Redox-sensing regulator Rex regulates aerobic metabolism, morphological differentiation, and avermectin production in *Streptomyces avermitilis*

Xingchao Liu, Yaqing Cheng, Mengya Lyu, Ying Wen, Yuan Song, Zhi Chen, & Jilun Li

State Key Laboratory of Agro-biotechnology and MOA Key Laboratory of Soil Microbiology, College of Biological Sciences, China Agricultural University, Beijing 100193, China. Correspondence and requests for materials should be addressed to Z.C. (email: chenzhi@cau.edu.cn)

Supplementary Table 1. Primers used in this study

Purpose	Primer	Sequence (5'--3')[a]
For construction of Drex mutant		
	rex-up-Fw	CCCAAGCTTCATCACCCAGGACGCGGAG (*Hin*dIII)
	rex-up-Rev	CGCGGATCCCTCGGAGGTACAGCGGAAGC (*Bam*HI)
	rex-dw-Fw	CGCGGATCCTCACCTCCATCCTGAACTTCG (*Bam*HI)
	rex-dw-Rev	CCGGAATTCCCCTCGCCCACGACCATC (*Eco*RI)
	rex-V-Fw	GGTGAGAATGGCCGACATGAGT
	rex-V-Rev	AACAGGTCGTTGAGGAGCCGT
	rex-V2-Fw	TGTGGGCTACGACGTCGAG
	rex-V2-Rev	AGCTCGTCCGTGTGCTGG
For complementation of Drex mutant		
	rex-E-Fw	CGGAATTCGGAACGAAGGCGCCTGTGT (*Eco*RI)
	rex-E-Rev	GCTCTAGACTGGAGCAGCTTGACCTGG (*Xba*I)
For overexpression of *wblE*		
	wblE-E-Fw	CGGAATTCGAGTCTCTACGTGGCGATCG (*Eco*RI)
	wblE-E-Rev	GCTCTAGACCAAGCTCAGGCTCGTTGC (*Xba*I)
For construction of His-tagged Rex		
	His-rex-Fw	CATGCCATGGCAACTGGCCGAACTCAC(*Nco*I)
	His-rex-Rev	CCCAAGCTTGAAGGCGAGGATCTGGAGCTC (*Hin*dIII)
For real-time RT-PCR		
	hrdB-QP-Fw	TACTGCGCAGCCTCAACCAG
	hrdB-QP-Rev	GCCGATCTGCTTGAGGTAGTC
	hemA-QP-Fw	TCTCTACGTGCACTACGAGG
	hemA-QP- Rev	GCGCGTCCTTGATCTGGC
	atpI-QP-Fw	CCAAGTGCTTCGCCCTGAC
	atpI -QP-Rev	GAGTCCGGCTCCACGTAGAGG
	cydA1-QP-Fw	CTCACGCAGGTCTTCCACAC
	cydA1-QP-Rev	AGCGAGGTCTTCATCACGGG
	nuoA1-QP-Fw	GGAGATGCTGCTCTTCGTGC
	nuoA1-QP-Rev	TCAGTCCCATTCCAGACCGC
	wblE-QP-Fw	ATGGACTGGCGTCACAACG
	wblE-QP-Rev	GGCTTCCTCGATCTGCAGC
	aveR-QP-Fw	CAGAAGAACTCACGCTCGTC
	aveR-QP-Rev	ACTCTTTCCACAGCCCATTC
	aveA1-QP-Fw	CGGACAGGACTACGCACTTC
	aveA1-QP-Rev	ACGAGATACGACCGGAGATG
	aveD-QP-Fw	GGACTACTACGACCGTTTGACC
	aveD-QP-Rev	CTCAGCTTGCCGATGAGGAG

Purpose	Primer	Sequence (5'--3')[a]
	olmRI- QP-Fw	GAGAGAAGGCACACGAGGTC
	olmRI- QP-Rev	ATGTCGAGTAAGCGGGAGAG
	olmRII- QP-Fw	GGAGGAACTCAGCCTCGACT
	olmRII- QP-Rev	ATCTCCCAGCCGATCTTCAC
For EMSA and ChIP assay		
	rexp-Fw	GAGTAGTCCAAACAGCCCGG
	rexp-Rev	CAGTTGCCACGGTGCTCCT
	atpIp-Fw	CGGTGTCATCTCGCTGAGC
	atpIp-Rev	GGTGGGCAGAGCACAGTG
	cydA1p-Fw	CGTTGTCCAGGTGTTCTACGC
	cydA1p-Rev	GGTGGTGATGCCGAACTGC
	nuoA1p-Fw	GCTTCATGATCCTTCGCCAG
	nuoA1p-Rev	TTCACGCTCCTCGCTCCTC
	wblEp-Fw	GAGTCTCTACGTGGCGATCG
	wblEp-Rev	TCCATGGCTGCTACCTCTCC
	aveRp-Fw	GATGGCCTTCTCCTCCGG
	aveRp-Rev	CGTGAGTTCTTCTGGTTTCCG
	aveD-A1p-Fw	CCGTCCATCCTCTGCACCTG
	aveD-A1p-Rev	GCTGTCGTGGCGATCTACTCC
	olmRIp-Fw	CCGCACCCTCCCGACCTG
	olmRIp-Rev	GACCTCGCCCAATCAGCACC
	olmRIIp-Fw	AATTGCTGGCCGAAGCCC
	olmRIIp-Rev	CGAGGACCACACCGCCTT
	amfRp-Fw	GGTCAAGGCTCCCTCCTG
	amfRp-Rev	CATTGTTGGCGGATGCGAC
	amfTp-Fw	GAGCGATTTGAGGGCAAGTT
	amfTp-Rev	GTCGGGCGTCTCGTAGAAG
	bldMp-Fw	CGGGAAGGCGAGAGAAATCT
	bldMp-Rev	CAGACGAGGACGGATGTCA
For footprinting assay		
	rexp-FAM-Fw	GAGTAGTCCAAACAGCCCGG
	rexp-Rev	CAGTTGCCACGGTGCTCCT
	aveRp-FAM-Fw	CCTCTGGACCCTTGCTCG
	aveRp-FAM-Rev	CGTGAGTTCTTCTGGTTTCCG
	aveD-A1p-FAM-Fw	CCGTCCATCCTCTGCACCTG
	aveD-A1p-Rev	GCTGTCGTGGCGATCTACTCC
	olmRIp-FAM-Fw	CCGCACCCTCCCGACCTG
	olmRIp-Rev	GACCTCGCCCAATCAGCACC
	cydA1p-FAM-Fw	CGTTGTCCAGGTGTTCTACGC
	cydA1p-Rev	GGTGGTGATGCCGAACTGC
	atpIp-FAM-Fw	AGTCCGGGCAGGGTTTCTAC
	atpIp-Rev	GGTGGGCAGAGCACAGTG
	nuoA1p-FAM-Fw	CTCGGATGCTTCGCGTATGA

Purpose	Primer	Sequence (5'--3')[a]
For 5'RACE assay	nuoA1p-Rev	TTCACGCTCCTCGCTCCTC
	oligo (dT)-anchor primer	GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTT
	sp1-rex	GATCACAACCGGCCAGTCCT
	sp2-rex	ACGAGAAGTCCTTGCGCAGC
	sp1-aveD	GTGATGCCTCTCAGCTTGCCG
	sp2-aveD	TGCGGCCAGTATCCGAGGTG
	sp1-aveA1	GATCATGACGGCGAACAGGGT
	sp2-aveA1	AGTTCCCTTCCCATGCCCGG
	sp1-olmRI	TCGTCCGGGAAAGGAGCCC
	sp2-olmRI	CAGGATCTCGCTCTTGCCGC

[a]The restriction enzyme sites are underlined.

Fig. S1

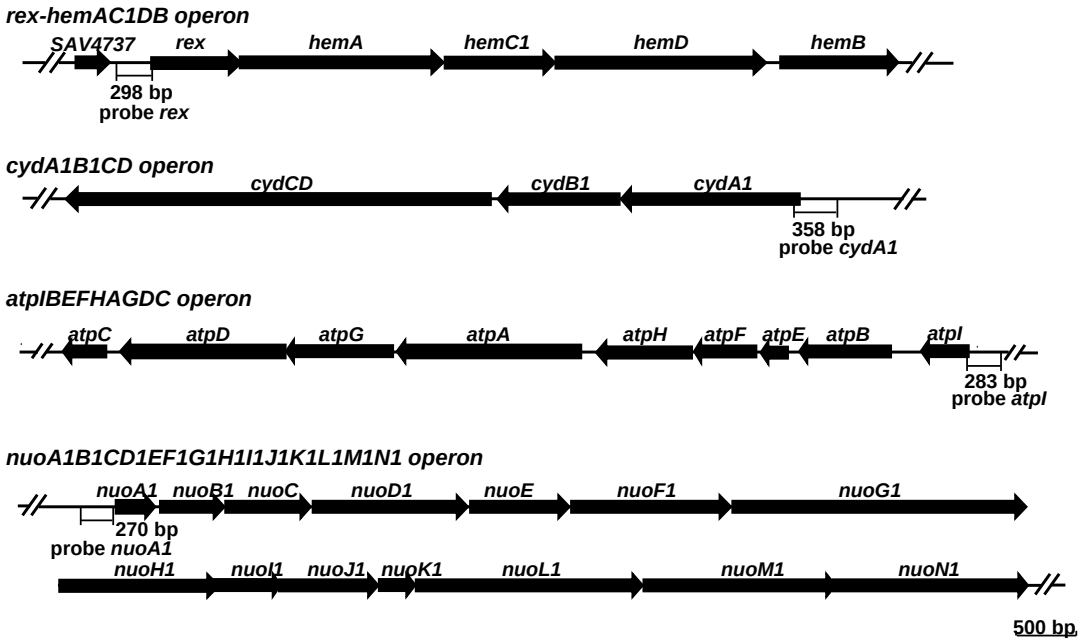

Fig. S1. Organization of *rex-hemAC1DB, cydA1B1CD, atpIBEFHAGDC,* and *nuoA1-N1* operons in *S. avermitilis*. Gene notations are based on the Genome Project of *S. avermitilis* (http://www.ls.kitasato-u.ac.jp/). Lengths and positions of probes used for EMSAs are shown.

Fig. S2

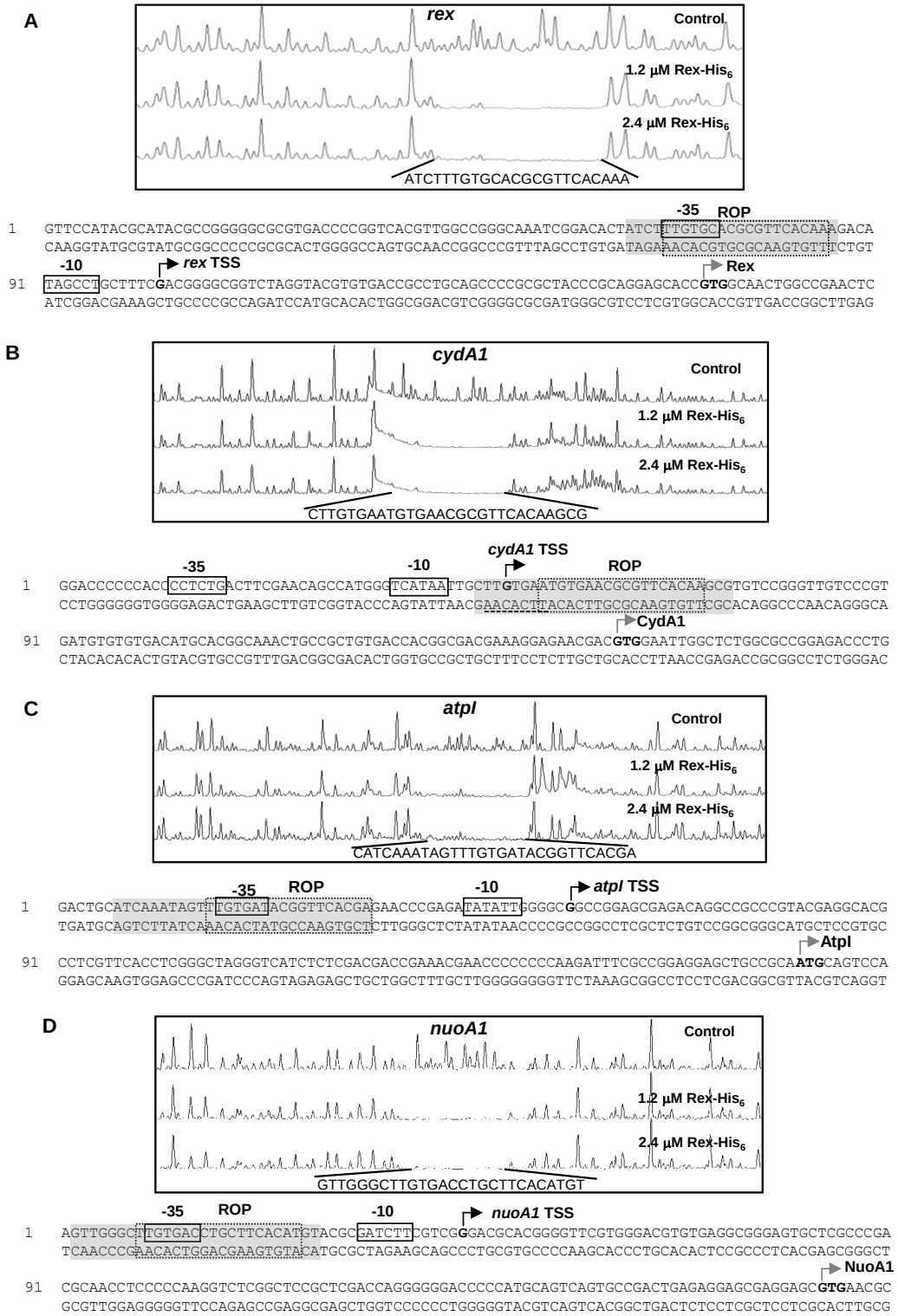

Fig. S2. Determination of Rex binding sites on promoter regions of *rex* (A), *cydA1* (B), *atpI* (C), and *nuoA1* (D) by DNase I footprinting assay. Fluorograms correspond to control DNA fragment and to protected reactions (with 1.2 and 2.4 μM Rex-His$_6$). Nucleotide sequences of *rex*, *cydA1*, *atpI*, and *nuoA1* promoter regions are shown below fluorograms. Notations as in Fig. 7.

Fig. S3

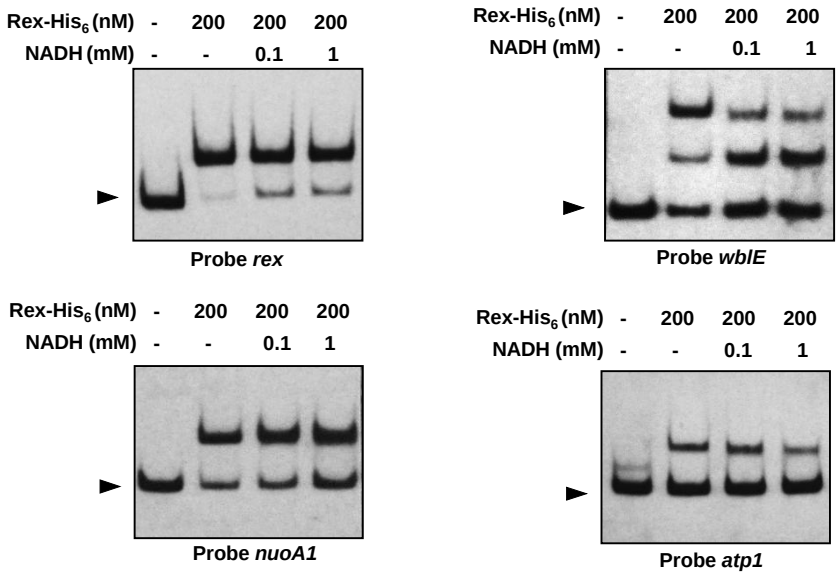

Fig. S3. DNA-binding activity of Rex is inhibited by NADH. EMSAs of promoter regions of *rex*, *wblE*, *nuoA1*, and *atpI* using Rex-His_6 and NADH. Arrow: free probe.

Fig. S4

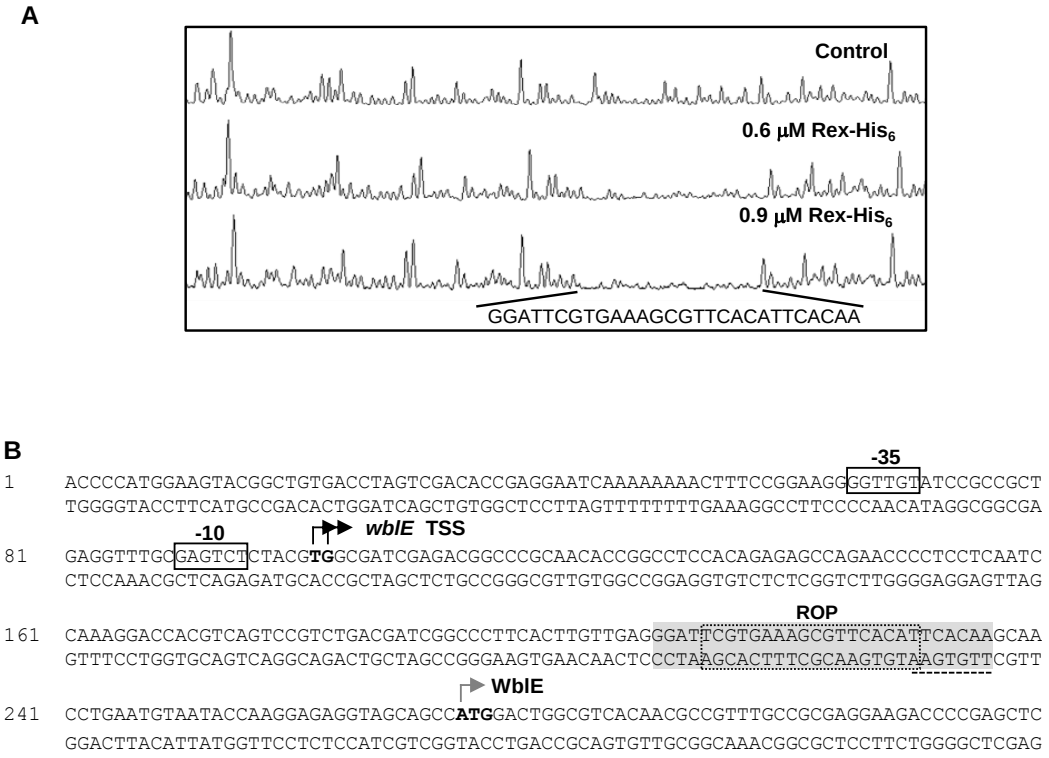

Fig. S4. Determination of Rex binding site on *wblE* promoter region by DNase I footprinting assay. (**A**) Fluorograms correspond to control DNA fragment and to protected reactions (with 0.6 and 0.9 μM Rex-His_6). (**B**) Nucleotide sequence of *wblE* promoter region. Notations as in Fig. 7.

Fig. S5

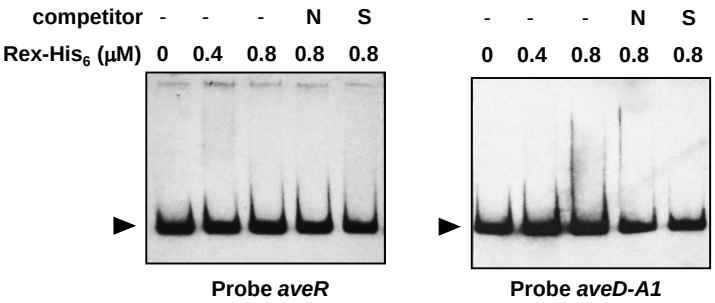

Fig. S5. Binding of Rex-His$_6$ to *aveR* promoter region and to *aveD-aveA1* intergenic region. Arrow: free probe. Specificity of band shifts was verified by adding 200-fold excess of unlabeled non-specific competitor DNA (N) and unlabeled specific probe (S).