

Supplementary Material

Transcriptional regulator DasR represses daptomycin production through both direct and cascade mechanisms in *Streptomyces roseosporus*

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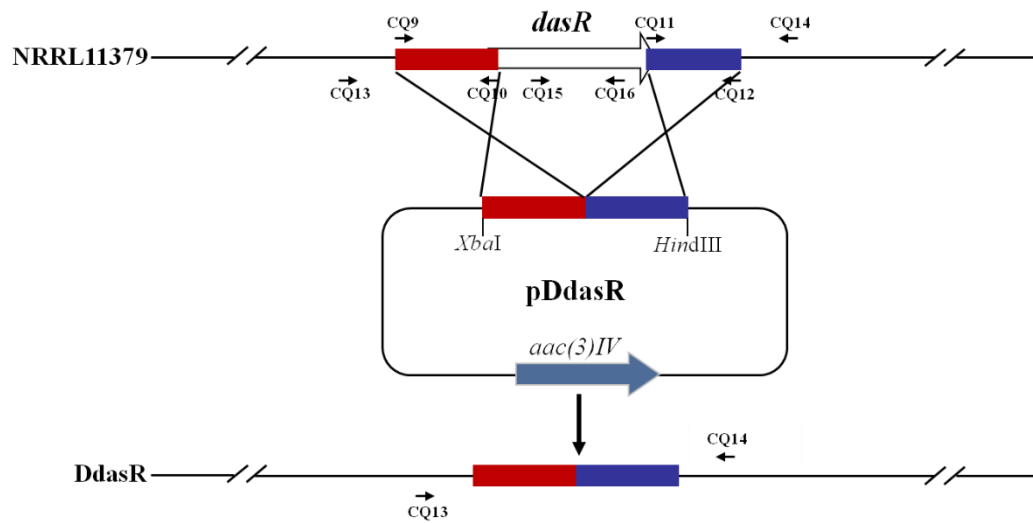
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Figure S1.

A



B

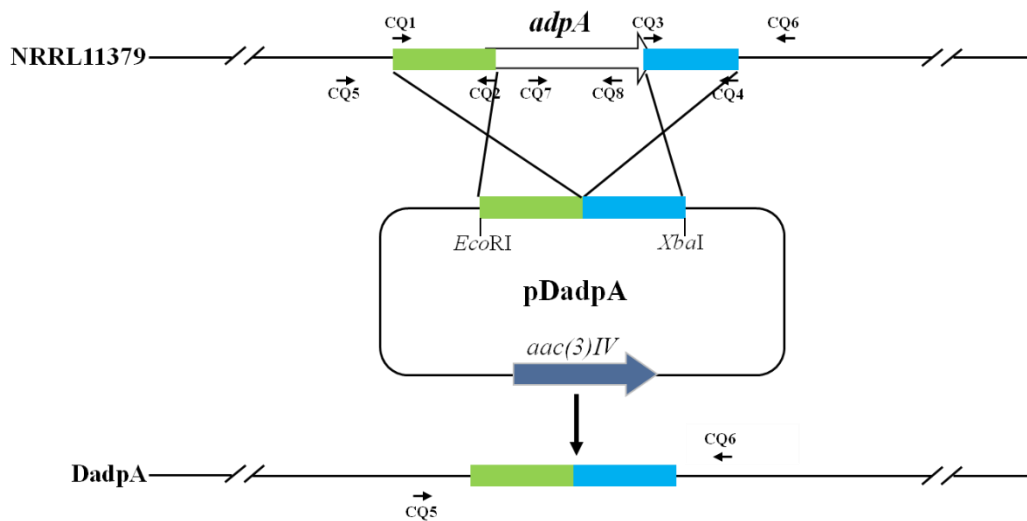


Figure S1. Strategy for deletion of *dasR* (A) and *adpA* (B) in WT (schematic). Large arrows: genes and their directions. Small arrows: positions of primers. Rectangles: homologous exchange regions used for gene deletion.

A

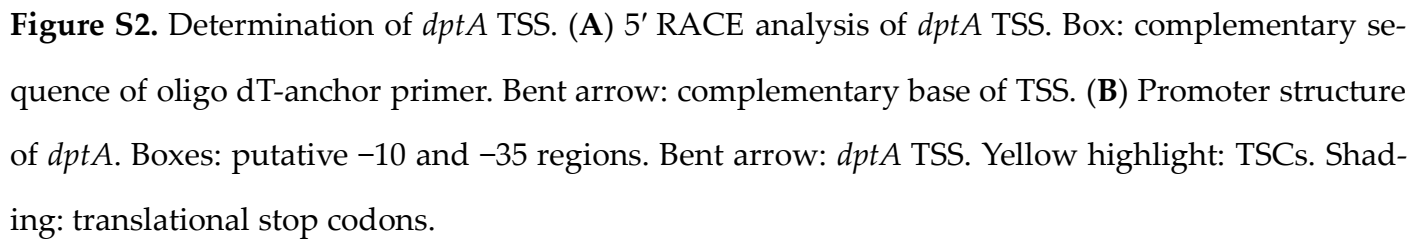


Figure S3.

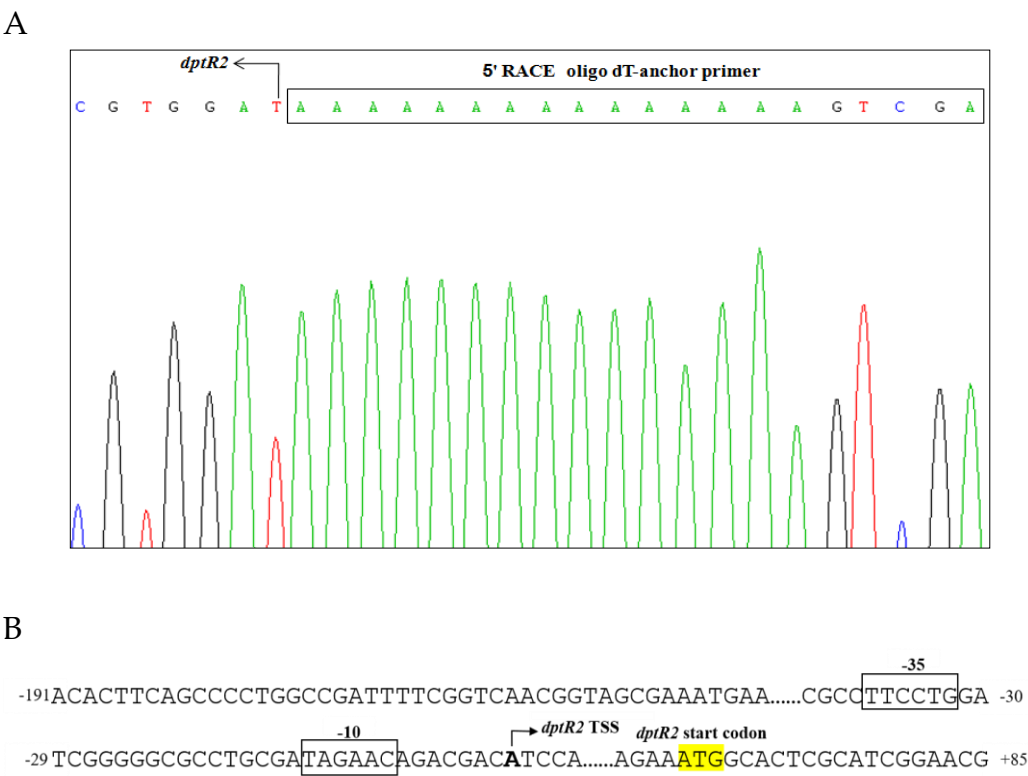


Figure S3. Determination of *dptR2* TSS. **(A)** 5' RACE analysis of *dptR2* TSS. Box: complementary sequence of oligo dT-anchor primer. Bent arrow: complementary base of TSS. **(B)** Promoter structure of *dptR2*. Boxes: putative -10 and -35 regions. Bent arrow: *dptR2* TSS. Yellow highlight: *dptR2* TSC.

Table S1. Strains and plasmids used in this study.

Strains or plas- mids	Description	Source or reference
Strains		
<i>S. roseosporus</i>		
NRRL11379	WT strain, daptomycin producer	Laboratory stock
DdasR	<i>dasR</i> deletion mutant	This study
CdasR	<i>dasR</i> complemented strain	This study
OdasR	<i>dasR</i> overexpression strain	This study
DadpA	<i>adpA</i> deletion mutant	This study
CadpA	<i>adpA</i> complemented strain	This study
OadpA	<i>adpA</i> overexpression strain	This study
WT/pKC1139	WT strain carrying empty vector pKC1139	This study
WT/pSET152	WT strain carrying empty vector pSET152	This study
<i>E. coli</i>		
JM109	General cloning host	Laboratory stock
ET12567	Methylation-deficient strain	[1]
BL21 (DE3)	Host for protein overexpression	Novagen
Plasmids		
pKC1139	Multiple-copy <i>E. coli-Streptomyces</i> shuttle vector	[2]
pSET152	Integrative <i>E. coli-Streptomyces</i> shuttle vector	[2]
pET-28a (+)	Vector for His ₆ -tagged protein overexpression in <i>E. coli</i>	Novagen
pJL117	pIJ2925 derivative carrying promoter <i>ermE</i> * <i>p</i>	[3]
pDdasR	<i>dasR</i> deletion vector based on pKC1139	This study
pCdasR	<i>dasR</i> complemented vector based on pSET152	This study
pOdasR	<i>dasR</i> overexpression vector based on pKC1139	This study
pET28-dasR	His ₆ -DasR overexpression vector based on pET-28a (+)	This study
pDadpA	<i>adpA</i> deletion vector based on pKC1139	This study
pCadpA	<i>adpA</i> complemented vector based on pSET152	This study
pOadpA	<i>adpA</i> overexpression vector based on pKC1139	This study
pET28-adpA	His ₆ -AdpA overexpression vector based on pET-28a (+)	This study

Table S2. Primers used in this study.

Primer	DNA sequence * (5'-3')	Purpose
CQ9	GCTCTAGATTTCCTTGC GGTCCTTCGG (<i>Xba</i> I)	Deletion of <i>dasR</i> gene
CQ10	CTTCAGACGCGCCACGAAGGTCTCGCTCCCCGTACT	
CQ11	AGTACGGGGAGCGAGACCTTCGTGGCGCGTCTGAAG	
CQ12	CCCAAGCTTCCCGTCGGTCGGTGGGAT (<i>Hind</i> III)	Confirmation of <i>dasR</i> deletion in mutant DdasR
CQ13	CGAGGGAGGTGTCCAGCTTC	
CQ14	CGTCGCGCGCCATCTGAC	
CQ15	ACCTTCGTCGCCAAGCCG	
CQ16	GGGACAGCATGAGCATCGG	
CQ21	CCCAAGCTTACGCAAGCGTCGGGAAGG (<i>Hind</i> III)	Overexpression of <i>dasR</i> in <i>S. roseosporus</i>
CQ22	GCTCTAGACGGTTCACGTGATCCTGC (<i>Xba</i> I)	
CQ75	CGGAATTC CCCACCGCGAAGACATTC (<i>Eco</i> RI)	Complementation of <i>dasR</i> in DdasR
CQ76	GCTCTAGAGTTCACGTGATCCTGCTCC (<i>Xba</i> I)	
CQ25	CGGAATTCAGTACGGGGAGCGAGACC (<i>Eco</i> RI)	
CQ26	CCCTCGAGCGGTTACGTGATCCTGC (<i>Xho</i> I)	Overexpression of His ₆ -DasR in <i>E. coli</i>
CQ1	CGGAATTC TGACCCCTTCGCGGGATG (<i>Eco</i> RI)	
CQ2	GGAACGAACCGGTCAGGATGAACAGCAGCACGGCAA	
CQ3	TTGCCGTGCTGCTGTTTCATCCTGACGCGTTCGTTCC	Deletion of <i>adpA</i> gene
CQ4	GCTCTAGAGTCACGGATGTCCGCCAG (<i>Xba</i> I)	
CQ5	ACACGCCATGAGGCCGC	
CQ6	CACAACCAGGTGCTCTGCC	Confirmation of <i>adpA</i> deletion in mutant DadpA
CQ7	GAACTCACCACGCCGTACG	
CQ8	GCACATCGCCGTCGTCCA	
CQ17	CCAAGCTTCACCGAGGGGGGCTTAGC (<i>Hind</i> III)	Overexpression of <i>adpA</i> in <i>S. roseosporus</i>
CQ18	GCTCTAGAGCGGGCGGATCACGATGT (<i>Xba</i> I)	
CQ19	CGGAATTC TGACCCCTTCGCGGGATG (<i>Eco</i> RI)	
CQ20	GCTCTAGAGCGGGCGGATCACGATGT (<i>Xba</i> I)	Complementation of <i>adpA</i> in DadpA
CQ23	CGGAATTCATGAGCCAGGACTCCGCC (<i>Eco</i> RI)	
CQ24	CCCTCGAGCGCAGTCGATCCACACCAT (<i>Xho</i> I)	
CQ27	GCTTAGCGTCATGAGCCAG	Overexpression of His ₆ -AdpA in <i>E. coli</i>
CQ28	TGAACAGCAGCACGGCAA	
ZQL75	TCCCGGGAATCGCGAGAG	
ZQL76	CCCGGATTCCCCCGACTC	Amplification of <i>dptR3</i> ORF for RT-qPCR
ZQL79	GAACAGACCAACCCTCCTCG	
ZQL80	CTGTGGCCGATGGGGTAG	
ZQL81	CGGCGTACATCATCCAGACC	Amplification of <i>dptBC</i> ORF for RT-qPCR
ZQL82	GTCATGCTCAGTTCGGAGACG	
ZQL83	GAGTGAGAGCCGCTGTGC	
ZQL84	GGTGTCCCGTACGAGAACC	Amplification of <i>dptA</i> ORF for RT-qPCR
ZQL85	CCAGATCCTCTCGACGGTG	
ZQL86	CCGATACAGGCGCGTACC	
ZQL87	TTCCGGTACGAGCGGCTG	Amplification of <i>dptE</i> ORF for RT-qPCR
ZQL88	CGTCAGATCGAAGCGGCG	
ZQL89	TCCGCACCATCACGTTTAC	
ZQL90	GACTTCCTGGGCCACCTG	Amplification of <i>dptD</i> ORF for RT-qPCR

ZQL93	GGCCAGGATCGTGACGTC	Amplification of <i>dptR2</i> ORF
ZQL94	CGGAACGGCAGGAGTTCATC	for RT-qPCR
ZQL115	GACAGATCGCCGAGTTCGT	Amplification of <i>dptF</i> ORF for
ZQL116	GACGCTCCACAGCAGCTC	RT-qPCR
ZQL117	CTACCACGTGACCGTCAAGG	Amplification of <i>dptI</i> ORF for
ZQL118	GTCGTCGAACTCGTTTCCCTC	RT-qPCR
ZQL119	CAACGACGGCAGCTACCTC	Amplification of <i>dptG</i> ORF for
ZQL120	GGCGCATATCGGTCCAGTTC	RT-qPCR
ZQL121	CAATCCGCCGTACCAGGC	Amplification of <i>dptP</i> ORF for
ZQL122	GGACACGCCCCGTGTTGG	RT-qPCR
GJ91	CCAAGGGCTACAAGTTCTCC	Amplification of <i>hrdB</i> ORF
GJ92	TTGATGACCTCGACCATGTG	for RT-qPCR and probe <i>hrdB</i>
		for EMSA
CQ49	ATGGCCGGACAACCCACC	Amplification of probe <i>adpAp</i>
CQ50	CTGGCTCATGACGCTAAGC	for EMSA
ZQL39	GGCGTGGAACATACTGGCG	Amplification of probe <i>dptEp</i>
ZQL40	GCACAGCGGCTCTCACTC	for EMSA
ZQL43	CGAGGTCACCGCGTGGAT	Amplification of probe <i>dptAp</i>
ZQL44	GTCCATCTCTCGCGGATCC	for EMSA
ZQL51	CCGTTCCGATGCGAGTGC	Amplification of probe <i>dptR2p</i>
ZQL52	CGTGCAGGAAGGTGTTCCG	for EMSA
ZQL37	TCCCGGGAATCGCGAGAG	Amplification of probe <i>dptR3p</i>
ZQL38	GAAGAGGCGGAGGATGCG	for EMSA
ZQL47	GCGCGGTCAACAAGATTCTT	Amplification of probe <i>dptIp</i>
ZQL48	CCTTGACGGTCACGTGGTAG	for EMSA
ZQL57	CGGTCCGAACCGGCTCTTG	Amplification of probe <i>dptP-M</i>
ZQL58	AGGCGCTGCGGATCGATG	for EMSA
CQ61	ACCCGCCTTCCCTGACCC, 5' FAM	Amplification of <i>adpAp</i> for
CQ62	CCTGGCTCATGACGCTAAGC	DNase I footprinting
CQ63	CGCACCGGACGCCCTGAC, 5' FAM	Amplification of <i>dptEp</i> for
CQ64	GGCACAGCGGCTCTCACTC	DNase I footprinting
CQ65	CCGTTCCGATGCGAGTGC	Amplification of <i>dptR2p</i> for
CQ66	AACAGCCGGTGAGTGTGC, 5' FAM	DNase I footprinting
CQ67	CGAGGTCACCGCGTGGAT, 5' FAM	Amplification of <i>dptAp</i> for
CQ68	GGTTTCGAGGATCTGCAGCAG	DNase I footprinting
Oligo	GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTTV	For 5' RACE
dT-anchor		
primer		
Anchor	GACCACGCGTATCGATGTCGAC	For 5' RACE
primer		
CQ72	GGGGACGCTCCACAGCAG	dptA-SP1 for 5' RACE
CQ73	CGCAGAGGGCAACTCCGG	dptA-SP2 for 5' RACE
CQ74	GATCCGGTCGGGTGTCTC	dptA-SP3 for 5' RACE
ZQL69	GACGTTTCAGCGAGCCCAG	dptR2-SP1 for 5' RACE
ZQL70	GCGTCGTAGGCGGAAGCG	dptR2-SP2 for 5' RACE
ZQL71	CGCGATAGGGGAGGGTGACG	dptR2-SP3 for 5' RACE

* Underlining: sequence of restriction enzyme shown in parentheses.

References

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3. Li, L.; Guo, J.; Wen, Y.; Chen, Z.; Song, Y.; Li, J. Overexpression of ribosome recycling factor causes increased production of avermectin in *Streptomyces avermitilis* strains. *J. Ind. Microbiol. Biotechnol.* **2010**, *37*, 673-679.