Supplementary information

Transcriptional regulator AcrR increases ethanol tolerance through regulating fatty acid synthesis in *Lactobacillus plantarum*

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Running Head: AcrR enhances ethanol tolerance of *L. plantarum*

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Table S1. Promoters and genes that may be regulated by AcrR during ethanol tolerance in *L. plantarum* NF92

Promoters	Genes	Locus_tags[a]	Products[b]	EMSA results[c]
P_{lp_0752}	*pspC*	EAV94_RS09020	PspC domain-containing protein	☆☆☆☆
P_{cfa2}	*cfa2*	EAV94_RS08825	Cyclopropane-fatty-acyl-phospholipid synthase	☆☆☆☆
P_{tagE6}	*tagE6*	EAV94_RS07265	Glycosyltransferase	☆☆☆☆
	tagE5	EAV94_RS07260	Accessory Sec system glycosyltransferase Asp1	
P_{fabZ1}	*fabZ1*	EAV94_RS01865	3-hydroxyacyl-ACP dehydratase FabZ	☆☆☆☆
	fabH2	EAV94_RS01870	Ketoacyl-ACP synthase III	
	acpP	EAV94_RS01875	Acyl carrier protein	
	fabD	EAV94_RS01880	ACP S-malonyltransferase	
	fabG1	EAV94_RS01885	SDR family oxidoreductase	
	fabF	EAV94_RS01890	Beta-ketoacyl-[acyl-carrier-protein] synthase II	

	accB2	EAV94_RS01895	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	
	fabZ2	EAV94_RS01900	Beta-hydroxyacyl-ACP dehydratase	
	accC1	EAV94_RS01905	Acetyl-CoA carboxylase biotin carboxylase subunit	
	accD1	EAV94_RS01910	Acetyl-CoA carboxylase carboxyl transferase subunit beta	
	accA1	EAV94_RS01915	Acetyl-CoA carboxylase carboxyl transferase subunit alpha	
	fabI	EAV94_RS01920	Enoyl-[acyl-carrier-protein] reductase FabI	
	sfp	EAV94_RS01925	4'-phosphopantetheinyl transferase superfamily protein	
P_{murA1}	*murAB*	EAV94_RS01085	UDP-N-acetylglucosamine1-carboxyvinyltransferase	☆☆☆☆
P_{cfa1}	*cfa1*	EAV94_RS01990	Cyclopropane-fatty-acyl-phospholipid synthase	☆☆☆☆
P_{alr}	*alr*	EAV94_RS01145	Alanine racemase	☆☆☆☆
P_{lp_3292}	_	EAV94_RS16380	Hypothetical protein	☆☆☆
P_{nrdH}	*nrdH*	EAV94_RS09265	Redoxin NrdH	☆☆☆
P_{lplA2}	*lplA2*	EAV94_RS03535	Lipoate--protein ligase	☆☆☆

P_{fum}	*fum*	EAV94_RS10395	Class II fumarate hydratase	☆☆☆
P_{tmcA}	*tmcA*	EAV94_RS00530	tRNA(Met) cytidine acetyltransferase TmcA	☆☆☆
P_{fabH1}	*fabH1*	EAV94_RS13665	Ketoacyl-ACP synthase III	☆☆☆
	accB1	EAV94_RS13660	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	
	accC2	EAV94_RS13655	Acetyl-CoA carboxylase biotin carboxylase subunit	
	accD2	EAV94_RS13650	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	
	accA2	EAV94_RS13645	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	
P_{clpE1}	*clpE1*	EAV94_RS13950	ATP-dependent Clp protease ATP-binding subunit	☆☆☆
P_{lp_1233}	*tuaA*	EAV94_RS10110	Putative undecaprenyl-phosphate N-acetylgalactosaminyl 1-phosphate transferase	☆☆☆
P_{potA}	*potA*	EAV94_RS00360	ABC transporter ATP-binding protein	☆☆☆
P_{trmFO}	*trmFO*	EAV94_RS05580	FADH(2)-oxidizing Methylenetetrahydrofolate-tRNA-(uracil(54)-C(5))-methyltransferase	☆☆☆

			TrmFO	
P_{lplA1}	*lplA1*	EAV94_RS03905	Lipoate--protein ligase	☆☆☆
P_{ftsA}	*ftsA*	EAV94_RS08305	Cell division protein FtsA	☆☆☆
	ftsZ	EAV94_RS08300	Cell division protein FtsZ	
	lp_2192	EAV94_RS08295	Cell division protein SepF	
	lp_2191	EAV94_RS08290	YggT family protein	
	lp_2190	EAV94_RS08285	RNA-binding protein	
P_{glyQ}	*glyQ*	EAV94_RS14145	Glycine--tRNA ligase subunit alpha	☆☆☆
P_{murD}	*murD*	EAV94_RS08320	UDP-N-acetylmuramoyl-L-alanine--D-glutamate ligase	☆☆☆
	murG	EAV94_RS08315	N-acetylglucosamine transferase	
	ftsQ	EAV94_RS08310	Cell division protein FtsQ	
P_{ribD}	*ribD*	EAV94_RS04290	Bifunctional diaminohydroxyphosphoribosylaminopyrimidine deaminase	☆☆☆

			RibD	
	ribE	EAV94_RS04295	Riboflavin synthase	
	ribBA	EAV94_RS04300	Bifunctional 3,4-dihydroxy-2-butanone-4-phosphate synthase/GTP cyclohydrolase II	
	ribH	EAV94_RS04305	6,7-dimethyl-8-ribityllumazine synthase	
P_{dak1A}	*dhaQ*	EAV94_RS03075	DhaKLM operon coactivator DhaQ	☆☆☆
P_{murB}	*murB*	EAV94_RS09980	UDP-N-acetylenolpyruvoylglucosamine reductase	☆☆☆
P_{racD}	*racD*	EAV94_RS04565	Aspartate/glutamate racemase family protein	☆☆☆
P_{accB3}	*accB3*	EAV94_RS00545	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	☆☆
P_{ezrA}	*ezrA*	EAV94_RS12610	Septation ring formation regulator EzrA	☆☆
P_{lp_1659}	*pncA*	EAV94_RS01820	Cysteine hydrolase	☆☆
P_{lp_2866}	_	EAV94_RS07380	Hypothetical protein	☆☆
P_{ica2}	*ica2*	EAV94_RS08070	Putative glycosyltransferase, exosortase G system-associated	☆☆

P_{murE}	*murE*	EAV94_RS06000	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2, 6-diaminopimelate ligase	☆☆
P_{aad}	*ddpX*	EAV94_RS08950	D-alanyl-D-alanine dipeptidase	☆☆
P_{acm2}	*acm2*	EAV94_RS03900	Cell wall hydrolase	☆☆
P_{fabK}	*fabK*	EAV94_RS10650	2-nitropropane dioxygenase	☆☆
P_{plsC}	*plsC*	EAV94_RS07845	1-acyl-sn-glycerol-3-phosphate acyltransferase	☆☆
P_{tagA}	*tagA*	EAV94_RS13755	Glycosyltransferase	☆
P_{hsp3}	*hsp3*	EAV94_RS11830	Hsp20/alpha crystallin family protein	☆
P_{dps4}	*dps4*	EAV94_RS16820	DNA starvation/stationary phase protection protein	☆
P_{lp_1457}	*cotS*	EAV94_RS04410	Phosphotransferase	☆
P_{lp_3245}	–	EAV94_RS08525	Hypothetical protein	☆
P_{lp_1454}	*hit*	EAV94_RS04395	HIT family protein	☆
	ecsA	EAV94_RS04390	Membrane protein	

P_{clpL}	*clpL*	EAV94_RS12915	ATP-dependent Clp protease ATP-binding subunit	☆
P_{hsp1}	*hsp1*	EAV94_RS02930	Hsp20/alpha crystallin family protein	☆
P_{lp_1812}	_	EAV94_RS05445	Hypothetical protein	☆
P_{pyrP}	*pyrP*	EAV94_RS15225	Uracil transporter	☆
P_{cggR}	*cggR*	EAV94_RS15830	Central glycolytic genes regulator	☆
P_{mraZ}	*mraZ*	EAV94_RS08345	Transcriptional regulator MraZ	☆
	rsmH	EAV94_RS08340	16S rRNA (cytosine(1402)-N(4))-methyltransferase RsmH	
	ftsL	EAV94_RS08335	Cell division protein FtsL	
	pbpB	EAV94_RS08330	PASTA domain-containing protein	
	mraY	EAV94_RS08325	Phospho-N-acetylmuramoyl-pentapeptide-transferase	
P_{cdsA}	*cdsA*	EAV94_RS07895	Phosphatidate cytidylyltransferase	☆
P_{oatA}	*oatA*	EAV94_RS09795	Acetyltransferase	☆
P_{oatB}	*oatB*	EAV94_RS10605	Acetyltransferase	☆

P_{asnB1}	*asnB*	EAV94_RS06010	Asparagine synthase (glutamine-hydrolyzing)	☆
P_{tagE4}	*tagE4*	EAV94_RS04880	Glycosyltransferase	×
P_{tarI}	*tarI*	EAV94_RS05465	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	×
	tarJ	EAV94_RS05470	Ribitol-5-phosphate 2-dehydrogenase	
	tarK	EAV94_RS05475	Ribitol phosphotransferase	
	tarL	EAV94_RS05480	Ribitol phosphotransferase	
P_{dnaJ}	*dnaJ*	EAV94_RS08005	Molecular chaperone DnaJ	×
P_{groES}	*groEL*	EAV94_RS09120	Co-chaperone GroES	×
	groES	EAV94_RS09115	Chaperonin GroEL	
P_{pbpX2}	*pbpX2*	EAV94_RS08010	Serine-type D-Ala-D-Ala carboxypeptidase	×
	dltX	EAV94_RS08015	Teichoic acid D-Ala incorporation-associated protein DltX	
	dltA	EAV94_RS08020	D-alanine-poly(phosphoribitol) ligase subunit DltA	
	dltB	EAV94_RS08025	D-alanyl-lipoteichoic acid biosynthesis protein DltB	

	dltC	EAV94_RS08030	D-alanine--poly(phosphoribitol) ligase subunit DltC	
P_{hrcA}	*hrcA*	EAV94_RS07990	Heat-inducible transcriptional repressor HrcA	×
	grpE	EAV94_RS07995	Nucleotide exchange factor GrpE	
	dnaK	EAV94_RS08000	Molecular chaperone DnaK	
P_{mae}	*mae*	EAV94_RS06530	NADP-dependent malic enzyme	×
	citc	EAV94_RS06535	[citrate (pro-3S)-lyase] ligase	
	citD	EAV94_RS06540	Citrate lyase acyl carrier protein	
	citE	EAV94_RS06545	Citrate (pro-3S)-lyase subunit beta	
	citF	EAV94_RS06550	Citrate lyase subunit alpha	
P_{lp_1357}	_	EAV94_RS06860	Membrane protein	×
P_{lp_1773}	_	EAV94_RS02315	Hypothetical protein	×
P_{lp_2526}	_	EAV94_RS05000	GNAT family N-acetyltransferase	×
P_{lp_3351}	_	EAV94_RS11835	Hypothetical protein	×

P_{oppb}	*oppb*	EAV94_RS13920	ABC transporter permease	×
P_{rodZ}	*rodZ*	EAV94_RS11085	Helix-turn-helix domain-containing protein	×
	pgsA	EAV94_RS11080	CDP-diacylglycerol--glycerol-3-phosphate-3-phosphatidyltransferase	
P_{dhaT}	*dhaT*	EAV94_RS09500	1,3-propanediol dehydrogenase	×
P_{suf}	*sufC*	EAV94_RS04455	Fe-S cluster assembly ATPase SufC	×
	sufD	EAV94_RS04460	Fe-S cluster assembly protein SufD	
	sufS	EAV94_RS04465	Cysteine desulfurase	
	iscu	EAV94_RS04470	SUF system NifU family Fe-S cluster assembly protein	
	sufB	EAV94_RS04475	Fe-S cluster assembly protein SufB	
P_{ctsR}	*ctsR*	EAV94_RS06180	CtsR family transcriptional regulator	×
	clpC	EAV94_RS06185	ATP-dependent Clp protease ATP-binding subunit	
P_{cps4A}	*cps4A*	EAV94_RS11555	Polysaccharide biosynthesis protein	×
	cps4B	EAV94_RS11560	CpsD/CapB family tyrosine-protein kinase	

	cps4C	EAV94_RS11565	polysaccharide biosynthesis protein	
	cps4D	EAV94_RS11570	NAD-dependent epimerase/dehydratase family protein	
	cps4E	EAV94_RS11575	Sugar transferase	
	cps4F	EAV94_RS11580	Glycosyltransferase family 4 protein	
	cps4G	EAV94_RS11585	Glycosyltransferase	
	cps4H	EAV94_RS11590	Polysaccharide polymerase	
	cps4I	EAV94_RS11595	Glycosyltransferase	
	cps4J	EAV94_RS11600	Oligosaccharide flippase family protein	
P_{tagD1}	*tagD1*	EAV94_RS00115	Glycerol-3-phosphate cytidylyltransferase	×
	tagF1	EAV94_RS00120	CDP-glycerol glycerophosphotransferase family protein	
	tagF2	EAV94_RS00125	Glycosyltransferase	
P_{murA2}	*murA*	EAV94_RS15180	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	×
P_{tagE1}	*tagE1*	EAV94_RS07055	Glycosyltransferase	×

P_{pyrB}	*pyrB*	EAV94_RS03650	Aspartate carbamoyltransferase catalytic subunit	×
	pyrC	EAV94_RS03655	Dihydroorotase	
	pyrAA	EAV94_RS03660	Carbamoyl phosphate synthase small subunit	
	pyrAB	EAV94_RS03665	Carbamoyl-phosphate synthase large subunit	
	pyrD	EAV94_RS03670	Dihydroorotate dehydrogenase	
	pyrF	EAV94_RS03675	Orotidine-5'-phosphate decarboxylase	
	pyrE	EAV94_RS03680	Orotate phosphoribosyltransferase	
P_{lp_1535}	*yceD*	EAV94_RS04620	DNA-binding protein	×
P_{nusG}	*nusG*	EAV94_RS15345	Transcription termination/antitermination protein NusG	×
P_{lp_2743}	*ccmA*	EAV94_RS03490	ABC transporter ATP-binding protein	×
P_{fthC}	*fthC*	EAV94_RS01425	5-formyltetrahydrofolate cyclo-ligase	×
P_{lp_1521}	*adh*	EAV94_RS04555	Zinc-binding alcohol dehydrogenase family protein	×
P_{mreB}	*mreB*	EAV94_RS12655	Rod shape-determining protein	×

	mreC	EAV94_RS12660	Rod shape-determining protein MreC	
	mreD	EAV94_RS12665	Rod shape-determining protein MreD	
	minC	EAV94_RS12670	Septum site-determining protein MinC	
	minD	EAV94_RS12675	Septum site-determining protein MinD	
P_{rsmG}	*rsmG*	EAV94_RS08710	16S rRNA (guanine(527)-N(7))-methyltransferase RsmG	×
	noc	EAV94_RS08715	Nucleoid occlusion protein	
	parA	EAV94_RS08720	ParA family protein	
	parB	EAV94_RS08725	ParB/RepB/Spo0J family partition protein	
	—	EAV94_RS08730	DUF951 domain-containing protein	
	ychF	EAV94_RS08735	Redox-regulated ATPase YchF	
	—	EAV94_RS08740	DUF1129 domain-containing protein	
P_{gidA}	*mnmE*	EAV94_RS12760	tRNA uridine-5-carboxymethylaminomethyl(34) synthesis GTPase MnmE	×

	mnmG	EAV94_RS12765	tRNA uridine-5-carboxymethylaminomethyl (34) synthesis enzyme MnmG	
P_{lp_0995}	—	EAV94_RS06070	Hypothetical protein	×
P_{tagE2}	*gtf1*	EAV94_RS07030	Glycosyl transferase family 1	×
	tagE3	EAV94_RS07025	Glycosyltransferase	
	tagE2	EAV94_RS07020	Accessory Sec system glycosyltransferase Asp1	
	—	EAV94_RS07015	Hypothetical protein	
P_{tgt}	*tgt*	EAV94_RS11005	tRNA guanosine (34) transglycosylase Tgt	×
	yajC	EAV94_RS11000	Preprotein translocase subunit YajC	
P_{pyrR1}	*pyrR*	EAV94_RS03645	Bifunctional pyr operon transcriptional regulator PyrR	×
P_{murC}	*murC*	EAV94_RS04435	UDP-N-acetylmuramate--L-alanine ligase	×
P_{murF}	*murF*	EAV94_RS01125	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D- alanine ligase	×
P_{murI}	*racE*	EAV94_RS10940	Glutamate racemase	×

P_{ftsW}	*ftsW*	EAV94_RS08060	FtsW/RodA/SpoVE family cell cycle protein	×

[a] The locus_tags of these genes in *L. plantarum* NF92.

[b] The description about these genes was based on their genome annotation data.

[c] EMSA selecting results. Stars, minimum concentration of AcrR binding to promoters. ☆☆☆☆, 10 nM; ☆☆☆, 50 nM; ☆☆: 100 nM; ☆, 500 nM; ×, No combination even the concentration of AcrR up to 1000 nM; —, Unkown.

Color code for genes that significantly expressed under 8% ethanol stress in ref. 21. Red color: these genes were up-regulated under 8% ethanol stress for 30 min and 24 h. Green color: these genes were down regulated under 8% ethanol stress for 30 min and 24 h. Cyan: these genes were up-regulated at 30 min and down regulated at 24 h under 8% ethanol. Gray: these genes were down regulated at 30 min and up-regulated at 24 h under 8% ethanol.

Table S2. Composition and percentage of fatty acids in *L. plantarum* NF92 under 9% (v/v) ethanol stress

Fatty acid[a]	[b]% of total fatty acids		
	WT	△*acrR*	P-*acrR*
C14:0	9.159±0.260	8.401±0.665	7.374±1.026
C15:0	0.155±0.004	0.196±0.016	0.207±0.093
C16:0	20.443±0.302	20.693±1.915	22.221±2.205
C18:0	0.928±0.099	0.782±0.039	1.232±0.221
C14:1	0.286±0.041	0.502±0.021**	0.214±0.043
C16:1	16.343±0.148	14.625±0.628	13.455±3.214
CycC17:0	0.450±0.090	0.480±0.048	0.587±0.0163
C18:1	17.318±0.278	15.365±0.075**	23.550±2.965*
C18:2	5.334±0.072	6.767±0.435**	4.944±0.773
CycC19:0	29.584±0.240	32.189±1.016*	30.41±0.917
CFA/SFA	0.979±0.011	1.131±0.001**	1.046±0.085
CFA/UFA	0.765±0.012	0.888±0.014**	0.782±0.021
UFA/SFA	1.280±0.012	1.241±0.059	1.336±0.072

[a] C14:0, myristic acid; C15:0, pentadecanoic acid; C16:0, palmitic acid; C18:0, stearic acid; C14:1, myristoleic acid; C16:1, palmitoleic acid; C18:1, oleic acid; C18:2, octadecadienoic acid; cycC17:0 and cycC19:0, cyclopropane fatty acid.

[b] % of total fatty acids: the percentage of each fatty acid. values are expressed as mean standard deviation of three repeated samples. Significance test was carried out

between WT and △*acrR* by one-way analysis of variance (ANOVA). *, $P<0.05$; **, $P<0.01$.