyde dehydrogenase	-6.9992
Novel00039	-//-	-5.8409
Novel00038	-//-	-4.2944
SCO0774	cytochrome P450	-4.2725
sRNA00087	-//-	-4.1381
SCO2113	bacterioferritin	-4.0842
Novel00040	-//-	-4.061
SCO1866	L-ectoine synthase	-3.8707
SCO0773	ferredoxin	-3.8704
SCO6005	lipoprotein	-3.7677
SCO1456	hypothetical protein	-3.7447
SCO3953	RNA 2'-phosphotransferase-like protein	-3.7258
SCO3073	urocanate hydratase	-3.7078
Novel00072	-//-	-3.678
SCO6171	oxidoreductase	-3.6497
SCO1457	transporter	-3.6223
SCO3286	hypothetical protein	-3.5629
SCO7470	phenylacetic acid degradation protein Paal	-3.5432
SCO6170	oxidoreductase subunit	-3.4607
SCO1225	osmoprotectant transporter	-3.4528
SCO5223	cytochrome P450	-3.4251

SCO2114	hypothetical protein	-3.3982
SCO6173	permease SC6C509	-3.3663
Novel00345	-//-	-3.3457
SCO6172	oxidoreductase	-3.2994
SCO0165	hypothetical protein	-3.1387
SCO1454	amino oxidase	-3.0892
Novel00053	-//-	-3.0655
SCO3285	large glycine/alanine rich protein	-2.9456
Novel00027	-//-	-2.9402
Novel00161	-//-	-2.8693
Novel00052	-//-	-2.7652
SCO1224	sugar-phosphate isomerase	-2.7421
SCO1455	hydrolase	-2.6558
SCO4971	dehydrogenase	-2.4937
SCO0912	hypothetical protein	-2.4055
SCO1175	hypothetical protein	-2.4051
SCO1901	zinc-binding dehydrogenase	-2.2764
SCO0760	methyltransferase	-2.2371
SCO1341	lipoprotein	-2.1438
SCO6720	ABC transporter	-2.0997
Med-ORF5	unknown	-2.41
Med-ORF6	short-chain dehydrogenase/ reductase SDR	-2.45
Med-ORF11	transcriptional regulator, SARP family	-2.43
Med-ORF12	3-hydroxybutyryl-CoA dehydrogenase	-2.04
Med-ORF23	acyl carrier protein	-3.23
Med-ORF24	4'-phosphopantetheinyl transferase	-2.51
Med-ORF25	drug resistance transporter, EmrB/QacA	-2.35
Med-ORF26	unknown	-3.01
Med-ORF28	TetR family transcriptional regulator	-3.07
Med-ORF29	crotonyl-CoA reductase/ alcohol dehydrogenase	-2.26
Med-ORF-30	regulator	-6.34

Table S3. The upregulated genes in CH999/pIK340- Δ med10 ($\log_2(\text{fold change}) > 1$ or < -1 and P-value < 0.05) using transcriptomics.

Name	Putative gene product	Fold-change
SCO4359	ABC transporter ATP-binding protein	5.984
Novel00105	-/-	5.4971
Novel00106	-/-	5.264
SCO4360	ABC transporter	5.2572
Novel00208	-/-	5.175
SCO5390	alkanal monooxygenase	3.9793
SCO4361	hypothetical protein	3.8669
SCO6394	IS element ATP binding protein	3.3109
Novel00373	-/-	3.148
SCO6393	transposase	3.1001
SCO0682	hypothetical protein	3.0914
SCO4412	regulatory protein	2.9698
Novel00131	-/-	2.7363
SCO1161	hypothetical protein	2.4752
SCO2954	RNA polymerase sigma factor SigL	2.2696

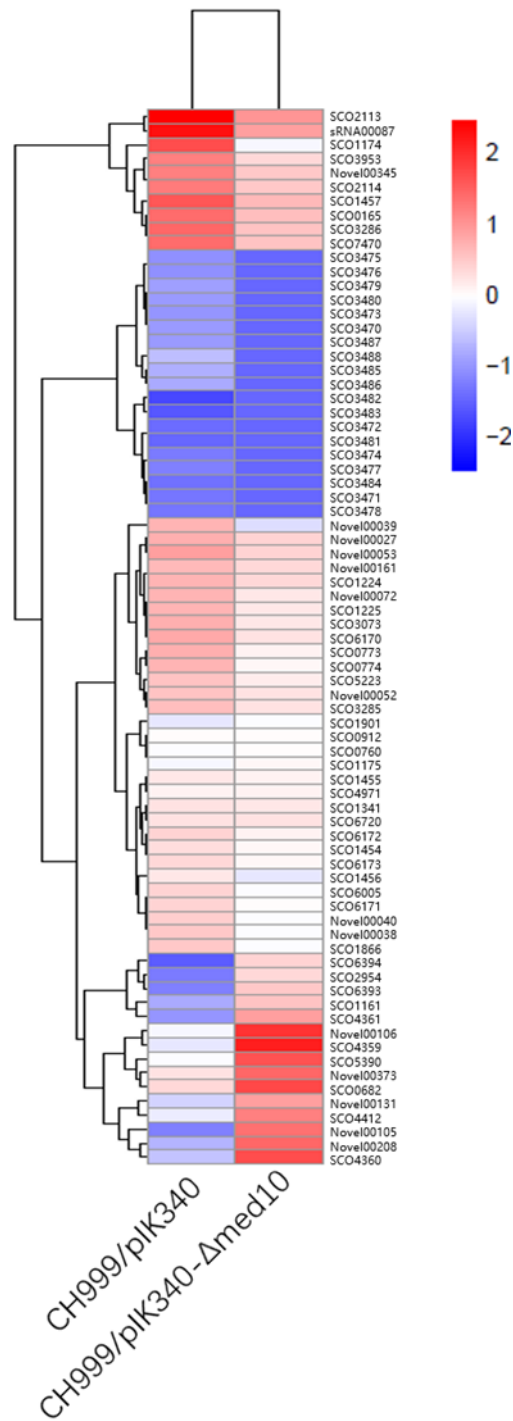


Fig. S1. The overall FPKM hierarchical clustering diagram. The $\log_{10}(\text{FPKM} + 1)$ value is normalized and converted (scale number) and clustered. Red indicates high-expressed genes, and blue indicates low-expressed genes. The color is from red to blue, which means $\log_{10}(\text{FPKM} + 1)$ from large to small.

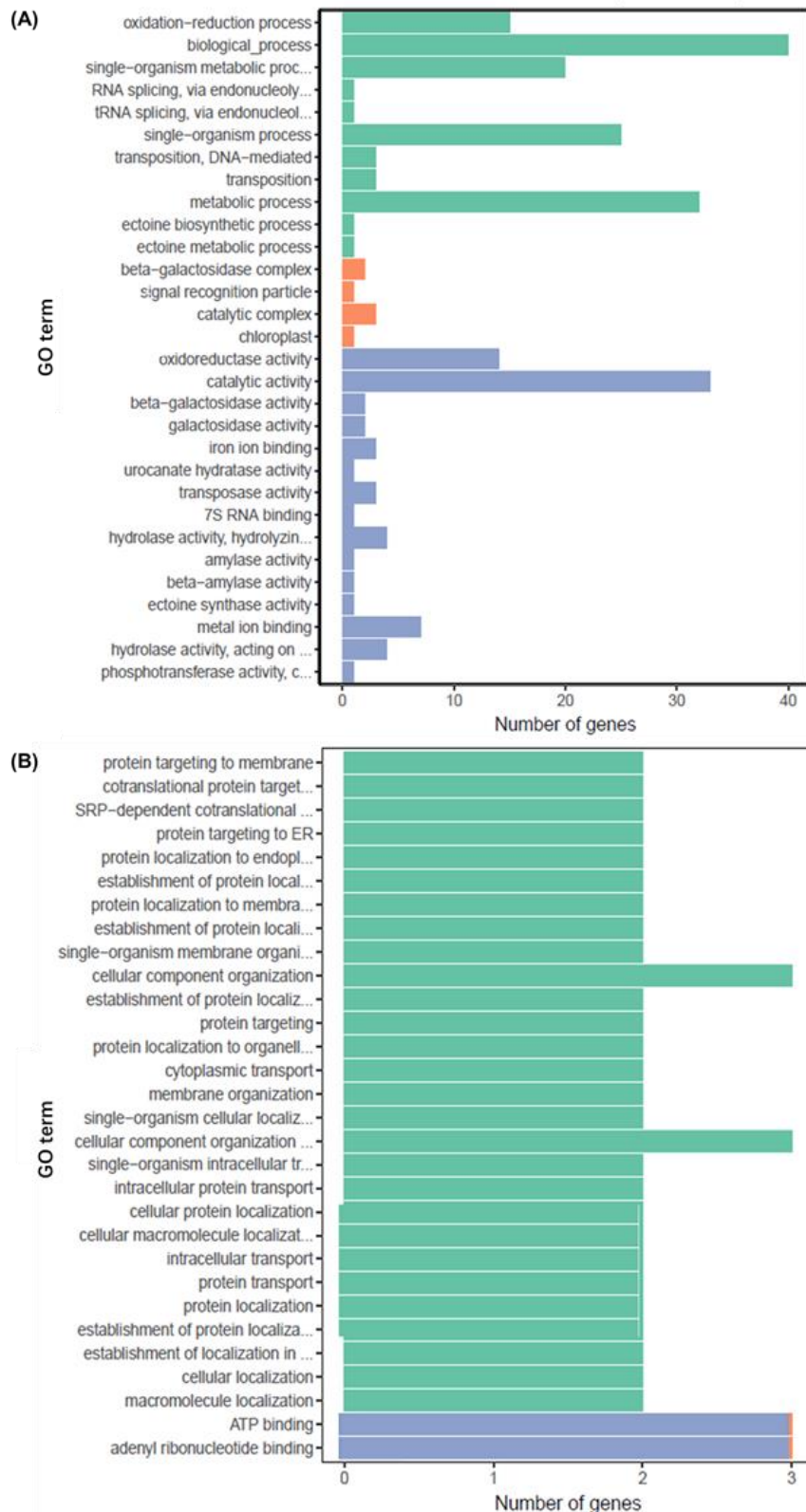


Fig. S2. The ordinate is the enriched GO term, and the abscissa is the number of differential genes in the term.

Different colors are used to distinguish biological processes, cellular components and molecular functions. Genes in (A) is down regulated and genes in (B) is up regulated.

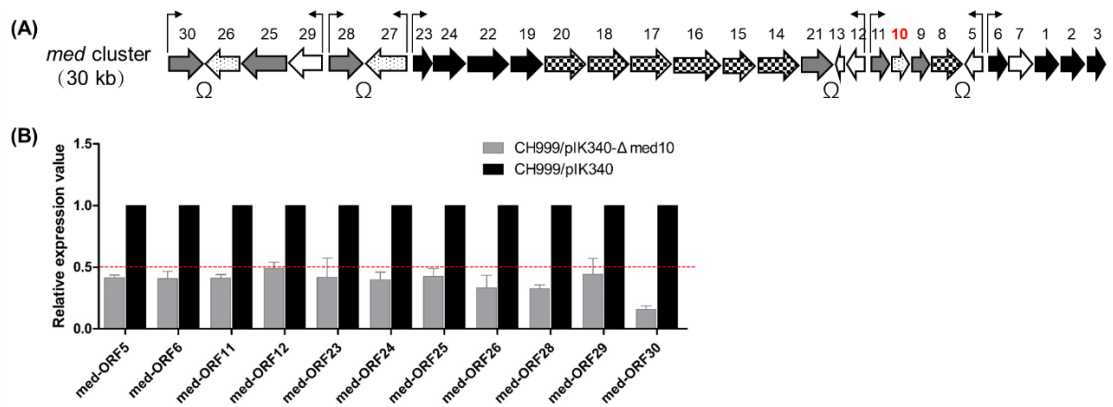


Fig. S3. The proposed operons in *med* gene cluster. (A) and differential expression of *med* genes using transcriptomics. (B) Ω : terminators; arrow: promoters. Genes lower than that the dashed line indicated the differential expression. The y-axis indicated the expression difference ($P < 0.05$ and \log_2 (fold-change) > 1 or < -1) of *med* genes between CH999/pIK340 and CH999/pIK340- $\Delta med10$. The transcriptional level of different genes from CH999/pIK340 was normalized into 1.