TABLE S1 Putative targets of AveT.

#	Gene ID	Putative target sites	Function
1	sav_5081, tagG	CGAAACGGTTGCGTTCCG	putative ABC transporter permease protein
	sav_5082		putative TetR-family transcriptional regulator
2	sav_559	CGAAACGGCGTCCTTTCG	putative secreted protein
<mark>3</mark>	sav_2282, pks3-3	CGATTCGGTTCCGTTCCG	putative acyl carrier protein
	sav_2283		putative aldehyde dehydrogenase
<mark>4</mark>	sav_3240	GACAACGAAATGGGGTCG	hypothetical protein
	sav_3241		hypothetical protein
5	sav_5816	TGAAAGCATTTACTTCAG	putative secreted protein
6	sav_6398, ectA	CTAAACGCAAAGATTTCG	L-2,4-diaminobutyrate acetyltransferase
7	sav_6852, pabC2	CGATACGGTAACGTTCCG	putative aminodeoxychorismate lyase
8	sav_7048	GAAACGTAAACCGTTGCG	putative cation efflux system protein
<mark>9</mark>	sav_7490, aveM	CGAAACGTTGCCGTTTCG	putative transmembrane efflux protein
	sav_7491		hypothetical protein
10	sav_626	GGGAAATAACCTGTTCCG	hypothetical protein
<mark>11</mark>	sav_2577, lplA	CGCAACAACTCCGTTTCC	putative multiple sugar ABC transporter
			substrate-binding protein
	sav_2578		putative sugar hydrolase
12	sav_2712	CGGAACCTTGTCTTTCCC	hypothetical protein
13	sav_3026, nrdL	CACAAGTACACCGTTGCG	putative ribonucleoside-diphosphate reductase
			alpha chain
14	sav_3343, pmmB	CGAAACGACTCCGCCCCG	putative phosphomannomutase
15	sav_3560	CGATACCGGATGGTTTCG	putative two-component system sensor kinase
16	sav_4488	CGAAACGGCTACCCTTCC	putative simple sugar ABC transporter
			substrate-binding protein
17	sav_4630	GGAAACCGGACCGTTTCC	hypothetical protein
18	sav_5287	CCGAACTCAAACGTCTCG	putative integrin-like protein
19	sav_5485	CACCACCAAATCGTTTCC	hypothetical protein
20	sav_7123, pfkA3	CCGCATGAAACGGTTCCG	6-phosphofructokinase
<mark>21</mark>	sav_7270	TGGAAGTCAATGGATTCG	putative LacI-family transcriptional regulator
	sav_7271		putative multiple sugar ABC transporter
			substrate-binding protein
22	sav_t4, trn4	CGAAACCATCCGGTATCG	codon recognized: CAA anticodon
23	sav_420	CGAAACGGCACCTTCTAG	putative Tn3 family ISXc5-like transposase
24	sav_525	TGAAAACAAAGCGCTTCG	putative IS630 family ISX07-like transposase
25	sav_575, cyp2	CGAAACGTTCCTGTTTCT	putative cytochrome P450 /
			NADPH-ferrihemoprotein reductase
26	sav_618	GGAAACGACGACGCTTCC	hypothetical protein
27	sav_1907	CGAAACGGCCGCGGCCCG	putative acetyltransferase
28	sav_1953	CGAAACGTGATCGACTCC	putative hydrolase
29	sav_2410	GCGGAACCAATGGTTTCG	hypothetical protein
30	sav_2653	CGATCCGCTTCCGTCTCG	hypothetical protein
31	sav_2821	TAGGAACATACGGTTTCG	hypothetical protein

<mark>32</mark>	sav_3217	GGAAACCCTTTCCTCGCC	hypothetical protein
	sav_3218, fumB		putative fumarate hydratase class I
33	sav_3667	CGTTGCGGAATCGTTTCG	putative erythropoiesis-stimulating protein
34	sav_3677	CGTTACGGGCACGTTTCG	putative ABC transporter permease protein
<mark>35</mark>	sav_4223, pepD3	GGGAATATAACTGTTTAG	putative serine protease, secreted
	sav_4224, glpQ2		putative glycerophosphoryl diester
			phosphodiesterase
36	sav_4634	CGAATCGGAGCCGCTCCG	putative glycosyltransferase
37	sav_4695	CGAAACGCGCATGTTTCC	putative Lsr2-like protein
38	sav_5359	CGAAAAGTTGACGTTTCT	putative glycosyltransferase
39	sav_5361, melC1-2	CGAAACGTGCCCTTTTCG	putative tyrosinase co-factor protein
40	sav_5367	CGAACGGGTTTCCTTCGA	putative ABC transporter ATP-binding protein
41	sav_5420	GGAAACGGATCCGGCTCG	putative ROK-family transcriptional regulator
<mark>42</mark>	sav_5943, panB	CGATACGGAACCGTCTCG	putative 3-methyl-2-oxobutanoate
			hydroxymethyltransferase
	sav_5944		putative transmembrane efflux protein
43	sav_6177, trpB	CGGAACGCTCCCCTTCCG	putative tryptophan synthase beta subunit
<mark>44</mark>	sav_6709	CGAACATATCGCGTTTCG	putative amino acid permease
	sav_6710		putative PadR-like family transcriptional
			regulator
45	sav_6917	CGAACCGCGTCCGGTTCG	putative TetR-family transcriptional regulator
46	sav_6976, pgsA3	GAAAACAAAAAAGCTCCG	putative
			CDP-diacylglycerolglycerol-3-phosphate
			3-phosphatidyl-transferase
47	sav_7362, pks1-3	GGGAAACAATGCGTTTCC	putative modular polyketide synthase
48	sav_7400, sig57	CGTTACGATTCCGATTCG	putative RNA polymerase sigma factor
49	sav_t66, trn66	CGAACCCAAAACGTGTCG	codon recognized: CUC anticodon

Yellow highlighting of numbers indicates that similar binding sites of AveT protein were found in bidirectional promoter regions of the divergently transcribed genes. Red front of numbers indicates the putative targets selected and confirmed experimentally by EMSAs.

A



FIG S1 Organization of *aveM* and its adjacent genes on the chromosome of *S. avermitilis* (A), and schematic method used for *aveM* deletion (B). (A) Gene notations are based on the *S. avermitilis* genome database (<u>http://avermitilis.ls.kitasato-u.ac.jp/</u>). White block: in-frame deletion in *aveM* gene. (B) Large arrows: genes and their directions. Short arrows: positions of primers used for cloning exchange regions and confirming gene deletion. Rectangles: homologous exchange regions used for deletion of *aveM*.

FIG S2

ATVRRSRITPEREAELYSAVLDLLREVGYDALTMDAVASRT 70 S. avermitilis SSKATLYRQ MTETATIVRSSKI PERELELISAVLULLE VOIDALIMUAVASKI SSKATLI RUNGGVELV MTEAATTRSSKI PEREAELYAAVLULLREVGYDALIMUAVAARTRSSKATLYRONGGVELV MTETATVRSSKI PEREAELYAAVLULLREVGYDALIMUAVAARTRSSKATLYRONGGVELV MTEVATARSSKI PEREAELYAAVLULLREVGYDALIMUAVAARTRSSKATLYRONGGVELV MTETATARSSKI PEREAELYBAVLULLREVGYDALIMUAVAARTRSSKATLYRONGGVELV S. sp. HGB0020 70 S. scabiei 70 S. prunicolor 70 S. coelicolor A3(2) 70 MTE mte at rrsritpereaely avldllrevgydaltmdava rt sskatlyrqwggk elv ka r Consensus K.HSHFGEVDTGSLRGDFHALVTRENNOEMEONSALMRGLAVAVHANPDLLDABRDLIIDPETNEMORLI KPGSSAGEIDTGSLRGDLHALTMRSDDCOMEONSALMRGLAVAVHGNPDLLKABKEHLIEPELABFRRL KPRPDFGSVDTGSLRGDMHALIMSODDCTIEONSALMRGLAVAIHGNPDLLKABRELIIEPELLEIRGM KPGASAAAVDTGSLRGDFHALTMRSDDCEMEONSALMRGLAVAIHGNPDLLKABREHLVEPEMABFOTM K.PGEIGDVDTGSLRGDFHALTMRSDDCEMEONSALMRGVAVAIHONPDLRGABREOLIEPEMABFORV S. avermitilis 139 S. sp. HGB0020 140 140 S. scabiei S. prunicolor 140 S. coelicolor A3(2) 139 Consensus dtgslrgd hal c equisalmrg a a h npdl af pe e ORAVORGEIRADNENTOYVUHALISADAARTLIDELEPENOEGITSYVDAVVIPALGA.... ORAIDRGEIRADNENTOYVUHALISADAARSLIDEOEPENOAGILSYIDAVVIPALGA.... ORAVORGEIREDNENTOYVUHAFYSADVARGMIDELEPENOAGILSYIDAVVIPALGAPVET... ORAIDRGEVRADNENTYVHAMIGGIAARTMIDEOEPENOAGILSYIDAVVIPALGAPVETT ORAVORGEIRADOEPILDELVHAMYGGIAARTMIDEOEPENOAGILSYIDAVVIPALGAPVETT. S. avermitilis 196 S. sp. HGB0020 198 200 S. scabiei 202 S. prunicolor S. coelicolor A3(2) 199 QR. Consensus qra drge r d pa hm gf r d ppt f sy dav lpal

TetR N

FIG S2 Amino acid alignment of AveT and its homologous proteins in five *Streptomyces* species. AveT protein in *S. avermitilis* has high identity with its homologs in *S. sp. HGB0020* (78%), *S. scabiei* (78%), *S. prunicolor* (76%), and *S. coelicolor* (75%). Black line at top: TetR_N domain.

Α



FIG S3 Determination of TSSs of *aveT* (A) and *pepD2* (B) by 5'-RACE PCR. Boxed areas: complementary sequences of 5'-RACE oligo dT-anchor primers. Bent arrows: complementary bases of TSSs.

<i>sco316</i> 7	CGAAACCGTTTCGTTTCG
sgr_4317	CGAAACGATTTCGTTTCG
scab53291	CGAAACGGTTTCGTTTTC
sven_3001	CGAAACGGTTTCGTTTCG
sclav_2302	CGAAACGATTTAGTTTCG
sli_3521	CGAAACCGTTTCGTTTCG
strs4_04951	CGAAACCGTTTCGTTCAC
sav_3619	CGAAACGGTTTCGTTTCG

Consensus sequence CGAAACSRTTTMGTTYHS



FIG S4 Analysis of the consensus sequence of AveT-binding sites. The consensus bases in the AveT-binding sequence are boxed and conserved 7 bp inverted repeats are indicated by arrows. In the sequence logo of AveT binding consensus, the height of each letter is proportional to the frequency of the base appearance.



FIG S5 Determination of TSS of *aveM* by 5'-RACE PCR (A) and AveT-binding site in the promoter region of *aveM* (B). (A) Boxed area: complementary sequence of 5'-RACE oligo dT-anchor primer. Bent arrow: complementary base of TSS. (B) Numbers, distance (nt) from TSS of *aveM*. Solid line, AveT-binding site. Straight arrows, inverted repeats. Bent arrow, TSS and transcription orientation. Boxes, putative -10 and -35 regions. Shaded areas, translational start codons.

FIG S6



FIG S6 Effects of deletion and overexpression of *aveM* on morphological differentiation of *S. avermitilis*. WT strain ATCC31267, *aveM* deletion mutant Δ aveM, and *aveM* overexpression strain OaveM were grown on YMS or MM plates for 7 days at 28 °C.





FIG S7 Real-time RT-PCR analysis of *aveT* and *aveM* transcription levels in the WT and A-178 strains. RNA samples were isolated from 2- and 6-day fermentation cultures in FM-I. Relative transcription levels were obtained after normalization against internal reference *hrdB* at specific time points. Error bars, SD from three independent experiments. *, P<0.05; ***, P<0.001 by Student's *t*-test.





FIG S8 EMSAs of AveT (0.05 μ M) with concentrated fermentation broth of WT and *aveM* deletion mutant Δ aveM grown in FM-I for 10 days. Concentrated supernatant of fermentation medium FM-I was used as medium control. Each lane contained 0.15 nM labeled probe.

FIG S9



FIG S9 Phenotypes (day 10) of WT, ∆aveM, and OaveM grown on MM containing various carbon sources (1%).