

## Supplementary Materials

**SufR, a [4Fe-4S] cluster-containing transcription factor, represses the *sufRBDCSU* operon in the iron-sulfur cluster assembly in *Streptomyces avermitilis***

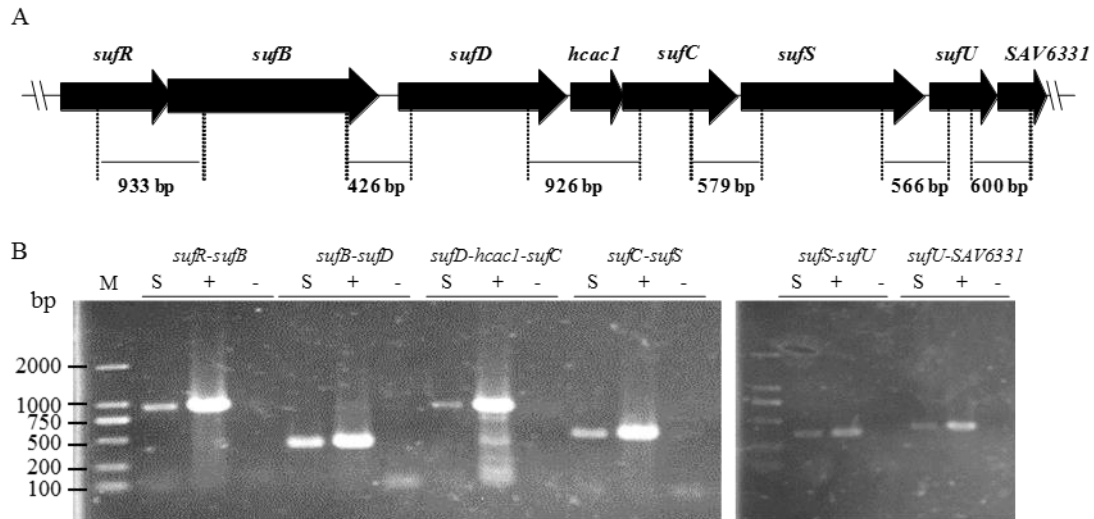
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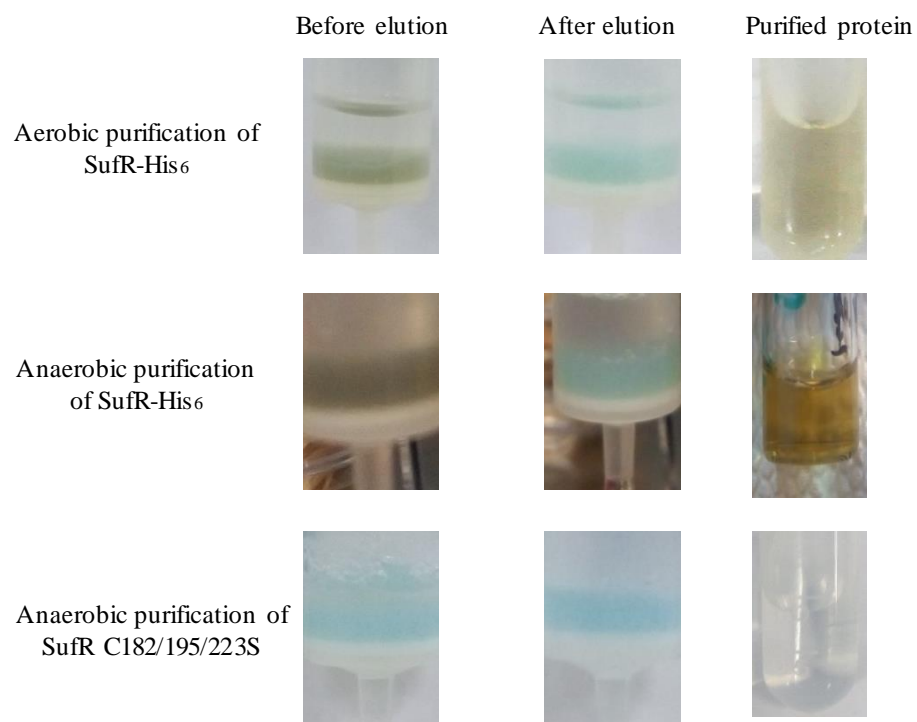
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Running title: Characterization of SufR in *Streptomyces avermitilis*

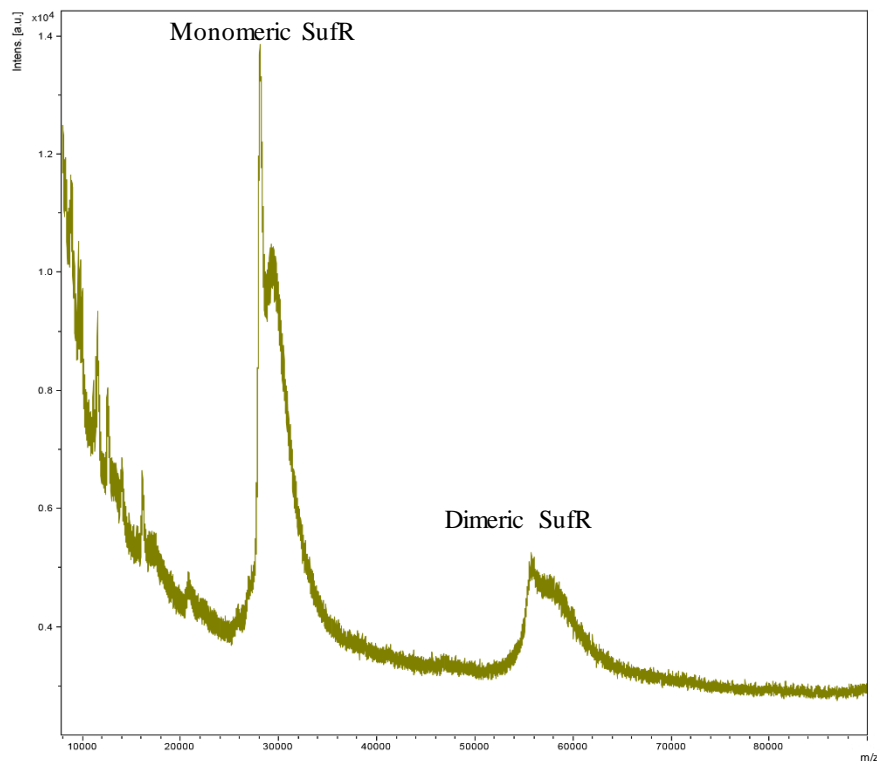
Keywords: Fe-S cluster homeostasis, SufR, [4Fe-4S] cluster, *suf* operon, *S. avermitilis*



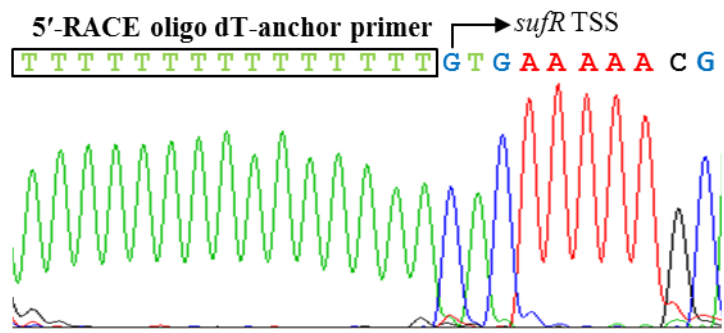
**Figure S1 RT-PCR analysis of the *suf* operon in *S. avermitilis*.** (A) The gene organization of the *suf* operon. The position of the primers used for PCR amplification and the lengths of DNA fragments are shown. (B) PCR amplification of the *suf* genes. The cDNA obtained by reverse transcription of WT RNA was used as template in lanes S. Lanes + are positive controls using the WT DNA as template. Lanes - are the negative controls using water as template. Lane M, 1-kb DNA marker.



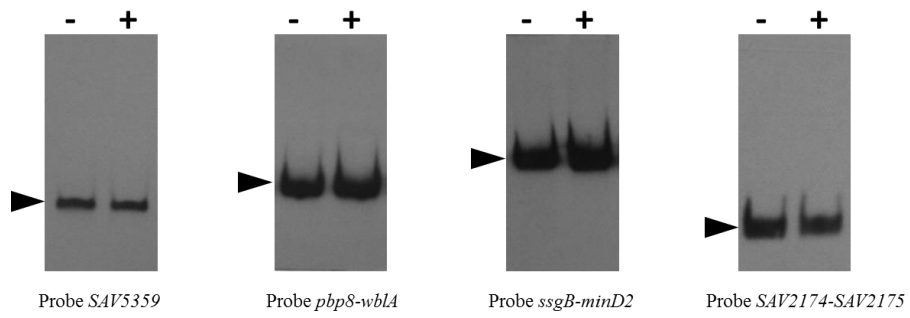
**Figure S2 The purification of SufR-His<sub>6</sub> and SufR C182/195/223S proteins under anaerobic or aerobic conditions.**



**Figure S3 MALDI-TOF analysis of anaerobically purified SufR-His<sub>6</sub>.** The observed mass peak is equal to the predicted mass of SufR.



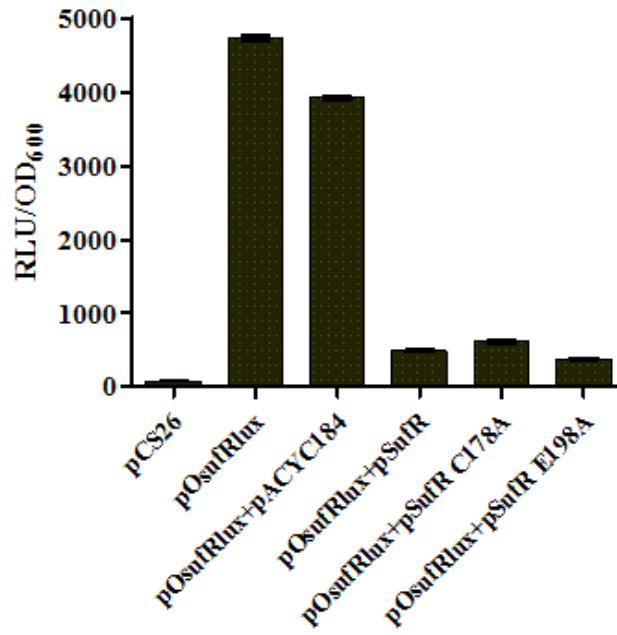
**Figure S4 Determination of the *sufR* TSS by 5'-RACE.** Boxed area: sequence of oligo dT-anchor primer. Bent arrow: *sufR* transcriptional start site.



**Figure S5 Binding of SufR to promoter regions of putative SufR targets.** Concentrations of SufR for probes were 120 nM. Arrow: free probe.

	-35	-10
<i>S. avermitilis</i>	CGCGGGTTGTCAGGCCCTGAGGAATTACGCAACAATGGCGTT <b>GTG</b>	
<i>S. coelicolor</i>	CGCGGGTTGCCCCCCCCGGAGGAATTACGCAACAATGGCGTT <b>GTG</b>	
<i>S. griseus</i>	CCGTGGTTGTCAGAGCCGAATGAATTACGCAACAATGGTGTT <b>GTG</b>	
<i>S. venezuelae</i>	CGGCGCTTGTCAAGCCGAGATTAATTACGCAACAATGGCGTT <b>GTG</b>	
<i>S. scabies</i>	CGCGGCTTGCCCCCTCTCCGAGGAATTACGCAACAATGGCGTT <b>GTG</b>	

**Figure S6 Alignment of *sufR* promoter regions in *Streptomyces* species (taken from StrepDB), revealing a conserved SufR binding site. Boxes: putative -10 and -35 regions of promoters.**

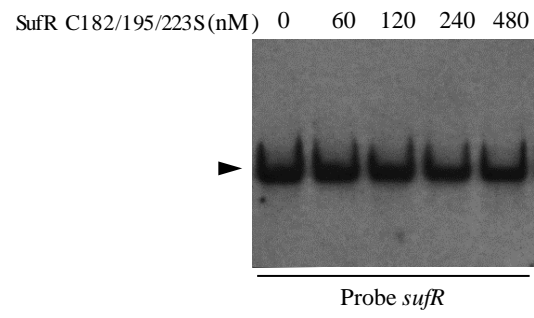


**Figure S7** The effect of SufRC178A and SufRE198A on bioluminescence level of *E. coli* containing reporter plasmid pOsufRlux. RLU (relative light units) represents bioluminescence level. Error bars: standard deviation of three technical replicates



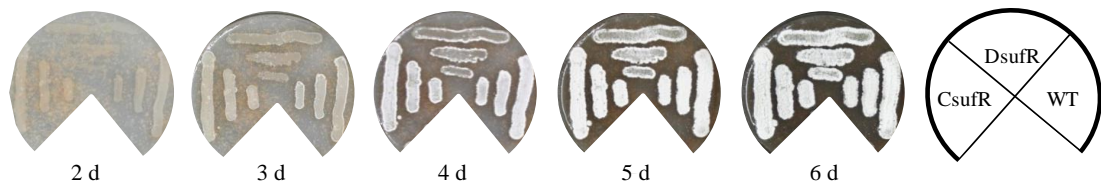
<i>Planktothrix agardhii</i>	80	HVYELTTQGREHFFNR	YDEF <b>A</b> ISFLD <b>T</b> L <b>T</b> Q <b>T</b> -WGHEQVSQILHQWQRKAIEYREKVGKGS-LKE-RVEKLVLRKT	153
<i>Mycobacterium fortuitum</i>	76	KRYRLTSA <b>G</b> RAKL <b>G</b> H <b>T</b>	YDDLAVAAIRQLREI-GGKDAVRTFARRRMDTILAGVTEGSG---GVA <b>d</b> TAERVADALTR	147
<i>Synechococcus</i> sp.	66	NIWRLTALGHQHFFDG [1]	-ESFALGLLNSMAAT-LSP <b>E</b> AMASLLSQALEKASRYRQRVGS <b>G</b> P-LRE-RIATLAE <b>L</b> RRQ	139
<i>Gloeotheca citriformis</i>	66	YVYGLSRQGRERYPHR	YGEF <b>A</b> VSFLD <b>T</b> L <b>V</b> ET-WGEKQWGEVLRHQWQRKAKEYRDRIGVGS-LKE-RVAKLVQLRQE	139
<i>Rippkaea orientalis</i>	66	YL <b>Y</b> HL <b>S</b> KQGRDRFFNR	YGEF <b>A</b> VSFLD <b>L</b> TA <b>E</b> T-WGKHQVTEVLRHQWQRKAKEYRQNI <b>G</b> EGT-LKE-RVDRLVKL <b>R</b> QE	139
<i>Crocospaera subtropica</i>	66	YIYSLKQGRDRFFNQ	YGEF <b>A</b> VSFLD <b>L</b> T <b>E</b> T-MG <b>S</b> QVKEVLRHQWQRKAD <b>D</b> YRQ <b>I</b> GKGS-LKE-RVERLVKL <b>R</b> QE	139
<i>Mycobacterium tuberculosis</i>	57	KRYRLTA <b>G</b> RAKL <b>D</b> H <b>S</b>	YDDL <b>A</b> SAAMRQLREI-GG <b>E</b> AVRTFARRRIDA <b>L</b> AD <b>V</b> APAD <b>G</b> PD <b>D</b> a <b>L</b> E <b>a</b> AAER <b>L</b> ATALSK	132
<i>Nostoc</i> sp.	67	HVYELSRQGRELHKS [4]	HGDF <b>A</b> VSLLD <b>L</b> TA <b>E</b> T-WQDQFKTILQHQWQRKAQ <b>E</b> YRDRVGN <b>G</b> S-L <b>E</b> E-RVANLV <b>L</b> RKA	144
<i>Cylindrospermopsis raciborskii</i>	67	HIYHLSFG <b>S</b> KEYL <b>Q</b> R <b>S</b> [7]	YGEF <b>A</b> VSLLD <b>L</b> TA <b>E</b> T-WGTEQVKTILQHQWQRKAQ <b>E</b> YQEKV <b>G</b> Q <b>C</b> -L <b>E</b> E-RVATLV <b>L</b> RKA	147
<i>Microcystis</i> sp.	66	HIYRLSRQGRGRFFHN	YDN <b>F</b> A <b>V</b> SFLN <b>L</b> T <b>L</b> V <b>E</b> T-WG <b>E</b> Q <b>V</b> GE <b>L</b> L <b>K</b> HQWQRKA <b>E</b> YQ <b>L</b> R <b>L</b> GKGS-L <b>G</b> E-RMRK <b>L</b> L <b>E</b> IRQE	139
<i>Bacillus cereus</i>	64	YL <b>V</b> GL <b>S</b> E <b>K</b> E <b>D</b> T <b>F</b> P <b>K</b> E	YK <b>F</b> A <b>I</b> DM <b>L</b> E <b>D</b> L <b>A</b> R <b>M</b> -G <b>D</b> E <b>K</b> IL <b>R</b> Y <b>V</b> L <b>K</b> E <b>R</b> T <b>K</b> R <b>M</b> E <b>B</b> Q <b>L</b> K <b>R</b> V <b>S</b> N <b>Q</b> K-N <b>L</b> A <b>g</b> K <b>V</b> Q <b>E</b> I <b>A</b> A <b>M</b> Q <b>E</b> K	138
<i>Streptomyces lividans</i>	80	K <b>V</b> F <b>A</b> L <b>T</b> D <b>C</b> R <b>D</b> A <b>F</b> D <b>S</b>	Y <b>D</b> K <b>L</b> A <b>A</b> D <b>A</b> L <b>R</b> W <b>I</b> A <b>E</b> Q <b>e</b> G <b>G</b> E <b>Q</b> A <b>I</b> A <b>A</b> F <b>A</b> R <b>A</b> R <b>I</b> A <b>A</b> Q <b>S</b> G <b>A</b> Y <b>R</b> A <b>A</b> V <b>S</b> V <b>P</b> -- <b>A</b> E <b>K</b> R <b>A</b> E <b>A</b> L <b>A</b> K <b>A</b> L <b>S</b> E	154
<i>Corynebacterium glutamicum</i>	80	K <b>T</b> Y <b>R</b> L <b>T</b> D <b>K</b> R <b>S</b> I <b>F</b> G <b>H</b> E	Y <b>D</b> S <b>L</b> A <b>A</b> A <b>A</b> L <b>A</b> T <b>L</b> R <b>E</b> V-G <b>D</b> D <b>A</b> V <b>R</b> Q <b>F</b> A <b>R</b> R <b>K</b> R <b>I</b> E <b>T</b> I <b>V</b> E <b>G</b> I <b>T</b> P <b>A</b> D <b>V</b> T <b>D</b> Q-S <b>I</b> E <b>t</b> A <b>K</b> S <b>L</b> V <b>E</b> A <b>F</b> S <b>R</b>	154
<i>Serratia quinivorans</i>	60	R <b>Y</b> W <b>S</b> L <b>T</b> Q <b>Q</b> G <b>H</b> A <b>Q</b> F <b>A</b> N <b>G</b>	H <b>D</b> R <b>L</b> A <b>T</b> E <b>L</b> L <b>G</b> S <b>A</b> H <b>Q</b> L-F <b>G</b> T <b>E</b> G <b>V</b> D <b>Q</b> L <b>L</b> S <b>A</b> R <b>E</b> D <b>K</b> L <b>Y</b> Q <b>R</b> Y <b>A</b> E <b>L</b> S <b>E</b> E <b>D</b> - <b>C</b> H <b>Q</b> - <b>K</b> L <b>A</b> R <b>L</b> V <b>R</b> L <b>R</b> Q <b>H</b>	134
<i>Planktothrix agardhii</i>	154	EGYMAEWYLL <b>E</b> SEF [6]	ERYILTEHHC <b>A</b> ISNV <b>A</b> ESF <b>P</b> SV <b>C</b> HE <b>L</b> EM <b>F</b> GE <b>L</b> -EDCTVERTHWIVNGEHRC <b>G</b> YL <b>I</b>	G 231
<i>Mycobacterium fortuitum</i>	148	AGYATT <b>N</b> FVD <b>G</b> P <b>I</b>	HGVQ <b>I</b> C <b>H</b> H <b>C</b> PSV <b>H</b> VA <b>E</b> EF <b>P</b> EL <b>C</b> ET <b>E</b> SE <b>A</b> FA <b>E</b> IL--G <b>T</b> H <b>Q</b> R <b>L</b> A <b>T</b> I <b>V</b> NG <b>D</b> CA <b>C</b> T <b>T</b> H <b>P</b> [ 9] <b>A</b>	227
<i>Synechococcus</i> sp.	140	EGYVTELE <b>F</b> DPD <b>G</b> H	-GW <b>L</b> SE <b>F</b> H <b>C</b> SV <b>R</b> IA <b>E</b> E <b>P</b> V <b>V</b> CD <b>Q</b> EL <b>Q</b> L <b>R</b> H <b>T</b> F-P <b>D</b> C <b>V</b> ER <b>V</b> H <b>V</b> R <b>L</b> E <b>K</b> H <b>S</b> <b>G</b> C <b>F</b> R <b>I</b> T	P 210
<i>Gloeotheca citriformis</i>	140	EGYMAEL-L <b>L</b> ME <b>K</b> N [4]	Q <b>K</b> Y <b>I</b> L <b>A</b> E <b>H</b> N <b>C</b> A <b>I</b> SE <b>V</b> A <b>E</b> SY <b>P</b> SV <b>C</b> HE <b>L</b> EM <b>F</b> SA <b>V</b> L-P <b>D</b> C <b>I</b> I <b>E</b> R <b>T</b> H <b>W</b> I <b>N</b> NG <b>E</b> H <b>R</b> C <b>G</b> YL <b>I</b> Q	P 214
<i>Rippkaea orientalis</i>	140	EGYMAELHLVNR <b>K</b> N [6]	EGY <b>I</b> L <b>A</b> E <b>H</b> H <b>C</b> A <b>I</b> SN <b>V</b> A <b>E</b> SY <b>P</b> TV <b>C</b> D <b>H</b> EL <b>Q</b> M <b>F</b> A <b>A</b> IL-P <b>D</b> C <b>T</b> V <b>E</b> R <b>T</b> H <b>W</b> I <b>N</b> E <b>G</b> E <b>H</b> N <b>C</b> G <b>Y</b> L <b>I</b> Q	K 217
<i>Crocospaera subtropica</i>	140	EGYMAELHRAN <b>E</b> K <b>N</b> [6]	EGY <b>L</b> L <b>A</b> E <b>H</b> H <b>C</b> A <b>I</b> SE <b>V</b> A <b>E</b> SY <b>P</b> TV <b>C</b> HE <b>L</b> EM <b>F</b> A <b>E</b> IL-P <b>D</b> C <b>M</b> I <b>E</b> R <b>T</b> H <b>W</b> I <b>N</b> E <b>G</b> E <b>H</b> N <b>C</b> G <b>Y</b> L <b>I</b> R	V 217
<i>Mycobacterium tuberculosis</i>	133	AGYVAT <b>T</b> TRV <b>G</b> G <b>P</b> I	HGVQ <b>F</b> Q <b>H</b> H <b>C</b> PSV <b>H</b> VA <b>E</b> EF <b>P</b> EL <b>C</b> ET <b>E</b> Q <b>Q</b> A <b>M</b> A <b>E</b> V <b>L</b> --G <b>T</b> H <b>Q</b> R <b>L</b> A <b>T</b> I <b>V</b> NG <b>D</b> CA <b>C</b> T <b>T</b> H <b>P</b> [10] <b>A</b>	213
<i>Nostoc</i> sp.	145	EGFMAEY <b>F</b> PVNS <b>P</b> H [4]	Q <b>R</b> F <b>I</b> F <b>M</b> E <b>H</b> N <b>C</b> A <b>I</b> SN <b>V</b> A <b>E</b> S <b>F</b> SV <b>C</b> HE <b>L</b> EM <b>F</b> A <b>A</b> V <b>L</b> -P <b>D</b> C <b>T</b> V <b>E</b> R <b>T</b> H <b>W</b> L <b>I</b> NG <b>E</b> H <b>R</b> C <b>G</b> YL <b>W</b> Q	A 220
<i>Cylindrospermopsis raciborskii</i>	148	EGFMAE <b>F</b> RPVE <b>E</b> K <b>D</b> [6]	H <b>G</b> F <b>L</b> F <b>M</b> E <b>H</b> N <b>C</b> A <b>I</b> SN <b>V</b> A <b>E</b> S <b>F</b> SV <b>C</b> HE <b>L</b> EM <b>F</b> A <b>A</b> IL-P <b>D</b> C <b>S</b> W <b>R</b> T <b>H</b> W <b>L</b> D <b>G</b> H <b>R</b> C <b>V</b> Y <b>L</b> I <b>K</b>	- 224
<i>Microcystis</i> sp.	140	EGYMAELVALE <b>A</b> E <b>N</b>	-SY <b>I</b> L <b>A</b> E <b>H</b> H <b>C</b> A <b>I</b> A <b>E</b> V <b>A</b> GS <b>Y</b> PS <b>I</b> C <b>H</b> E <b>L</b> EM <b>F</b> A <b>M</b> L <b>L</b> -P <b>D</b> C <b>T</b> I <b>E</b> R <b>T</b> H <b>W</b> I <b>N</b> Q <b>G</b> E <b>Q</b> R <b>C</b> G <b>Y</b> L <b>I</b> K	C 210
<i>Bacillus cereus</i>	139	NGY <b>M</b> V <b>Q</b> I <b>K</b> R <b>D</b> E <b>K</b> S	--F <b>V</b> I <b>E</b> K <b>Q</b> N <b>C</b> L <b>K</b> E <b>I</b> A <b>E</b> R <b>F</b> P <b>Q</b> <b>C</b> E <b>D</b> E <b>K</b> E <b>M</b> Y <b>K</b> R <b>L</b> F-T <b>N</b> A <b>E</b> V <b>K</b> T <b>L</b> A <b>N</b> M <b>C</b> E <b>G</b> D <b>C</b> S <b>C</b> S <b>Y</b> Q <b>I</b> K	E 208
<i>Streptomyces lividans</i>	155	D <b>G</b> Y <b>A</b> A <b>T</b> A <b>R</b> S <b>A</b> P <b>H</b> P <b>Q</b>	Q <b>G</b> E <b>Q</b> L <b>Q</b> H <b>H</b> C <b>P</b> V <b>A</b> H <b>V</b> A <b>E</b> Q <b>F</b> P <b>Q</b> L <b>C</b> E <b>A</b> E <b>T</b> E <b>F</b> F <b>A</b> E <b>L</b> L--G <b>T</b> H <b>Q</b> R <b>L</b> A <b>T</b> I <b>A</b> H <b>G</b> D <b>G</b> W <b>C</b> T <b>T</b> F <b>I</b> P [11] <b>S</b>	236
<i>Corynebacterium glutamicum</i>	155	H <b>G</b> Y <b>A</b> A <b>T</b> --V <b>D</b> A <b>T</b> R	N <b>G</b> L <b>Q</b> L <b>Q</b> H <b>H</b> C <b>P</b> I <b>S</b> T <b>V</b> A <b>T</b> E <b>F</b> P <b>E</b> L <b>C</b> E <b>A</b> E <b>H</b> Q <b>A</b> V <b>S</b> E <b>L</b> L--G <b>Q</b> H <b>T</b> Q <b>P</b> L <b>A</b> T <b>I</b> A <b>D</b> G <b>H</b> G <b>I</b> C <b>T</b> T <b>N</b> I <b>P</b> [ 6] <b>S</b>	228
<i>Serratia quinivorans</i>	135	D <b>G</b> Y <b>M</b> A <b>E</b> L--L <b>E</b> H <b>P</b> Q	-G <b>M</b> L <b>V</b> E <b>N</b> H <b>C</b> P <b>I</b> G <b>V</b> A <b>A</b> T <b>A</b> C <b>S</b> S <b>L</b> C <b>N</b> S <b>E</b> L <b>Q</b> L <b>F</b> N <b>R</b> L <b>F</b> <sub>g</sub> Q <b>Y</b> R <b>I</b> E <b>R</b> T <b>A</b> H <b>A</b> I <b>S</b> G <b>S</b> R <b>H</b> C <b>A</b> Y <b>L</b> I <b>R</b> [ 4]-	207

**Figure S8 Alignment of SufR proteins from bacteria.** Strictly conserved amino acids are shown in red.

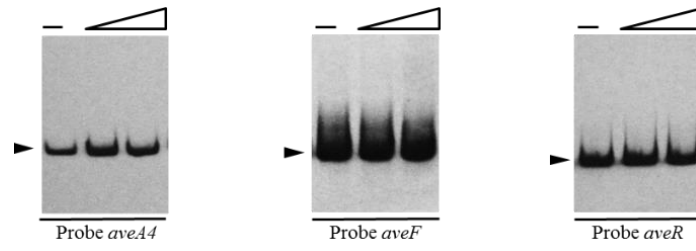


**Figure S9 Binding of SufR C182/195/223S to the *sufR* promoter region by EMSA.**

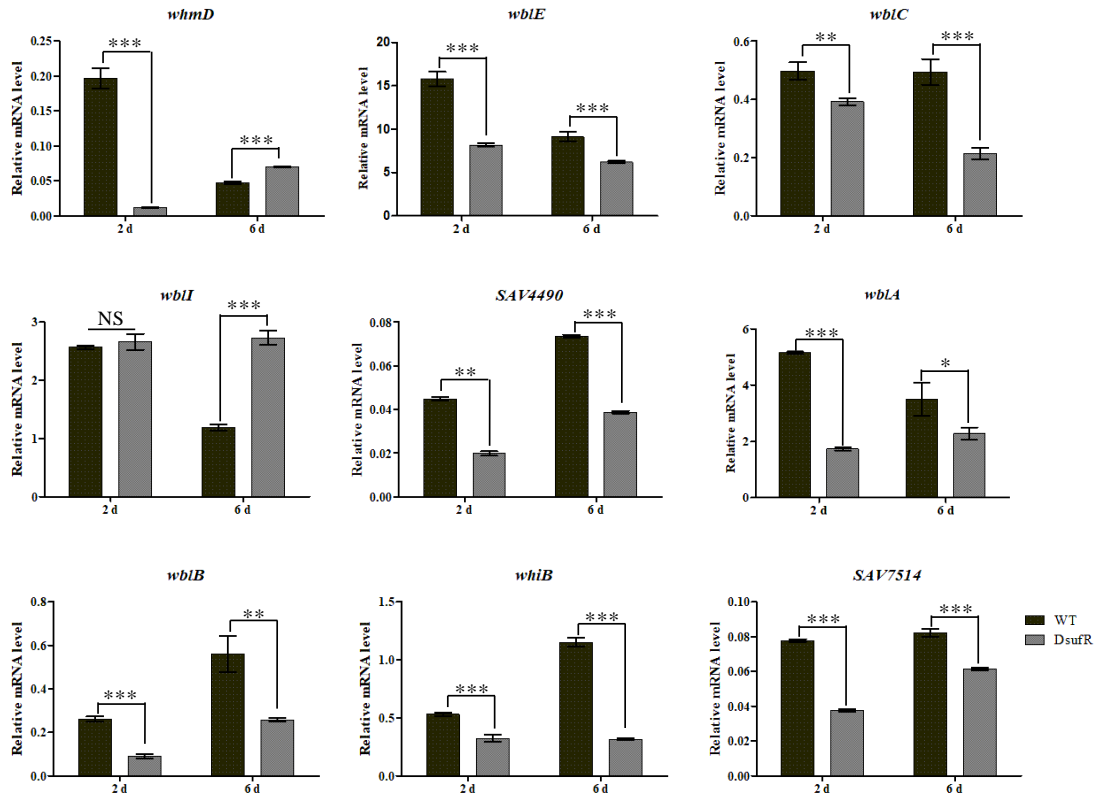
EMSA conditions as described for Figure 2. Arrow: free probe.



**Figure S10 Effects of *sufR* deletion on morphological differentiation.** WT, wild-type stain; DsufR, *sufR* deletion mutant; CsufR, *sufR* complement strain. The strains were grown on SFM agar.



**Figure S11 Binding of SufR to promoter regions of *aveA4*, *aveF* and *aveR*.** The concentration of SufR: 60 nM and 120 nM. Arrow: free probe.



**Figure S12 RT-qPCR analysis of transcription of *whiB*-like genes in the WT and DsufR.**

RNAs were prepared from cells grown on SFM agar for 2 or 6 days. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; NS, not significant. Error bars: standard deviation of three technical replicates.

**Table S1 Putative targets of SufR**

<b>Gene Name</b>	<b>Function</b>	<b>SEP Score</b>	<b>Sequence</b>
<i>SAV5359</i>	putative glycosyl transferase	-7.56	CAACTTTTTCGGTTG
<i>SAV5715</i>	hypothetical protein	-7.56	CAACAAGCGAGTTG
<i>pbp8 (SAV4583)</i>	putative penicillin-binding protein,	-7.59	CAACAAAAAAGTTG
<i>wblA (SAV4584)</i>	putative WhiB-family transcriptional regulator	-7.59	CAACAAAAAAGTTG
<i>ssgB (SAV4604)</i>	putative morphological differentiation-associated protein	-7.61	CAACCCAGTGGTTG
<i>minD2 (SAV4605)</i>	putative septum site determining protein	-7.61	CAACCCAGTGGTTG
<i>SAV2174</i>	hypothetical protein	-7.64	CAACTTGCCGGTTG
<i>SAV2175</i>	putative DNA-binding protein	-7.64	CAACTTGCCGGTTG
<i>SAV6323</i>	hypothetical protein	-7.64	CAACGCCATTGTTG
<i>sufR (SAV6324)</i>	putative ArsR-family transcriptional regulator	-7.64	CAACGCCATTGTTG
<i>smc (SAV2658)</i>	putative chromosome segregation protein	-7.84	CAACCGCTAAGTTG
<i>kdgK (SAV3280)</i>	putative 2-oxo-3-deoxygluconate kinase	-7.92	CAACGGTGCCGTTG
<i>SAV3281</i>	putative D-amino acid deaminase	-7.92	CAACGGTGCCGTTG
<i>SAV6620</i>	putative integral membrane protein	-7.95	CAACTGCCGGGTTG
<i>SAV6621</i>	hypothetical protein	-7.95	CAACTGCCGGGTTG
<i>SAV5256</i>	putative cellobiose ABC transporter substrate-binding protein	-8.03	CAACCATGCAGTTG
<i>SAV5257</i>	putative LacI-family transcriptional regulator	-8.03	CAACCATGCAGTTG
<i>SAV2213</i>	putative secreted protein	-8.04	CAACCTTCCTGTTG
<i>SAV4346</i>	hypothetical protein	-8.31	CAACTCCGCGGTTG

**Table S2 Predicted WhiB-family proteins in *S. avermitilis***

<b>Gene ID</b>	<b>Gene</b>	<b>Start</b>	<b>End</b>	<b>MW</b>	<b>Definition</b>
SAV1693	<i>whmD</i>	2072004	2071759	9150	putative WhiB-family transcriptional regulator
SAV3016	<i>wblE</i>	3771438	3771695	9671	putative WhiB-family transcriptional regulator
SAV3070	<i>wblC</i>	3832545	3832177	13190	putative WhiB-family transcriptional regulator
SAV3216	<i>wblI</i>	4007642	4007271	13658	putative WhiB-family transcriptional regulator
SAV4490		5487214	5485460	61647	putative WhiB-family transcriptional regulator
SAV4584	<i>wblA</i>	5599696	5600085	14257	putative WhiB-family transcriptional regulator; putative role in cell cycle control
SAV4997	<i>wblB</i>	6046508	6046179	11977	putative WhiB-family transcriptional regulator; putative role in cell cycle control
SAV5042	<i>whiB</i>	6104412	6104675	9892	putative WhiB-family transcriptional regulator
SAV7514		8958111	8957401	26526	putative WhiB-family transcriptional regulator