

TABLE S1 Putative targets of AveT.

#	Gene ID	Putative target sites	Function
1	<i>sav_5081, tagG</i> <i>sav_5082</i>	CGAAACGGTTGCGTTCCG	putative ABC transporter permease protein putative TetR-family transcriptional regulator
2	<i>sav_559</i>	CGAAACGGCGTCCTTTCG	putative secreted protein
3	<i>sav_2282, pks3-3</i> <i>sav_2283</i>	CGATTCGGTTCGGTTCCG	putative acyl carrier protein putative aldehyde dehydrogenase
4	<i>sav_3240</i> <i>sav_3241</i>	GACAACGAAATGGGGTCG	hypothetical protein hypothetical protein
5	<i>sav_5816</i>	TGAAAGCATTTACTTCAG	putative secreted protein
6	<i>sav_6398, ectA</i>	CTAAACGCAAAGATTTTCG	L-2,4-diaminobutyrate acetyltransferase
7	<i>sav_6852, pabC2</i>	CGATACGGTAACGTTCCG	putative aminodeoxychorismate lyase
8	<i>sav_7048</i>	GAAACGTAAACCGTTGCG	putative cation efflux system protein
9	<i>sav_7490, aveM</i> <i>sav_7491</i>	CGAAACGTTGCCGTTTCG	putative transmembrane efflux protein hypothetical protein
10	<i>sav_626</i>	GGGAAATAACCTGTTCCG	hypothetical protein
11	<i>sav_2577, lplA</i> <i>sav_2578</i>	CGCAACAACCTCCGTTTCC	putative multiple sugar ABC transporter substrate-binding protein putative sugar hydrolase
12	<i>sav_2712</i>	CGGAACCTTGCTTTTCCC	hypothetical protein
13	<i>sav_3026, nrdL</i>	CACAAGTACACCGTTGCG	putative ribonucleoside-diphosphate reductase alpha chain
14	<i>sav_3343, pmmB</i>	CGAAACGACTCCGCCCCG	putative phosphomannomutase
15	<i>sav_3560</i>	CGATACCGGATGGTTTCG	putative two-component system sensor kinase
16	<i>sav_4488</i>	CGAAACGGCTACCCTTCC	putative simple sugar ABC transporter substrate-binding protein
17	<i>sav_4630</i>	GGAAACCGGACCGTTTCC	hypothetical protein
18	<i>sav_5287</i>	CCGAACTCAAACGTCTCG	putative integrin-like protein
19	<i>sav_5485</i>	CACCACCAAATCGTTTCC	hypothetical protein
20	<i>sav_7123, pfkA3</i>	CCGCATGAAACGGTTCCG	6-phosphofructokinase
21	<i>sav_7270</i> <i>sav_7271</i>	TGGAAGTCAATGGATTCG	putative LacI-family transcriptional regulator putative multiple sugar ABC transporter substrate-binding protein
22	<i>sav_14, trn4</i>	CGAAACCATCCGGTATCG	codon recognized: CAA anticodon
23	<i>sav_420</i>	CGAAACGGCACCTTCTAG	putative Tn3 family ISXc5-like transposase
24	<i>sav_525</i>	TGAAAACAAAGCGCTTCG	putative IS630 family ISXo7-like transposase
25	<i>sav_575, cyp2</i>	CGAAACGTTCTGTTTCT	putative cytochrome P450 / NADPH-ferrihemoprotein reductase
26	<i>sav_618</i>	GGAAACGACGACGCTTCC	hypothetical protein
27	<i>sav_1907</i>	CGAAACGGCCGCGGCCCG	putative acetyltransferase
28	<i>sav_1953</i>	CGAAACGTGATCGACTCC	putative hydrolase
29	<i>sav_2410</i>	GCGGAACCAATGGTTTTCG	hypothetical protein
30	<i>sav_2653</i>	CGATCCGCTTCCGTCTCG	hypothetical protein
31	<i>sav_2821</i>	TAGGAACATACGGTTTTCG	hypothetical protein

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

FIG S1

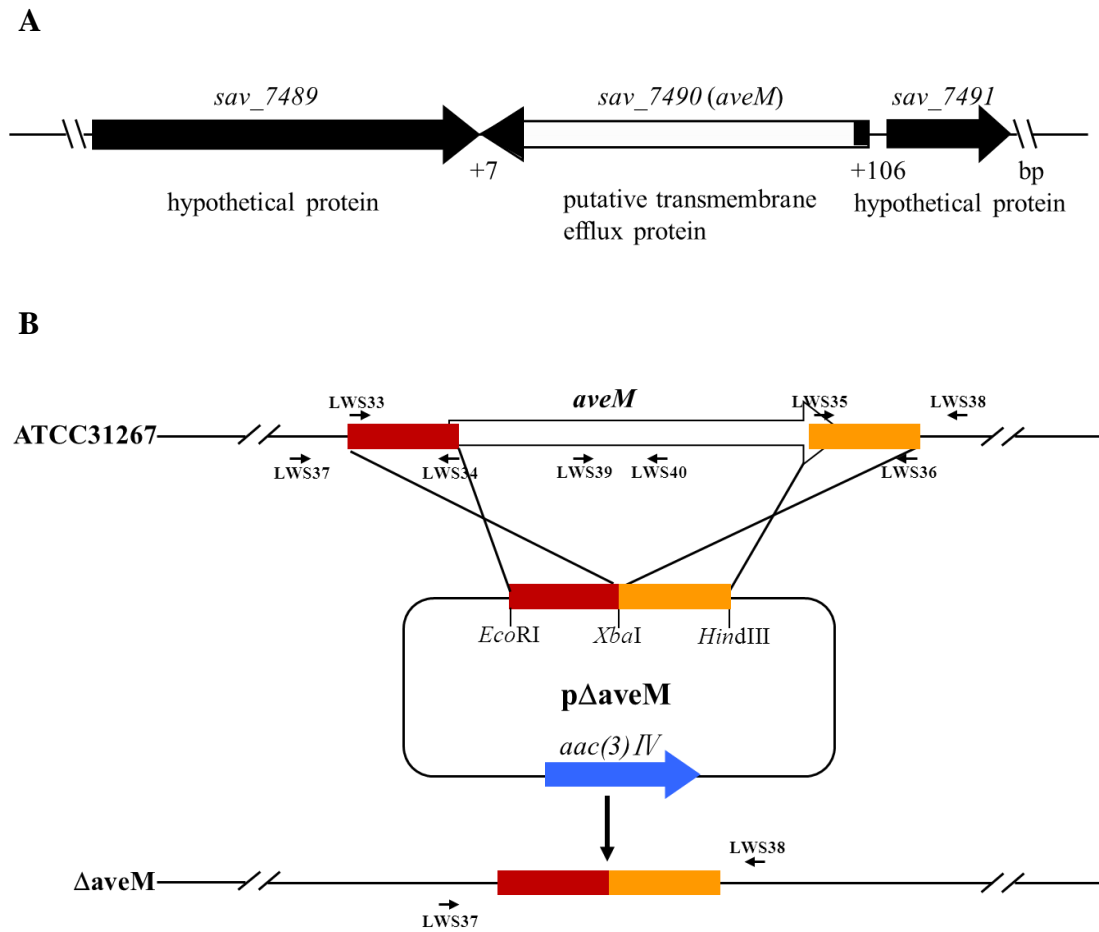


FIG S1 Organization of *aveM* and its adjacent genes on the chromosome of *S. avermitilis* (A), and schematic method used for *aveM* deletion (B). (A) Gene notations are based on the *S. avermitilis* genome database (<http://avermitilis.ls.kitasato-u.ac.jp/>). White block: in-frame deletion in *aveM* 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

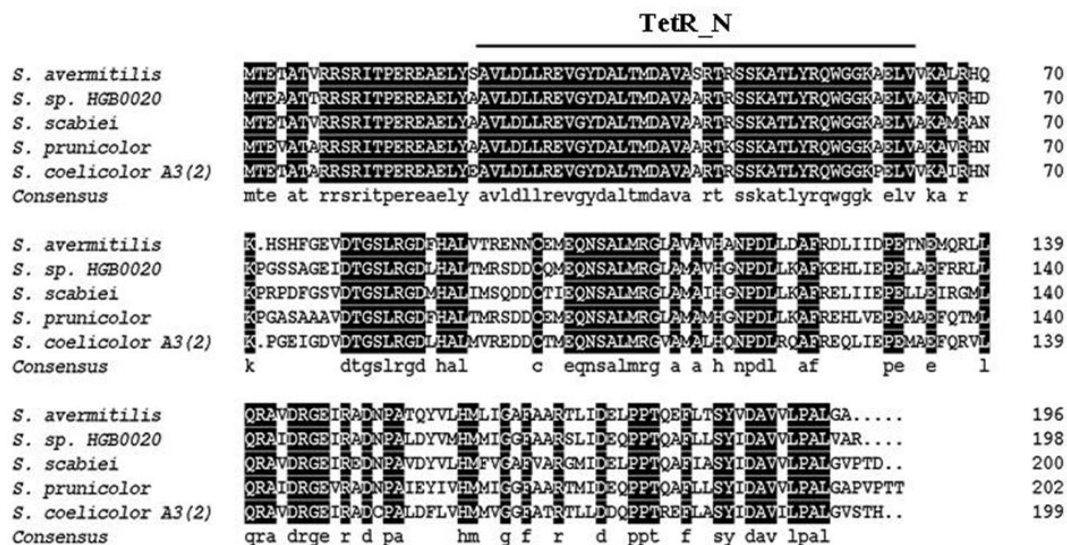
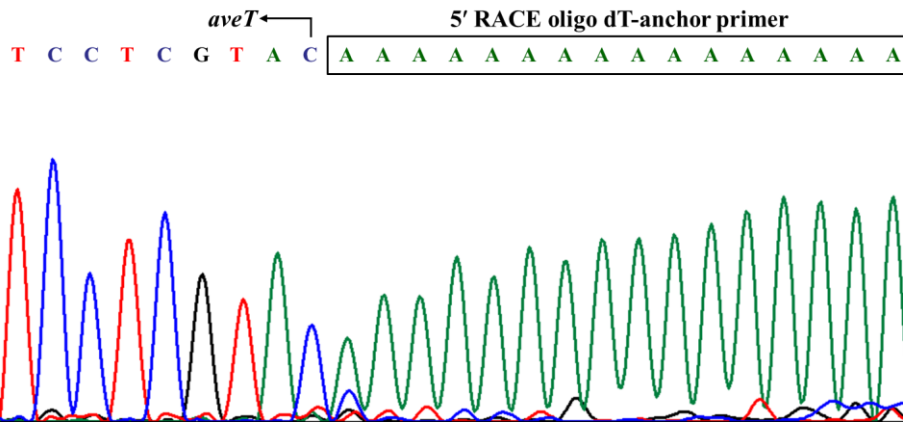


FIG S2 Amino acid alignment of AveT and its homologous proteins in five *Streptomyces* species. AveT protein in *S. avermitilis* has high identity with its homologs in *S. sp. HGB0020* (78%), *S. scabiei* (78%), *S. prunicolor* (76%), and *S. coelicolor* (75%). Black line at top: TetR_N domain.

FIG S3

A



B

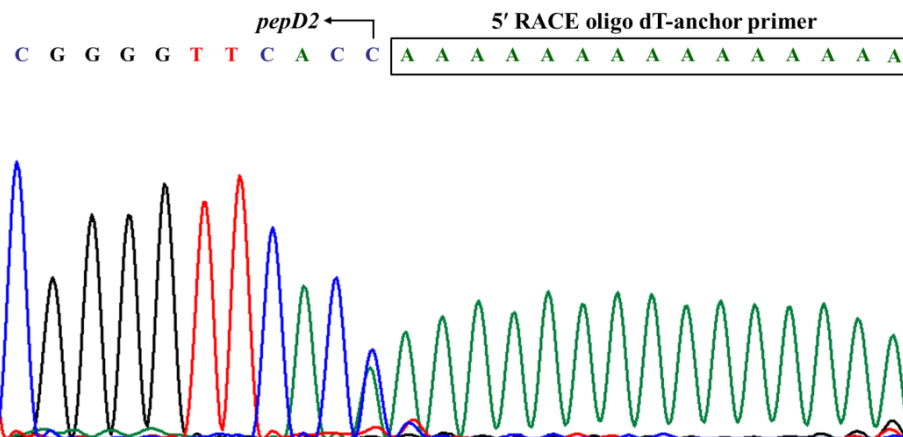


FIG S3 Determination of TSSs of *aveT* (A) and *pepD2* (B) by 5'-RACE PCR. Boxed areas: complementary sequences of 5'-RACE oligo dT-anchor primers. Bent arrows: complementary bases of TSSs.

FIG S4

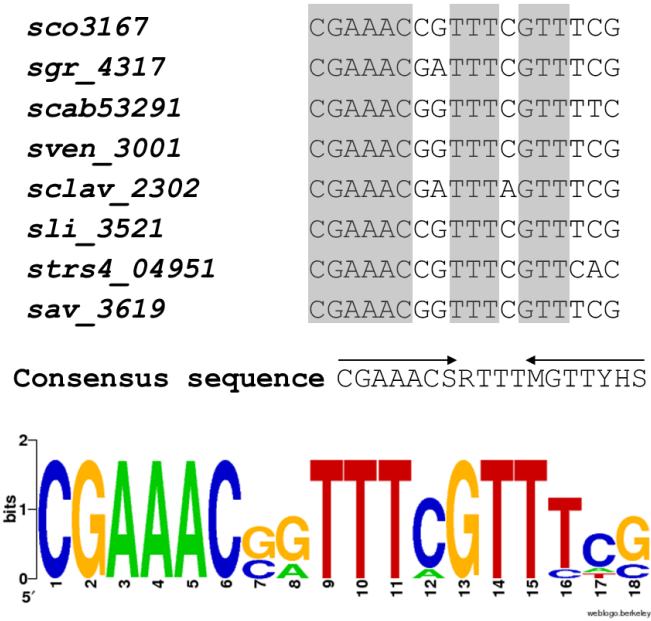


FIG S4 Analysis of the consensus sequence of AveT-binding sites. The consensus bases in the AveT-binding sequence are boxed and conserved 7 bp inverted repeats are indicated by arrows. In the sequence logo of AveT binding consensus, the height of each letter is proportional to the frequency of the base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

FIG S6

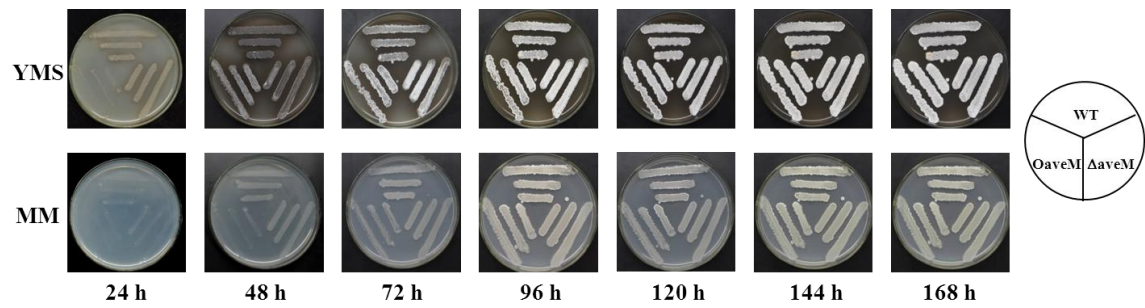


FIG S6 Effects of deletion and overexpression of *aveM* on morphological differentiation of *S. avermitilis*. WT strain ATCC31267, *aveM* deletion mutant Δ aveM, and *aveM* overexpression strain OaveM were grown on YMS or MM plates for 7 days at 28 °C.

FIG S7

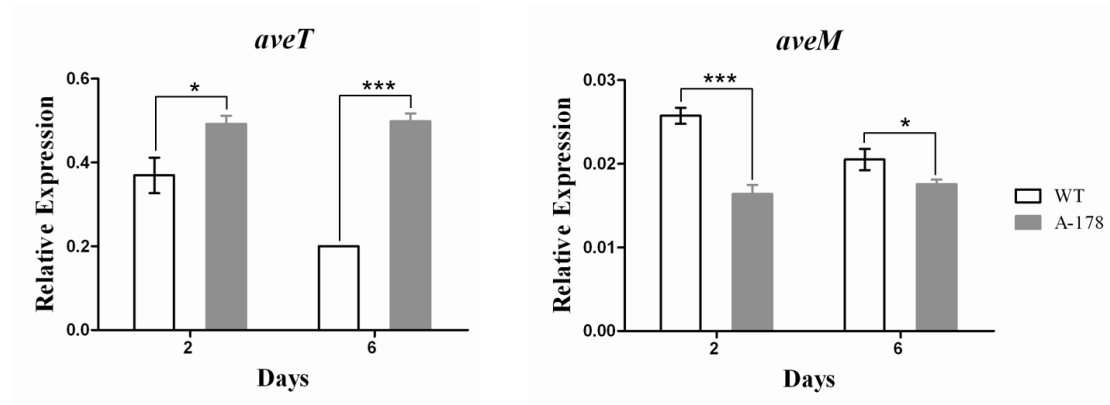


FIG S7 Real-time RT-PCR analysis of *aveT* and *aveM* transcription levels in the WT and A-178 strains. RNA samples were isolated from 2- and 6-day fermentation cultures in FM-I. Relative transcription levels were obtained after normalization against internal reference *hrdB* at specific time points.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

FIG S8

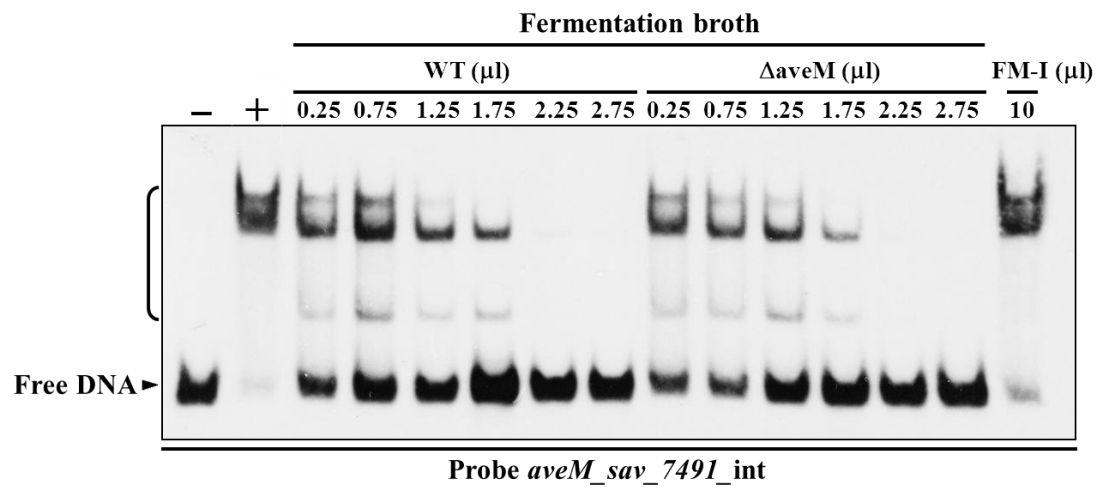


FIG S8 EMSAs of AveT (0.05 μM) with concentrated fermentation broth of WT and *aveM* deletion mutant ΔaveM grown in FM-I for 10 days. Concentrated supernatant of fermentation medium FM-I was used as medium control. Each lane contained 0.15 nM labeled probe.

FIG S9

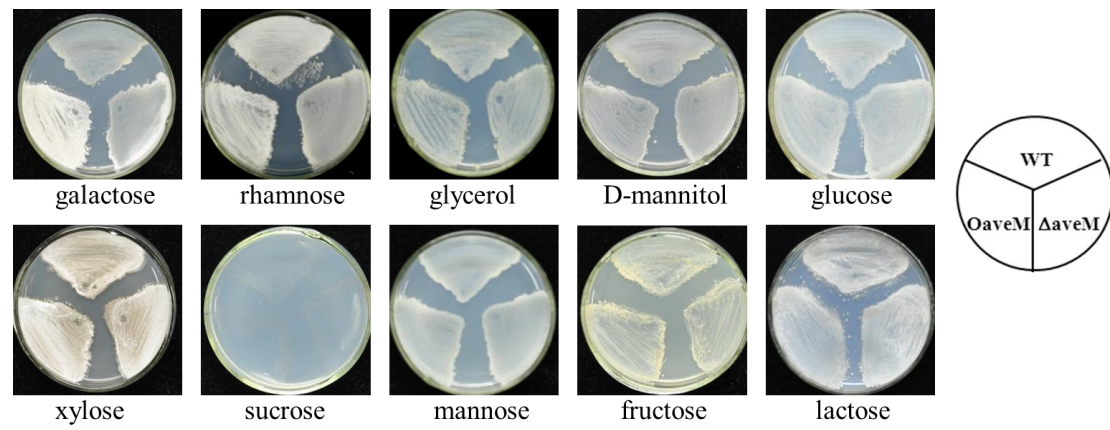


FIG S9 Phenotypes (day 10) of WT, Δ aveM, and OaveM grown on MM containing various carbon sources (1%).