Supplemental Materials

AccR, a TetR family transcriptional repressor, coordinates short-chain acyl-CoAs homeostasis in *Streptomyces avermitilis*

Mengya Lyu, Yaqing Cheng, Xiao Han, Ying Wen, Yuan Song, Jilun Li, Zhi Chen*

State Key Laboratory of Agrobiotechnology and Key Laboratory of Soil Microbiology, Ministry of Agriculture, College of Biological Sciences, China Agricultural University, Beijing 100193, China.

*Corresponding author. E-mail chenzhi@cau.edu.cn.

Table S1. Predicted target genes of AccR in *S. avermitilis*.

Classification	Gene	Function	Sequence	Position[a]	Score	EMSA[b]
Regulator						
	SAV5279	TetR/AcrR family transcriptional regulator	GTTAATGAGCGTTAAC	-67	22.3	+
	SAV5279	TetR/AcrR family transcriptional regulator	GTTAACGACCGCTAAC	-38	20.3	+
Fatty acid and lipid metabolism						
	SAV5278 accD1	acetyl/propionyl-CoA carboxylase β-subunit	GTTAATGAGCGTTAAC	-46	22.3	+
	SAV5278 accD1	acetyl/propionyl-CoA carboxylase β-subunit	GTTAACGACCGCTAAC	-75	20.6	+
	SAV6919 fadE2	acyl-CoA dehydrogenase	GTGAGCGCTCGTTAAC	-184	16.4	+
	SAV3327 echA9	enoyl-CoA hydratase	GTTAACGCGGGTTAAC	-28	13.9	+
	SAV2786 echA8	enoyl-CoA hydratase	GTTAACGGCGACTAAC	-24	11.5	+
	SAV5026 fadE1	acyl-CoA dehydrogenase	CTTAACGGTAATTAGC	-37	11	-
	SAV3460 icmB	isobutyryl-CoA mutase	GTGAACGAACGTTATC	-86	10.1	-
Carbohydrate metabolism						
	SAV6918 chiC2	chitinase	GTGAGCGCTCGTTAAC	-256	16.4	+
	SAV5025 udgA	UDP-glucose/GDP-mannose dehydrogenase family protein	CTTAACGGTAATTAGC	-154	11	-
Cell division and differentiation						
	SAV4026 amfC	AmfC protein	GTTAAGGCTAATTAAG	-113	12.2	ND
Transport						
	SAV1133	MFS transporter	GTGAATGGTCATTCAC	-79	10.1	-
RNA modification						
	SAV4027 dtd	D-aminoacyl-tRNA deacylase	GTTAAGGCTAATTAAG	-73	12.2	ND
Protein modification						
	SAV4118	M23 family peptidase	CTTACTGATCGTTAAC	-266	11.9	ND
	SAV4118	M23 family peptidase	GTTATCGAACGTTAAT	-222	11.1	ND

[a] Distance from translation start site.

[b] +: AccR binds to the promoter region of gene. -: no binding. ND: not detected.

Fig. S1

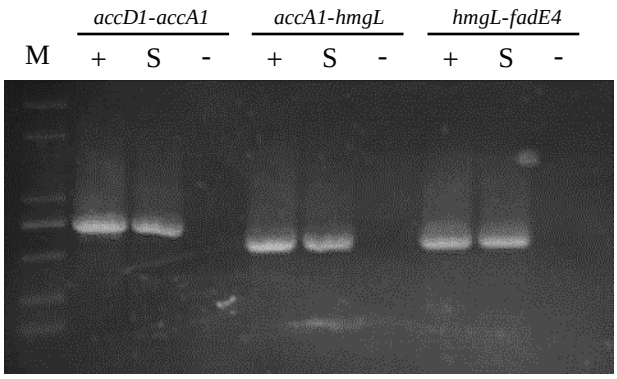

Fig. S1. RT-PCR analysis of *accD1-accA1-hmgL-fadE4* co-transcription in *S. avermitilis*. The cDNA obtained by reverse transcription of DaccR RNA was used as template (lane S) for determination of *accD1-accA1*, *accA1-hmgL*, and *hmgL-fadE4* co-transcription. WT DNA was used as positive control (lane +), and water was used as negative control (lane -). M: 2000-bp DNA marker.

Fig. S2

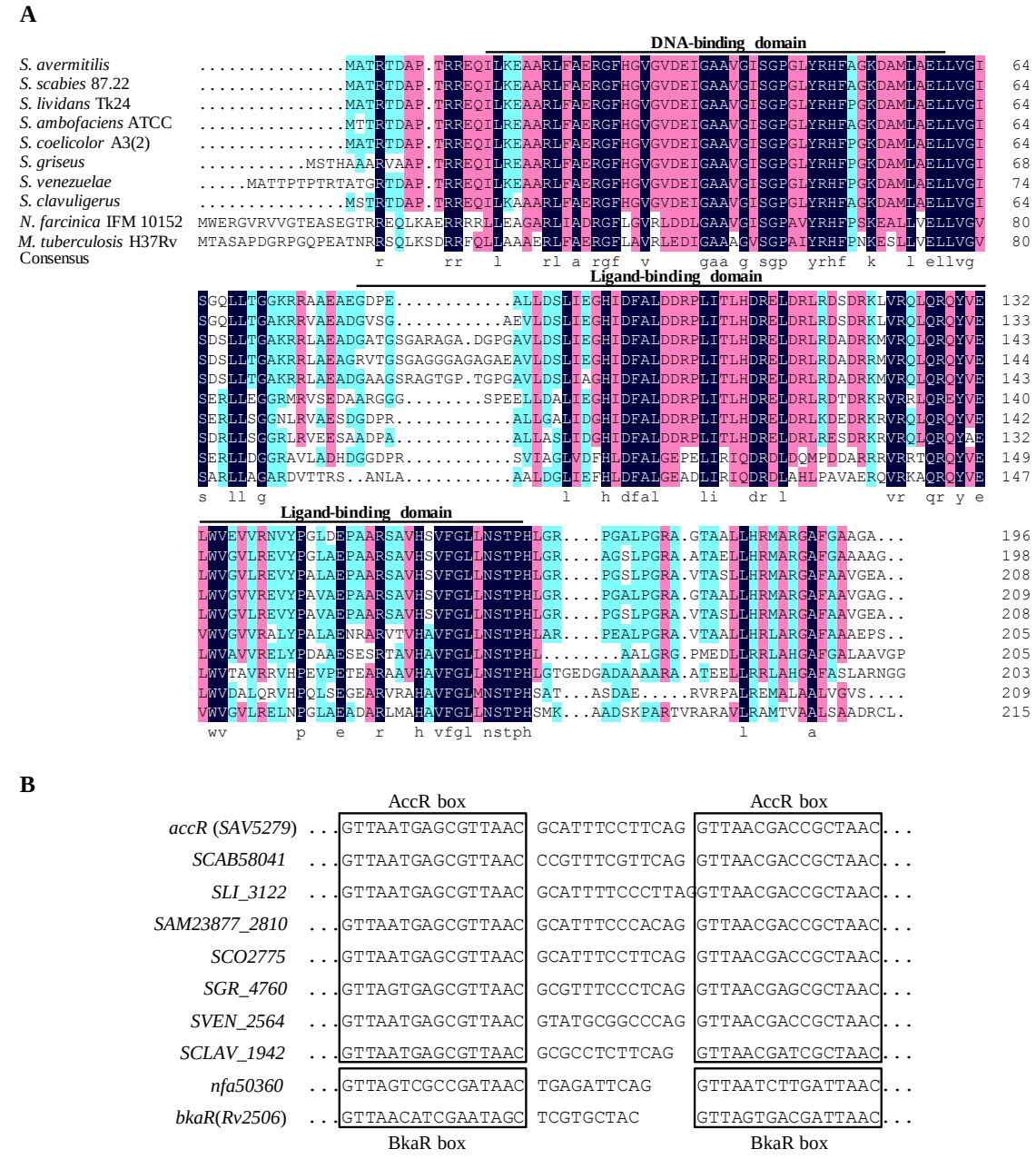

Fig. S2. Alignments of amino acid sequences (A) and binding sites in promoter regions (B) of AccR in *Streptomyces, Nocardia farcinica,* and *M. tuberculosis.*

Identities of AccR with its orthologs: SACB58041 (91%), SLI_3122 (84%),

SAM23877_2810 (84%), SCO2775 (83%), SGR_4760 (81%), SVEN_2564 (78%), SCLAV_1942 (76%), Nfa50360 (53%), BkaR (50%).

Fig. S3

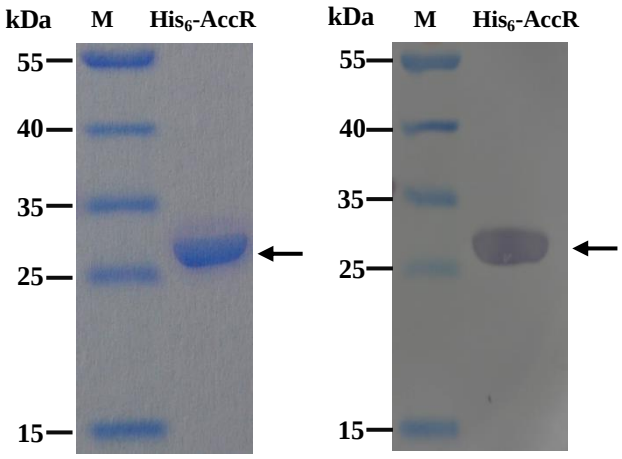

Fig. S3. SDS-PAGE (left) and Western blotting (right) of purified His_6-AccR.

Fig. S4

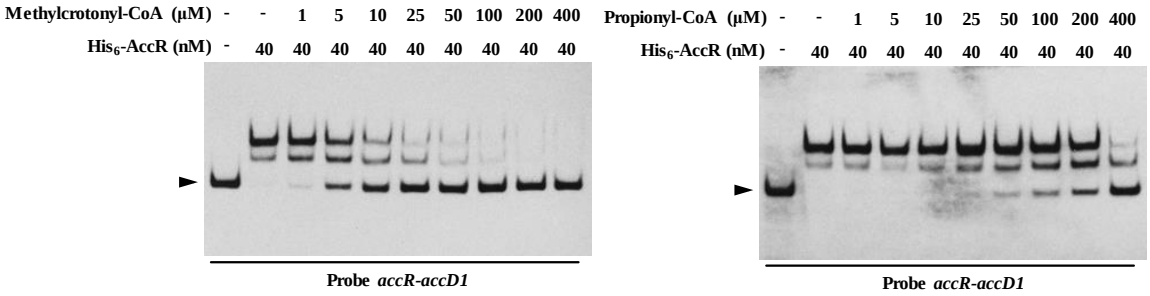

Fig. S4. Effects of methylcrotonyl- and propionyl-CoA at different concentrations on interaction between AccR and *accR-accD1* intergenic region. Arrows: free probes.

Fig. S5

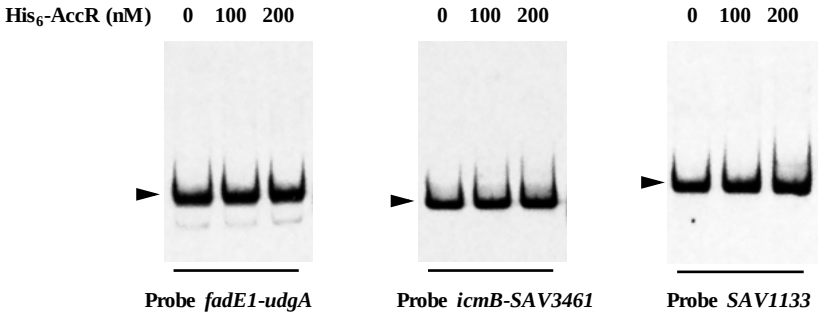

Fig. S5. Interaction of AccR with predicted target genes analyzed by EMSAs. Arrows: free probes.

Fig. S6

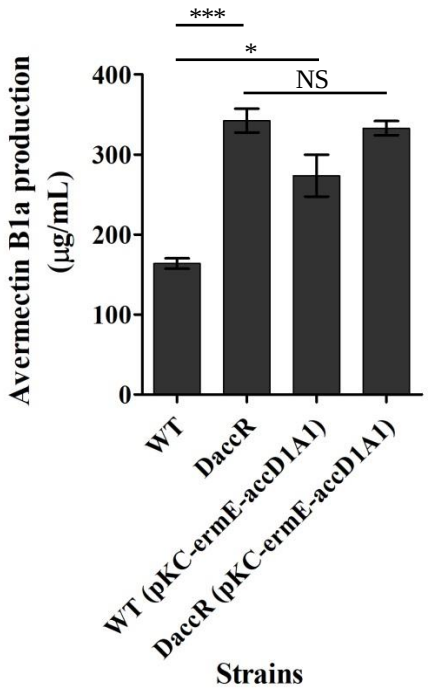

Fig. S6. Avermectin B1a production of WT, DaccR and *accD1A1* overexpression strains (in WT and DaccR) in FM-I. NS, not significant. *, $P < 0.05$; ***, $P < 0.001$ (Student's *t*-test).

Fig. S7

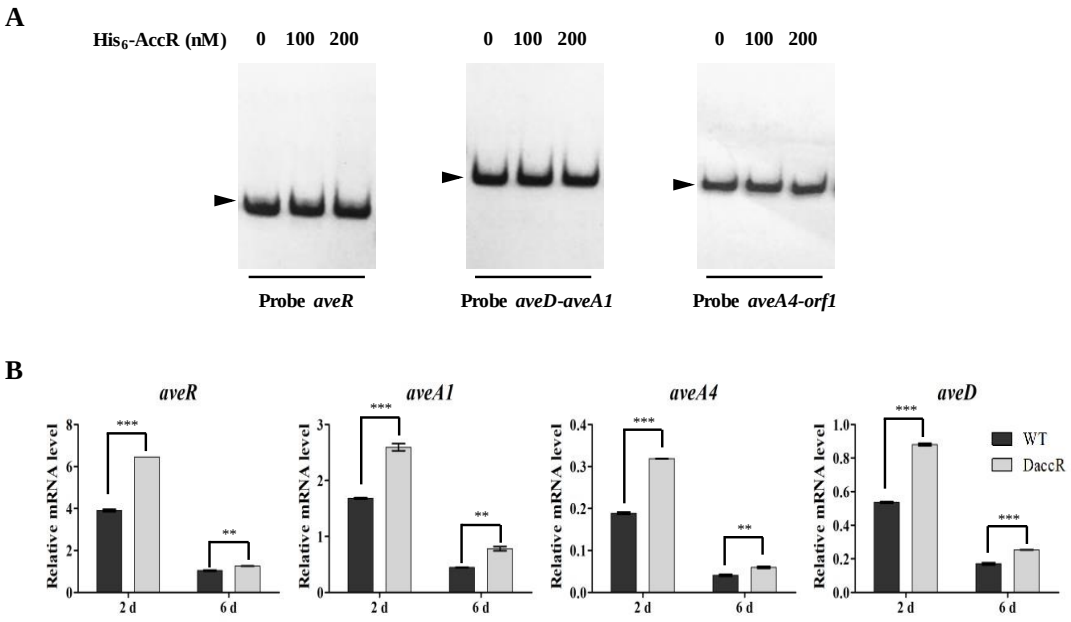

Fig. S7. Regulatory effect of AccR on *ave* genes. (A) EMSAs of AccR with promoter region of *aveR* and intergenic regions of *aveD-aveA1* and *aveA4-orf1*. Arrows: free DNA probes. **(B)** RT-qPCR analysis of transcription levels of *ave* genes in WT and DaccR. **, $P < 0.01$; ***, $P < 0.001$ (Student's *t*-test).

Fig. S8

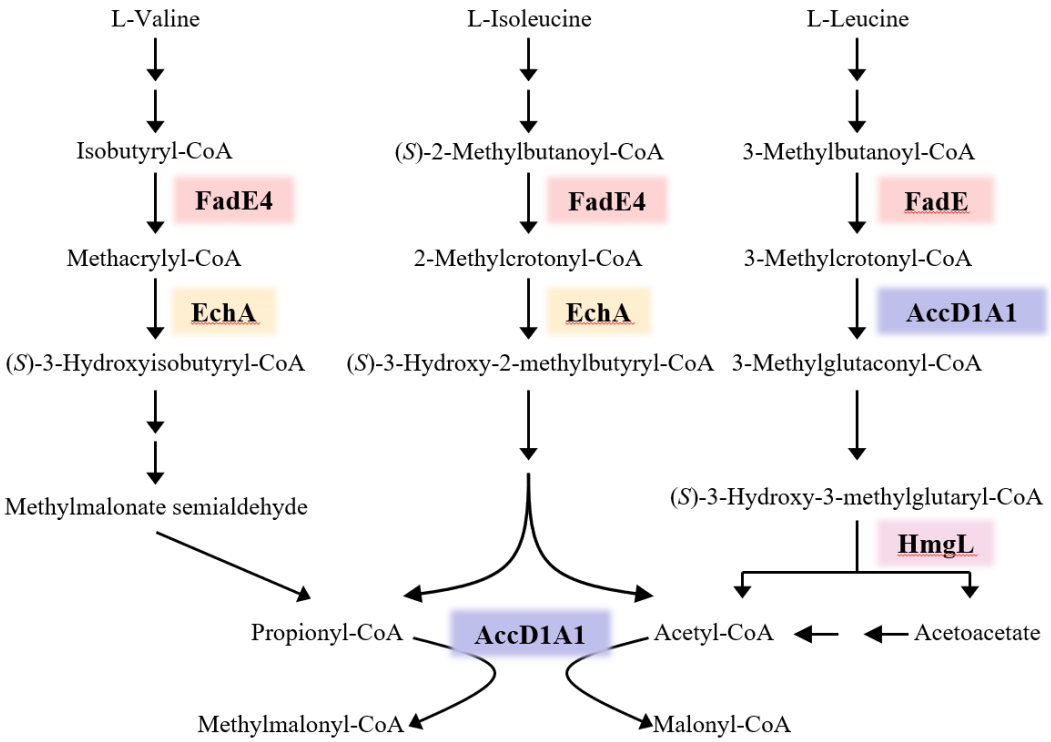

Fig. S8. AccR represses the transcription of genes involved in the branched-chain amino acid degradation pathway.

Fig. S9

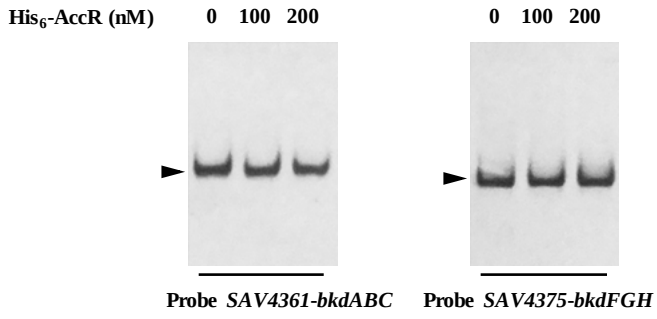

Fig. S9. EMSAs of AccR with intergenic regions of *SAV4361-bkdABC* and *SAV4375-bkdFGH.* Arrows: free DNA probes.