
Applied Microbiology and Biotechnology

Improving the production of carbamoyltobramycin by an industrial

Streptoalloteichus tenebrarius through metabolic engineering

Yun Feng¹, Yiqi Jiang¹, XuTong Chen¹, Li Zhu¹, Hailong Xue¹, Mianbin Wu¹, Lirong Yang^{1, 2}, Haoran Yu^{1, 2, *}, Jianping Lin^{1, *}

¹ Key Laboratory of Biomass Chemical Engineering of Ministry of Education,
College of Chemical and Biological Engineering, Zhejiang University, Hangzhou,
310058, China

² Hangzhou Global Scientific and Technological Innovation Center, Zhejiang
University, Hangzhou, 311200, China

*For correspondence: Haoran Yu, E-mail: yuhaoran@zju.edu.cn; Jianping Lin, E-mail: linjp@zju.edu.cn

Supplementary Figure

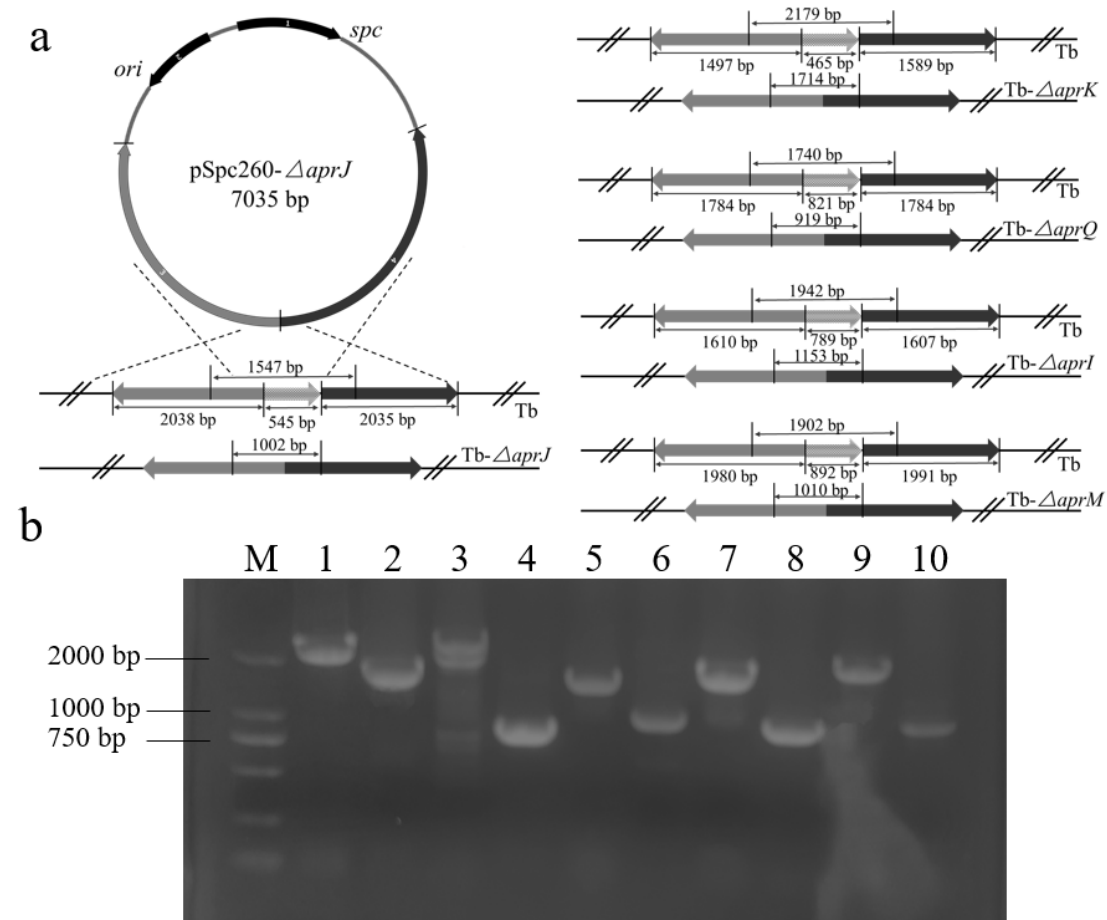


Figure S1. Construction of strains and plasmids for the elimination of byproduct apramycin. **a** Construction of inactivation plasmid pSpc260- $\Delta aprJ$ and the genotype of mutants Tb- $\Delta aprJ$, Tb- $\Delta aprK$, Tb- $\Delta aprQ$, Tb- $\Delta aprI$ and Tb- $\Delta aprM$. **b** Identification of Tb- $\Delta aprJ$, Tb- $\Delta aprK$, Tb- $\Delta aprQ$, Tb- $\Delta aprI$ and Tb- $\Delta aprM$ by PCR. The template of lane 1 was from Tb genomes with a 2179 bp band, the template of lane 2 was from $\Delta aprK$ mutant genomes with a 1714 bp band, primers di-aprK-F and di-aprK-R were used in this part, consistent with the expected deletion of 465 bp fragment within the region of the *aprK*. The template of lane 3 was from Tb genomes with a 1942 bp band, the template of lane 4 was from $\Delta aprI$ mutant genomes with a 1153 bp band, primers di-aprI-F and di-aprI-R were used in this part, consistent with the expected deletion of 789 bp fragment within the region of the *aprI*. The template of lane 5 was from Tb genomes with a 1547 bp band, the template of lane 6 was from $\Delta aprJ$ mutant genomes with a 1002 bp band, primers di-aprJ-F and di-aprJ-R were used in this part, consistent with the expected deletion of 545 bp fragment within the region of the *aprJ*. The template of lane 7 was from Tb genomes with a 1740 bp band, the template of lane 8 was from $\Delta aprQ$ mutant genomes with a 910 bp band, primers di-aprQ-F and di-aprQ-R were used in this part, consistent with the expected deletion of 821 bp fragment within the region of the *aprQ*. The template of lane 9 was from

Tb genomes with a 1902 bp band, the template of lane 10 was from $\Delta aprM$ mutant genomes with a 1010 bp band, primers di-*aprM*-F and di-*aprM*-R were used in this part, consistent with the expected deletion of 892 bp fragment within the region of the *aprM*.

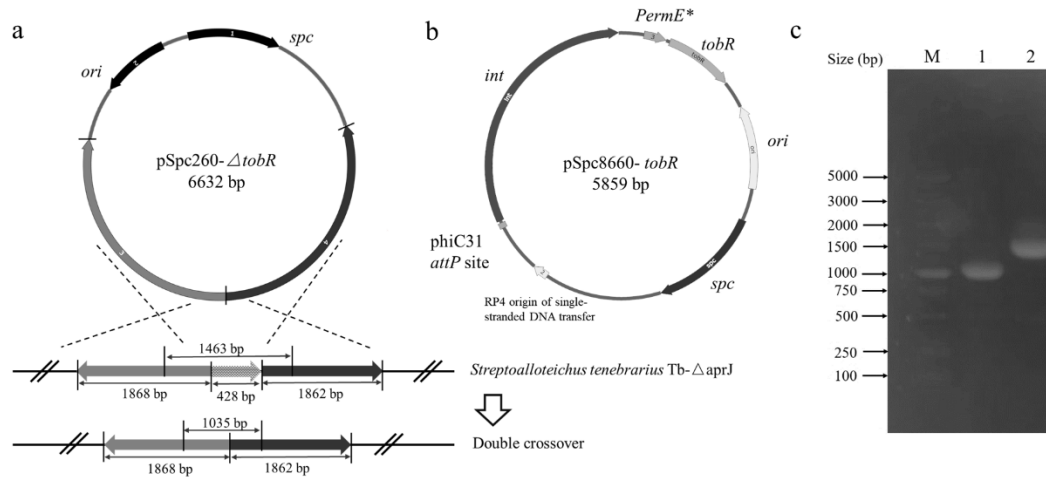


Figure S2. Construction of strains and plasmids for TobR research. **a** Construction of inactivation plasmid pSpc260- Δ tobR. **b** Construction of overexpression plasmid pSpc8660-tobR. **c** Identification of Δ tobR mutant by PCR. The template of lane 1 was from Tb- Δ aprJ- Δ tobR mutant genomes with a 1035 bp band and template of lane 2 was from Tb- Δ aprJ genomes with a 1463 bp band, consistent with the expected deletion of 428 bp fragment within the region of the *tobR*.

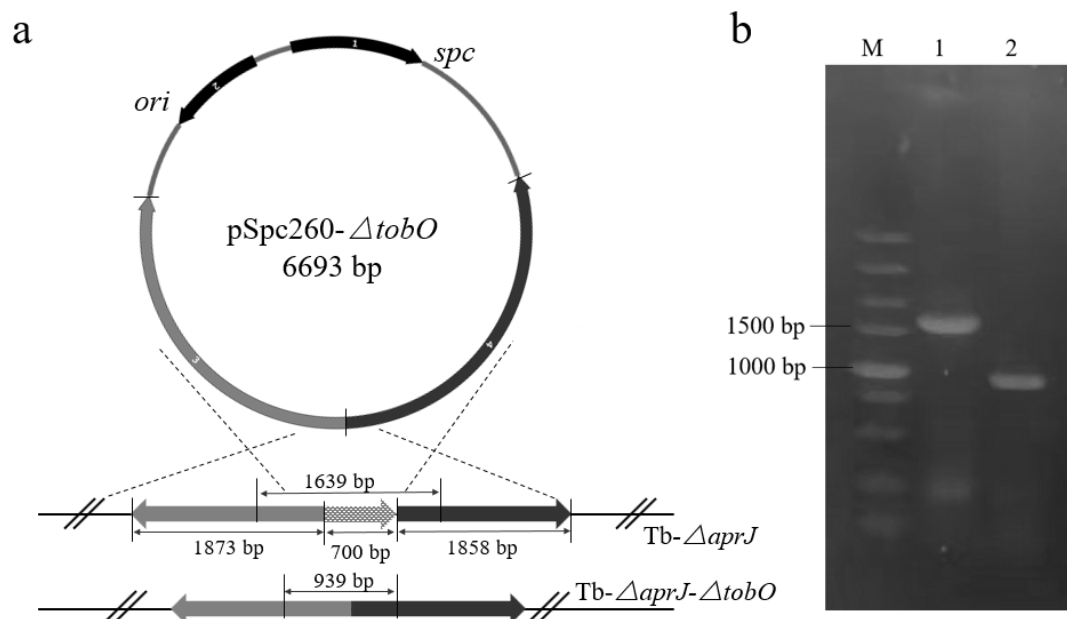


Figure S3. Construction of strain Tb- Δ aprJ- Δ tobO. **a** Construction of inactivation plasmid pSpc260- Δ tobO. **b** Identification of Δ tobO mutant by PCR. The template of lane 1 was from Tb- Δ aprJ genomes with a 1639 bp band, the

template of lane 2 was from Tb- $\Delta aprJ$ - $\Delta tobO$ mutant genomes with a 939 bp band, primers di-tobO-F and di-tobO-R were used in this part, consistent with the expected deletion of 700 bp fragment within the region of the *tobO*.

glycosyltransferase family 4 protein [Streptoalloteichus tenebrarius]

Sequence ID: [WP_253672175.1](#) Length: 439 Number of Matches: 1

[See 2 more title\(s\)](#) [See all Identical Proteins\(IPG\)](#)

Range 1: 1 to 439 [GenPept](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
857 bits(2213)	0.0	Compositional matrix adjust.	438/439(99%)	439/439(100%)	0/439(0%)
Query 1	VRVLRLLTPFFHDCVDSWPAEFDSVGGMQVQILRLSRQLAQRGVQEVFTVGFPGLPVR	60			
	+RVLRLTPFFHDCVDSWPAEFDSVGGMQVQILRLSRQLAQRGVQEVFTVGFPGLPVR				
Sbjct 1	MRVLRLLTPFFHDCVDSWPAEFDSVGGMQVQILRLSRQLAQRGVQEVFTVGFPGLPVR	60			
Query 61	EDSPGLVVRIITRAPMPRLRSELTLVGLNLAWFLGAMAECLRRRGPLPDLIQVHGQGQL	120			
	EDSPGLVVRIITRAPMPRLRSELTLVGLNLAWFLGAMAECLRRRGPLPDLIQVHGQGQL				
Sbjct 61	EDSPGLVVRIITRAPMPRLRSELTLVGLNLAWFLGAMAECLRRRGPLPDLIQVHGQGQL	120			
Query 121	WALLAGPLASAILRRPYSLVHCSRLGVYQPMSTRYDRWQHFRFAAVERWAVRRASGVCAL	180			
	WALLAGPLASAILRRPYSLVHCSRLGVYQPMSTRYDRWQHFRFAAVERWAVRRASGVCAL				
Sbjct 121	WALLAGPLASAILRRPYSLVHCSRLGVYQPMSTRYDRWQHFRFAAVERWAVRRASGVCAL	180			
Query 181	TTTADVVRKALRPHQVRVDVVPDSVDPDPAHSGVPVADRLRAAGLPDARVVGYYGVRV	240			
	TTTADVVRKALRPHQVRVDVVPDSVDPDPAHSGVPVADRLRAAGLPDARVVGYYGVRV				
Sbjct 181	TTTADVVRKALRPHQVRVDVVPDSVDPDPAHSGVPVADRLRAAGLPDARVVGYYGVRV	240			
Query 241	AHEKGWSHFVDVAERLAGGPAGERVVFVVGDGQPRPRMAERVAAAGLADRFFVTGFLPN	300			
	AHEKGWSHFVDVAERLAGGPAGERVVFVVGDGQPRPRMAERVAAAGLADRFFVTGFLPN				
Sbjct 241	AHEKGWSHFVDVAERLAGGPAGERVVFVVGDGQPRPRMAERVAAAGLADRFFVTGFLPN	300			
Query 301	QDIPLTMGGIDVLVMPVSVHEELGGSAIBAMVLGVVYVYGVGGLRDTVGRVTPSLAVRPQ	360			
	QDIPLTMGGIDVLVMPVSVHEELGGSAIBAMVLGVVYVYGVGGLRDTVGRVTPSLAVRPQ				
Sbjct 301	QDIPLTMGGIDVLVMPVSVHEELGGSAIBAMVLGVVYVYGVGGLRDTVGRVTPSLAVRPQ	360			
Query 361	DVGALTDVAVRDVLARTDEYRAQVRAGRPFWEENYDGVGVTRTVAHYHRLAGGRGGVTA	420			
	DVGALTDVAVRDVLARTDEYRAQVRAGRPFWEENYDGVGVTRTVAHYHRLAGGRGGVTA				
Sbjct 361	DVGALTDVAVRDVLARTDEYRAQVRAGRPFWEENYDGVGVTRTVAHYHRLAGGRGGVTA	420			
Query 421	GAGLPDDPARAGRSPASGR	439			
	GAGLPDDPARAGRSPASGR				
Sbjct 421	GAGLPDDPARAGRSPASGR	439			

Figure S4. Functional analysis of AprM through NCBI database

2yx4/1-150	1	MDEIDLRILKILQYNAYKSLDEIAREIRIPKSTLSY	36
2e1c/1-171	1	MGSSHHHHHHSSGLVPRGSHMRVPLDEIDKKIILKILQNDGKAPLREISKITGLAESTIHE	60
2p6s/1-162	1	GMPQLTLDKTDIKILQVLQENGRLTNVELSERVALSPSPCLR	43
2gqg/1-163	1	VDSKKRPGKDLRIDRNILNELQKDGRI SNVELSKRVGLSPTPCLE	46
1i1g/1-141	1	MIDERDKIILEILEKDAARTPFTEIAKKLGISETAVRK	37
2cg4/1-152	1	MENYLIDNLDRLGLEALMGNARTAYAEIAKQFGVSPETIHV	41
2vby/1-150	1	MNEALDDIDRILVRELAADGRATLSELATRAGLSVSAVQS	40
TobR/1-163	1	VPRDGVKLVDDVDHRLALLQEDSGRTLGLALGELVGLSASAVQR	43
2cfx/1-144	1	MKLDQIDLNIIIEELKKDSRLSMRELGRKIKLSPPSVTE	38
2ia0/1-171	1	MAHHHHHHGSSEIHLDDLDNRNLRLLKKDARLTISELSEQLKKPESTIHF	50
4pcq/1-190	1	MILFRGHMRDNSTEHKTRRAASSKDVRAELDEVDRRLSLSLHGDARMPNNALADTVGIAPSTCHG	67
2dbb/1-151	1	MDCMRKLDVRDMLVKILSENSRLTYRELAIDLNTTRQRIAR	42
2yx4/1-150	37	RIKKLEKDGVIKGYAYI....NPASLNLDYIVITSVKAKYGKNYHVELGN-KLAQIPGVWGVYFVL	98
2e1c/1-171	61	RIRKLRESGVIKKFTAIL....DPEALGYSMLAFILVKVKAGK-YSEVAS-NLAKYPEIVEVEYETT	120
2p6s/1-162	44	RLKQLEDAGIVRQYAALL....SPESVNLGLQAFIRVSIRK-AKDAREDFAAASVRKWPEVLSFCALT	105
2gqg/1-163	47	RVRRLERQGFIIQGYTALL....NPHYLDASLLVFVEITLNRGAPDVFEQNTAVQKLEEQECHLVS	109
1i1g/1-141	38	RVKALEEKGIIEGYTIKI....NPKKLGYSLVITITGVDTKPEK-LFEVAEKLKEYDFVKELYLSS	97
2cg4/1-152	42	RVEKMKQAGIITGARIDV....SPKQLGYDVGCFIGIILKSAD-DYPSALAKLESDEVTEAYTT	102
2vby/1-150	41	RVRRLESRGVVQGYSARI....NPEAVGHLLSAFVAITPLDPS-QPDDAPARLEHIEEVESCVSA	101
TobR/1-163	44	RVERYRASGILARYVAVL....EPRRGDLVLLAICLVTLERESGHAHEEFRRLRLAAPEVQQLYNVS	106
2cfx/1-144	39	RVRQLESFGIIKQYTLEV....DQKKLGLPVSCEIVATVKNAD-YERFKSYIQTLPNIEFCYRIA	98
2ia0/1-171	51	RIKKLQERGVIERYTIILGEQLKPKHLALIVLEVGPVIEDFLERYISYISSTLSALPGVLFVAK-S	116
4pcq/1-190	68	RVRRLVDLGVIRGFYTDI....DPVAVGLPLQAMISVNLQSSARGKIRSIQQIRRRKQVMDVYFLA	130
2dbb/1-151	43	RIDKLKKLGIIRKFTIIP.....DIDKLGMYAIVLIKSKVPSDADKVISEISDIEYVKSVEKGV	102
2yx4/1-150	99	GDNDFIVMARY-KTREEFMEKFLERVMSIPEVERTSAQVVVKIIKESPNIVIF.....	150
2e1c/1-171	121	GDYDMVVKIRT-KNSEELNN-FLDLIGSIPGVEGTHMIVLKTHKETTELPIK.....	171
2p6s/1-162	106	GETDYLLQAFF-TDMNAFSHFVLDTLSSHGVQDAQSSFVLKEIKHTTSLPLNHLLEK...	162
2gqg/1-163	110	GDFDYLLKTRV-PDMSAYRKLLGETLLRLPGVNDTRTYVVMEEVKQSNRLVIKTR.....	163
1i1g/1-141	98	GDMHIMAVIWA-KDGEDLAEIISNKIGKIEGVTKVCPAIILEKLK.....	141
2cg4/1-152	103	GHYSIFIKVMC-RSIDALQHVLIINKIQTIDEIQSTETLIVLQNPIMRTIKP.....	152
2vby/1-150	102	GEESYVLLVRV-ASARALED-LLQRIRTTANVTRSTIILNTFYSDRQHIP.....	150
TobR/1-163	107	GDSDYVVVLAT-TGMAHHRREVADRLLKDAPNVRRYSTLFVLDPVRTGSALPTRREGED...	163
2cfx/1-144	99	GAACYMILKINA-ESLEAVEDFINKTSPYAQTIVTHVIFSEIDTKNGRG.....	144
2ia0/1-171	117	GEDKIIALVGK-NNKDELVKFIEENITSIPNLKHIQIFPITEIKKGEDLTGFLAEV.....	171
4pcq/1-190	131	GADDFILHVAA-RDTEDLRSFVVENLNADADVAGTQTSLIFEHLRGAAPIAAALGHHHHH	190
2dbb/1-151	103	GRYNIIVRLLLPKDIKDAENLISEFLQRIKNAENVEVILISEVRKFEII.....	151

Figure S5. The amino acid sequences of TobR and the other Lrp/AsnC family transcription regulars. All sequences (PDB ID: 4PCQ, 2CFX, 2VBY, 2GQQ, 2P6S, 2DBB, 2IA0, 2CG4, 1I1G, 2E1C and 2YX4) were aligned by CLUSTALW. Blue depth indicates the percentage identity of conservation.

		10	20	30	40									
<i>TobR</i> /1-164	1	- - - - VPRDGKVL	DDV	DHRL	LALLQEDSGRTL	GALGELVGLSAS 39								
<i>Kribbella</i> /1-154	1	- - - - - - - - MTF	DEI	DHRL	LDTLQRDAS	RTLQALGEDVGLSAS 34								
<i>Nonomuraea</i> /1-163	1	- - - - MPKPIL	TLDDV	DHELL	DLQRD	SGRTLRELGD	LVALSPS 39							
<i>Streptoalloteichus</i> /1-164	1	- - - - MPRDGKVL	DEL	DHRL	LALLQEDSGRTL	GDLGEAVGLSAS 39								
<i>Actinokineospora</i> /1-157	1	- - - - - MTTVAL	DET	DHQL	LALLQRD	SARTLGELGELV	SLSPS 37							
<i>Streptoalloteichus</i> /1-163	1	MAQDYCV	TALRL	DET	DHLL	LALLQKDS	SRTLAE	LGEV	ISLSPS 43					
		50	60	70	80									
<i>TobR</i> /1-164	40	AVQRR	VERYRAS	GI	LARY	VAVLE	EP	RRGL	DVLL	AI	CLVTL	ERES 82		
<i>Kribbella</i> /1-154	35	AVQRR	INRYRSAG	VL	ARHV	VAVLE	EAR	TAD	I	V	LAVV	LVTL	ERES 77	
<i>Nonomuraea</i> /1-163	40	AVQRR	IDRYRK	HGV	LARH	VALL	DPS	R	LPT	ALL	AVCL	VTL	ERES 82	
<i>Streptoalloteichus</i> /1-164	40	AVQRR	VERYRAS	GI	LARC	VAVLE	EP	RHGL	DVLL	AVCL	VTL	ERES 82		
<i>Actinokineospora</i> /1-157	38	AVQRR	IDRYRK	AGL	IERH	VAVL	DPAQ	-	VDV	LL	AVCL	VTL	AKES 79	
<i>Streptoalloteichus</i> /1-163	44	AVQRR	VDRYRK	AGL	LL	DRQ	VAVL	DPVK	-	AD	ALL	AVCL	VTL	AKES 85
		90	100	110	120									
<i>TobR</i> /1-164	83	GHA	HEEF	RRRL	LAAP	EVQQL	YN	VSGDS	DYVV	V	LATTG	MAH	HRE 125	
<i>Kribbella</i> /1-154	78	SGH	HDAF	AQRL	LAVP	EVQQA	YD	VSGDW	YVV	V	LATIG	MAH	SE 120	
<i>Nonomuraea</i> /1-163	83	SRH	HHTF	RRRL	LAAP	EVQQL	YD	VSGDW	YVV	I	LACIG	MAH	HTA 125	
<i>Streptoalloteichus</i> /1-164	83	SHA	HEEF	RSRL	LAAP	EVQQL	YN	VAGDT	DYVV	V	LATTG	MAH	HRE 125	
<i>Actinokineospora</i> /1-157	80	RAL	HAAF	RRRL	LAAP	EVQQL	YS	VSGET	DF	VV	V	LATTG	MSH	RE 122
<i>Streptoalloteichus</i> /1-163	86	NAI	HAAF	RRRL	LAAP	EVQQL	YS	VSGDT	DYVV	V	LASTG	MAH	HRE 128	
		130	140	150	160									
<i>TobR</i> /1-164	126	VADR	LLKD	APNV	RRYST	LFV	LD	PVRT	GSAL	P	TRREGED	*	164	
<i>Kribbella</i> /1-154	121	LAQH	LFKD	APNV	KRYTT	MFV	LD	PLRT	GT	YL	PTR	* - - - -	154	
<i>Nonomuraea</i> /1-163	126	VADR	LFQE	APNV	RRYST	TLF	V	LD	PVRT	GCAL	P	TRTPDT	* -	163
<i>Streptoalloteichus</i> /1-164	126	VADR	LLKDS	PNV	LRYST	MFV	LD	PVRT	GSV	L	P	TRHAKED	*	164
<i>Actinokineospora</i> /1-157	123	VSE	RL	LKD	APNI	QRYST	MFV	LD	PVRT	GTAL	P	TRR	* - - - -	157
<i>Streptoalloteichus</i> /1-163	129	VADR	LLKD	APNI	QRYTT	MFV	LD	PVR	AGLS	L	P	TKR	* - - - -	163

Figure S6. The amino acid sequences of TobR and its homologous proteins. The homologous proteins were from *Streptoalloteichus hindustanus* (WP_073480789.1), *Actinokineospora alba* (WP_228769743.1), *Alloactinosynnema* sp. L-07 (CRK55752.1), *Nonomuraea* sp. KC401 (WP_138203008.1) and *Kribbella antibiotica* (WP_138203008.1) are 68.89%-88.27% similar. The alignment was analyzed by Clustal Omega. Blue depth indicates the percentage identity of conservation.

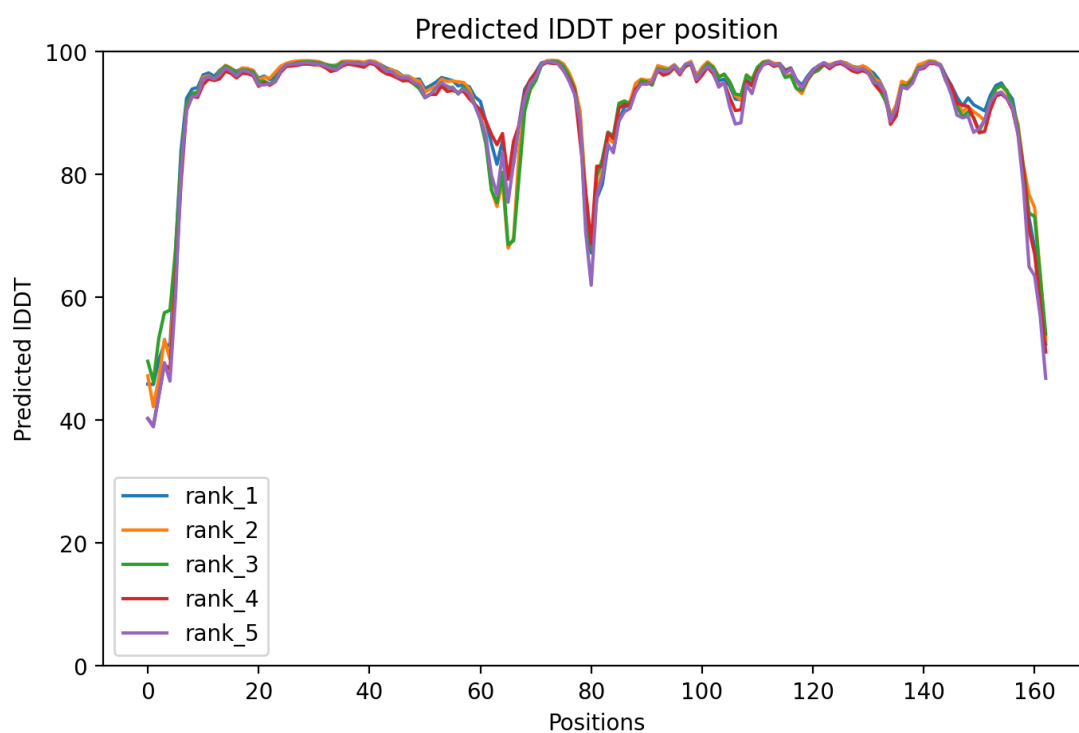


Figure S7. pLDDT score for the TobR structure predicted by AlphaFold 2.

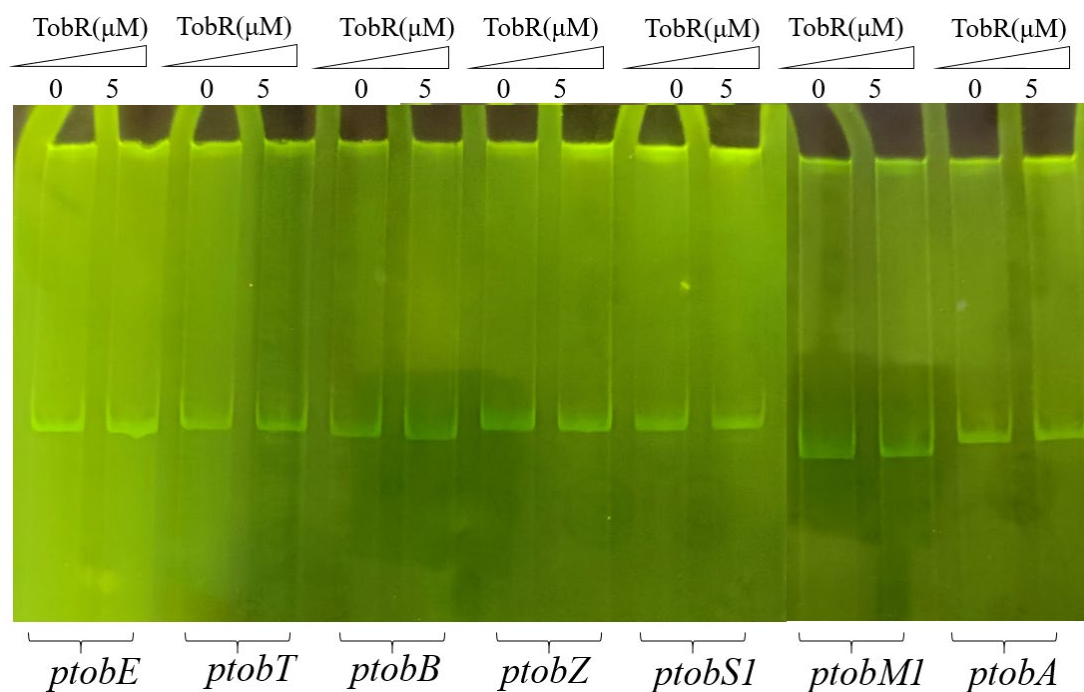


Figure S8. The regulatory targets of TobR on tobramycin biosynthesis gene cluster analyzed by EMSA. The protein concentrations in the experimental groups were 5 μ M.

Supplementary Tables

Table S1 Plasmids used in this study.

Plasmids	Relevant characteristic	Source
pOJ260	<i>E. coli</i> , cloning vector, containing pUC18 replicon, <i>oriT</i> , <i>aac3(IV)</i>	Changsha Yingrun Biotechnology Co.,Ltd (Hunan, China)
pSpc260	<i>E. coli</i> , cloning vector, containing pUC18 replicon, <i>oriT</i> , <i>spc</i> , derived from pOJ260	This study
pSpc260- $\Delta aprJ$	pSpc260 derivative for <i>aprJ</i> deletion	This study
pSpc260- $\Delta aprK$	pSpc260 derivative for <i>aprK</i> deletion	This study
pSpc260- $\Delta aprI$	pSpc260 derivative for <i>aprI</i> deletion	This study
pSpc260- $\Delta aprQ$	pSpc260 derivative for <i>aprQ</i> deletion	This study
pSpc260- $\Delta aprM$	pSpc260 derivative for <i>aprM</i> deletion	This study
pSpc260- $\Delta tobR$	pSpc260 derivative for <i>tobR</i> deletion	This study
pSpc260- $\Delta tobO$	pSpc260 derivative for <i>tobO</i> deletion	This study
pIJ8660	<i>aac3(IV)</i>	(Sun et al. 1999)
pIJ8660- <i>spc</i>	<i>spc</i> , originl, derived from pIJ8660	This study
pSpc8660	<i>spc</i> , <i>ermEp*</i> originl, derived from pIJ8660- <i>spc</i>	This study
pSpc8660- <i>tobR</i>	pSpc8660 derivative for expression of <i>tobR</i>	This study
pSpc8660- <i>tobO</i>	pSpc8660 derivative for expression of <i>tobO</i>	This study
pSpc- <i>kasOp*</i>	<i>spc</i> , <i>kasOp*</i> originl, derived from pSpc8660	This study
pSpc- <i>kasOp*-tobO</i>	pSpc- <i>kasOp*</i> derivative for expression of <i>tobO</i>	This study
pET28a(+)	<i>kan</i> , P _{T7} , His-tag, vector for protein expression	Sangon Biotech (Shanghai, China)
pET28a- <i>tobR</i>	pET28a(+) derivative carrying <i>tobR</i>	This study

Table S2 Primers used in this study.

Primers	Sequences (5'→3')
aprJ-F1	atgcgctccatcaagaagagcgatcggtgagggacatgacg
aprJ-R1	ccacgtcgccggggatgggcccgtggtcgt
aprJ-F2	gcccattccccggcgacgtggcggtggtact
aprJ-R2	tcctctagagtcgacctgcatccaccagactcggtggactc
aprK-F1	atgcgctccatcaagaagaggtgctcggcgaggtccacta
aprK-R1	aagtcgtctcgtgctggagtcggggaggtaa
aprK-F2	gactccagcacgagacgacttttggcgaagta
aprK-R2	tcctctagagtcgacctgcagctacgtgatcgtcctgctgaa
aprQ-F1	atgcgctccatcaagaagagccgggtcggtcacgtccatcg
aprQ-R1	gcggggcaggcacgccccagtggtcgtaggc
aprQ-F2	ctggggcgctgcctgccccgcgtggtgatggacta
aprQ-R2	tcctctagagtcgacctgcatggtctccggctcctcggg
aprI-F1	atgcgctccatcaagaagagcggggaagaacggtgatcattc
aprI-R1	tcgttgggccacggtcgtcctccagtcgtc
aprI-F2	ggacgaccgtggcccaacgagtgaaggactc
aprI-R2	tcctctagagtcgacctgcagtgatcagctccacgaagactg
aprM-F1	atgcgctccatcaagaagagccgcccgttcttcaccgagg
aprM-R1	ctcgtggccgcggtgcacgaggaactgggc
aprM-F2	tcgtgcaccgcgccacgagtcacgcagtc
aprM-R2	tcctctagagtcgacctgcatctaccggggccgcaaggtc
di-aprJ-F	cggccggttcgacgtgatct
di-aprJ-R	cacgtccaccacctgacgca
di-aprK-F	gacgtgatctacgcgaacctg
di-aprK-R	gttcgtcttcaccggcttc
di-aprQ-F	gacccggacagttgggcctacg
di-aprQ-R	cggggctgcggatgctcgtgta
di-aprI-F	tgaccgtgatcagcaacgag
di-aprI-R	gatcagcgctcgagcagtagc
di-aprM-F	ctgctggcgagctgttcct
di-aprM-R	cctggagtacggccacaccg
28a-V-F	caaagcccgaagggaagctgag
28a-V-R	gcgacccatttgctgtccac
28-tobR-F	gtggacagcaaatgggtcgcgtgcctcgtgacgggaaggt
28-tobR-R	cagcttccttcgggctttgtcagtcctccccctcacgcc
ptobO-F	agtgtcccacaaccggattc
ptobO-R	aggcagatcgccagcaggac
v1139-F	tgcaggtcgactctagaggatc
v1139-R	ctcttcttgatggagcgcattg
tobR-F1	atgcgctccatcaagaagaggggtcagcgaccggatccacg
tobR-R1	tccttcagcagccacctggggagcgggttat
tobR-F2	cccagggtggctgctgaaggacgcgccgaac
tobR-R2	tcctctagagtcgacctgcaaaccgacggctacaccttc

Primers	Sequences (5'→3')
di-tobR-F	cagcgggtggtggacgttct
di-tobR-R	ccgccgctgcaatgttcac
v152-F	atgtccgcctcctttggtcac
v152-R	tttgctattgggcgctctt
tobO-F1	atgcgctccatcaagaagagggtacggcgtgtcctccacg
tobO-R1	ccgcggacgctggctgcggcggttctactc
tobO-F2	accgccgcagccagcgtccgcggcgaaacc
tobO-R2	tcctctagagtcgacctgcaggaggcaccgttcggacacc
di-tobO-F	atctccgggaacgcgtggtg
di-tobO-R	tcctgcaacagggcgagcaa
ermE-tobR-F	tgaccaaaggaggcggacatgtgcctcgtgacgggaaggt
ermE-tobR-R	aagagcgcccaatacgcaaactcagtcctccccctcacgc
ptobE-F	gtactcccccccttcgag
ptobE-R	gctgccgcagatcgagttgt
ptobT-F	cgtgaaggcgttgacgatcag
ptobT-R	gctcgtgcagcccgaagaac
ptobB-F	ctcggtcggcttctacctggc
ptobB-R	ggtgaggtcgaggtaggcgtt
ptobZ-F	ggttggccgtgtgtccgag
ptobZ-R	ttccgtgtgaaccgctctc
ptobS1-F	gcacgaggactggtccacc
ptobS1-R	ccgttggtgatcgcgaggtg
ptobM1-F	ggcggaccgaggacatcca
ptobM1-R	cgggacagccgcaggatct
ptobA-F	gcgtactggaagaccgcgac
ptobA-R	tccggtgtggacgtggtcaa
ptobO-F	agtgtcccacaaccggattc
ptobO-R	aggcagatcgccagcaggac
ermE-tobO-F	tgaccaaaggaggcggacatggaccgacagtggaggaaat
ermE-tobO-R	aagagcgcccaatacgcaaactcaccacaggcctcgat
V-kasOp-tobO-F	atttctccactgtcggtccaaactccccagtcctgcac
tobO-F	ggaccgacagtggaggaaat
qp-gapA-F	gccaacgaggagaagtacga
qp-gapA-R	ggttctggctcctgcgtgta
qp-tobO-F	cctgtggttcctcagcaacta
qp-tobO-R	cgtggtggagaaggtgtgt
qp-tobB-F	ccgaccaggcgttgttcta
qp-tobB-R	cgtggaactccgtggcata
qp-tobE-F	tggcacggcttcagttacc
qp-tobE-R	tcctggagggttctcgagag
qp-tobM1-F	ctggctgaccgacctctc
qp-tobM1-R	ctattctgaggcgaggac
qp-tobS1-F	cgcattggaactggtggaga

Primers	Sequences (5'→3')
qp-tobS1-R	gaggagggtgttgagcagtt
qp-tobT-F	tgagccgtccacagagga
qp-tobT-R	tgggcaacaggatgtccag
qp-tobZ-F	agcgggtcacacggaagaa
qp-tobZ-R	tgggcaacaggatgtccag

Table S3: Analysis of the gene products of the tobramycin gene cluster of *Streptoalloteichus tenebrarius* Tb. The table presents the results of BlastP analysis (Altschul et al. 1990).

Gene	Protein	Length (bp)	Predicted function	BlastP-Hits (Accession, percent identity)
<i>tobE</i>	TobE	1020	2-deoxy-scylo-inosamine dehydrogenase	WP_253672087.1 (99.71)
<i>tobT</i>	TobT	1311	MFS transporter	WP_253672088.1 (99.77)
<i>tobB</i>	TobB	1188	glutamate-1-semialdehyde 2, 1-aminomutase	WP_253672089.1 (100%)
<i>tobQ</i>	TobQ	1527	paromamine 6'-oxidase	WP_253672090.1 (100%)
<i>tobZ</i>	TobZ	1713	carbamoyltransferase	WP_253672091.1 (100%)
<i>tobS1</i>	TobS1	1381	DegT/DnrJ/EryC1/StrS family aminotransferase	WP_253672092.1 (100%)
<i>tobC</i>	TobC	1161	2-deoxy-scylo-inosose synthase	WP_253672093.1 (100%)
<i>tobD2</i>	TobD2	1044	Gfo/Idh/MocA family oxidoreductase	WP_253672094.1 (100%)
<i>tobM2</i>	TobM2	1263	putative 6-glucosyltransferase	WP_253672095.1 (100%)
<i>tobN</i>	TobN	786	PIG-L family deacetylase	WP_253672096.1 (100%)
<i>tobS2</i>	TobS2	1251	DegT/DnrJ/EryC1/StrS family aminotransferase	WP_253672097.1 (99.74%)
<i>orf16</i>	Orf16	372	Unknown	WP_253672098.1 (100%)
<i>tobM1</i>	TobM1	1266	glycosyltransferase involved in cell wall bisynthesis	MCP2261261.1 (100%)
<i>tobL</i>	TobL	1233	ATP-grasp domain-containing protein	WP_253672100.1 (99.76%)
<i>tobU</i>	TobU	981	DMT family transporter	WP_253672101.1 (100%)
<i>tobA</i>	TobA	1119	PLP-dependent aminotransferase	WP_253672102.1 (100%)
<i>tobD3</i>	TobD3	1077	alpha-hydroxy-acid oxidizing protein	WP_253672103.1 (100%)
<i>tobO</i>	TobO	984	TauD/TfdA family dioxygenase	WP_253672104.1 (100%)
<i>tobR</i>	TobR	492	Lrp/AsnC family transcriptional regulator	WP_253672105.1 (100%)

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic Local Alignment Search Tool. *J Mol Biol* 215(3):403-410 doi:DOI 10.1006/jmbi.1990.9999
- Sun JH, Kelemen GH, Fernández-Abalos JM, Bibb MJ (1999) Green fluorescent protein as a reporter for spatial and temporal gene expression in *A3(2)*. *Microbiol-Uk* 145:2221-2227 doi:Doi 10.1099/00221287-145-9-2221