SUPPLEMENTAL MATERIAL

Physiological, Genetic, and Transcriptomic Analysis of Alcohol-Induced Delay of Bacterial Death in *Escherichia coli*

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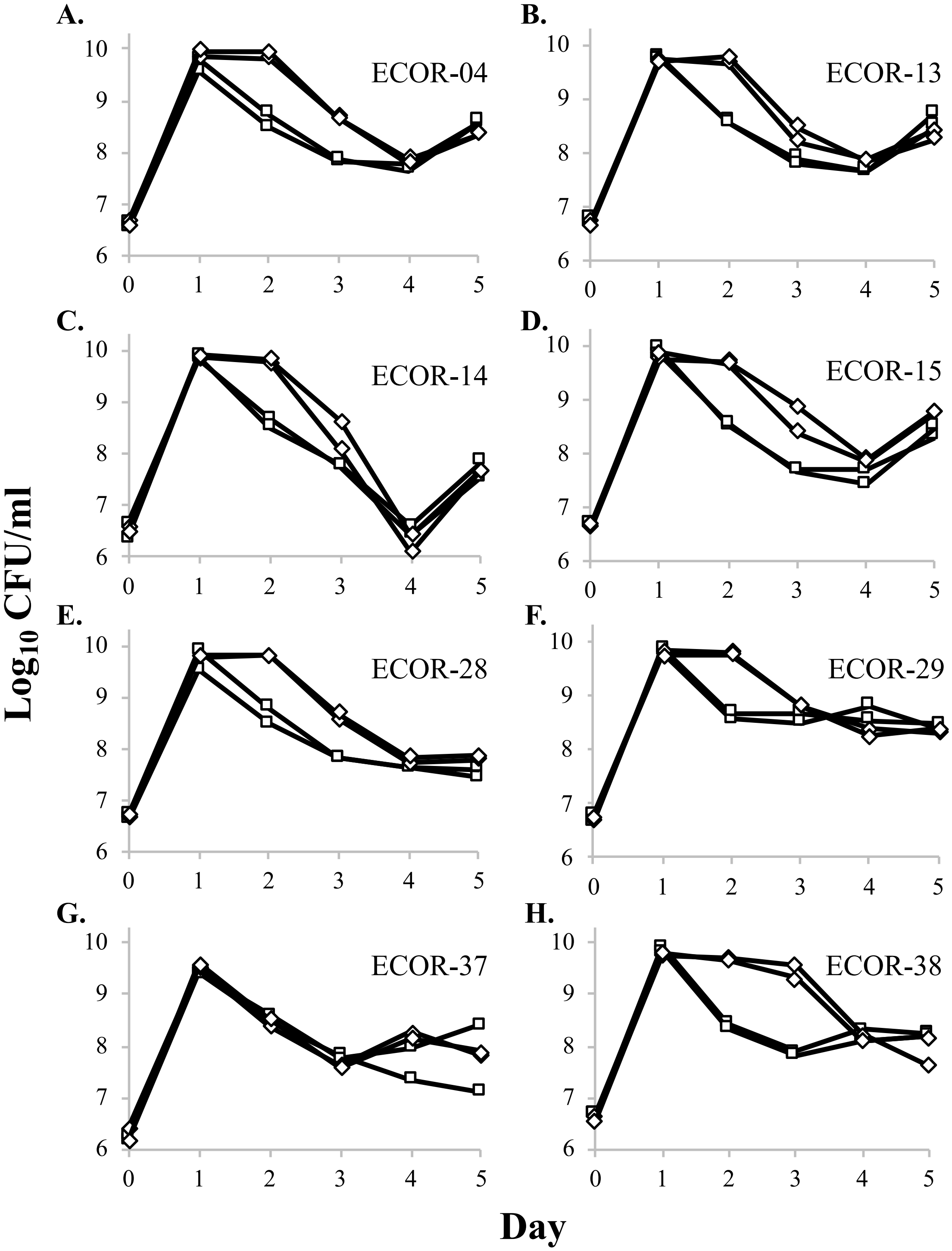
Table S1 | Significantly upregulated genes one hour after ethanol addition

Gene	Synonym(s)	Description	Fold Change	*p*-Value
glxR	*glxB1*, *ybbQ*	Tartronate semialdehyde reductase 2	36.25	7.10e-57
hyi	*ybbG*, *gip*	Hydroxypyruvate isomerase	19.83	1.28e-36
gcl		Glyoxylate carboligase	12.05	2.88e-75
glcD	*yghM*, *gox*	Glycolate dehydrogenase, putative FAD-linked subunit	11.46	2.14e-94
glcE	*yghL*, *gox*	Glycolate dehydrogenase, putative FAD-linked subunit	9.17	6.83e-50
cysA		Sulfate/thiosulfate ABC transporter ATP binding subunit	4.78	1.65e-34
cysI		Sulfite reductase, hemoprotein subunit	4.76	1.30e-20
frmR	*yaiN*	DNA-binding transcriptional repressor	4.63	7.96e-49
cysJ		Sulfite reductase, flavoprotein subunit complex	4.30	2.57e-30
asnA		Asparagine synthetase A	3.73	3.83e-28
cysD		Sulfate adenylyltransferase subunit 2	3.71	9.94e-32
cysH		Phosphoadenosine phosphosulfate reductase	3.69	3.82e-25
frmA	*adhC*	*S*-(hydroxymethyl) glutathione dehydrogenase, formaldehyde dehydrogenase	3.67	4.40e-23
allA	*glxA2*, *ybbT*	Ureidoglycolate lyase	3.47	2.67e-07
yciW		Putative oxidoreductase	3.42	9.62e-35
yeeD		Putative sulfurtransferase	3.11	3.65e-25
cysN		Sulfate adenyltransferase subunit 1	3.10	3.22e-27
cysW		Sulfate/thiosulfate ABC transporter inner membrane subunit	3.08	5.32e-18
mgtA	*atmA*, *corB*, *mgt*, *mtg*	Mg^{2+}-importer P-type ATPase	2.96	2.51e-24
cysU		Sulfate/thiosulfate ABC transporter inner membrane subunit	2.95	7.23e-13

14 **Table S2 | Significantly downregulated genes one hour after ethanol addition**

Gene	Synonym(s)	Description	Fold Change	*p*-Value
kdpF		K^+-transporting P-type ATPase subunit	-51.91	0.000183
elbA	*iraM*, *elb1*, *ycgW*	Anti-adaptor protein, inhibitor of σ^S proteolysis	-4.42	0.00126
ygeL		Uncharacterized protein	-4.30	0.0121
yadK		Putative fimbrial protein	-4.18	0.000641
yehC		Putative fimbrial chaperone	-4.04	0.00234
yhhH		PF15631 family protein	-3.92	0.00434
ydeM		Putative anaerobic sulfatase maturation enzyme	-3.89	0.000370
ybcQ		DLP12 prophage, putative antitermination protein	-3.86	0.00208
ygiZ		Conserved inner membrane protein	-3.62	0.00438
ypjC		DUF5507 domain-containing protein	-3.54	0.00779
ynfO		Qin prophage	-3.53	0.00105
ydeQ		Putative fimbrial adhesin protein	-3.48	0.00419
ydcC		H repeat-associated putative transposase	-3.39	0.000994
bglH	*yieC*	Carbohydrate-specific outer membrane porin, cryptic	-3.36	0.000515
yddL		Putative uncharacterized protein	-3.33	0.0185
yhaC		Uncharacterized protein	-3.33	0.00305
yohH	*mdtQ*, *yohG*	Putative multidrug resistance outer membrane protein	-3.31	0.00779
tdcR		DNA-binding transcriptional activator	-3.20	0.0191
ydeS		Putative fimbrial protein	-3.20	0.0181
yiaB		Conserved inner membrane protein	-3.17	0.0113
ycaK		Putative NAD(P)H-dependent oxidoreductase	-3.16	0.00487
yddK		Leucine-rich repeat domain-containing protein	-3.15	0.0180
agaB	*yraD*	Galactosamine-specific PTS enzyme IIB component	-3.14	0.0156
ygcW		Putative deoxygluconate dehydrogenase	-3.14	0.0112
yaiS		Putative deacetylase	-3.11	0.00508
ycjM	*ggaP*	Glucosylglycerate phosphorylase	-3.10	0.00389
ychS		Putative uncharacterized protein	-3.09	0.00479
essQ	*ydfS*	Qin prophage, putative S lysis protein	-3.08	0.00721

fixX	*yaaT*	Putative ferredoxin	-3.08	0.0171
yddJ		Uncharacterized protein	-3.07	0.00495
yqeK		Uncharacterized protein	-3.05	0.0105
yafT		Lipoprotein	-3.03	0.00859
yjfM		DUF1190 domain-containing protein	-3.00	0.00819
ygeK		Putative DNA-binding transcriptional regulator	-2.98	0.0200
nrfB	*yjcI*	Periplasmic nitrite reductase penta-heme *c*-type cytochrome	-2.97	0.00347
yafU		Putative inner membrane protein	-2.97	0.0108
ydfR		Qin prophage	-2.94	0.0183
ybcO		DLP12 prophage, putative nuclease	-2.93	0.0362
yhcA		Putative fimbrial chaperone	-2.92	0.0142
yhhZ		Putative endonuclease	-2.91	0.00417
sfmF	*ybcG*	Putative fimbrial protein	-2.89	0.0225
ybbD		Putative uncharacterized protein	-2.89	0.00623
mokC	*gefL*	Regulatory protein	-2.88	0.00399
pin	*argU*, *dnaY*	tRNA-Arg(UCU)	-2.88	0.00784
yigE		DUF2233 domain-containing protein	-2.85	0.00122
ynbB		Putative CDP-diglyceride synthase	-2.84	0.00514
ydeT	*fmlC*	Fimbrial usher domain-containing protein	-2.83	0.00298


A.
ECOR-04
B.
ECOR-13
C.
ECOR-14
D.
ECOR-15
E.
ECOR-28
F.
ECOR-29
G.
ECOR-37
H.
ECOR-38
6
7
8
9
10
0
1
2
3
4
5
Log$_{10}$ CFU/ml
Day

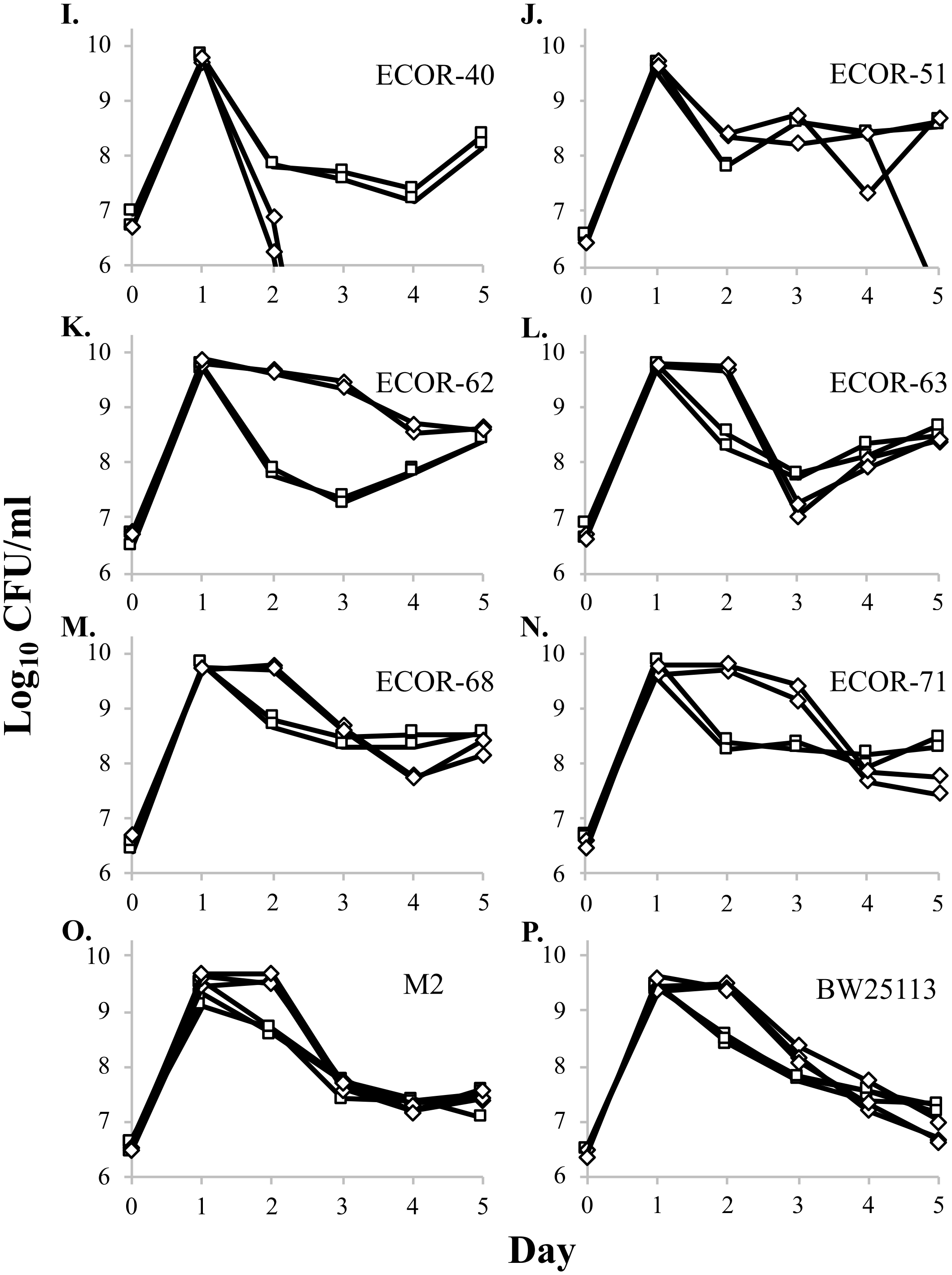


Figure S1 | The majority of natural isolate and laboratory strains show the delayed death effect induced by the addition of ethanol. The effect was identified across multiple different ECOR (1) strains and laboratory strains (2-4). Cultures were either left untreated (squares) or treated (diamonds) on Day 1. (A) ECOR-04; (B) ECOR-13; (C) ECOR-14; (D) ECOR-15; (E)

ECOR-28; (F) ECOR-29; (G) ECOR-37; (H) ECOR-38; (I) ECOR-40; (J) ECOR-51; (K) ECOR-62; (L) ECOR-63; (M) ECOR-68; (N) ECOR-71; (O) M2; and (P) BW25113 strains.

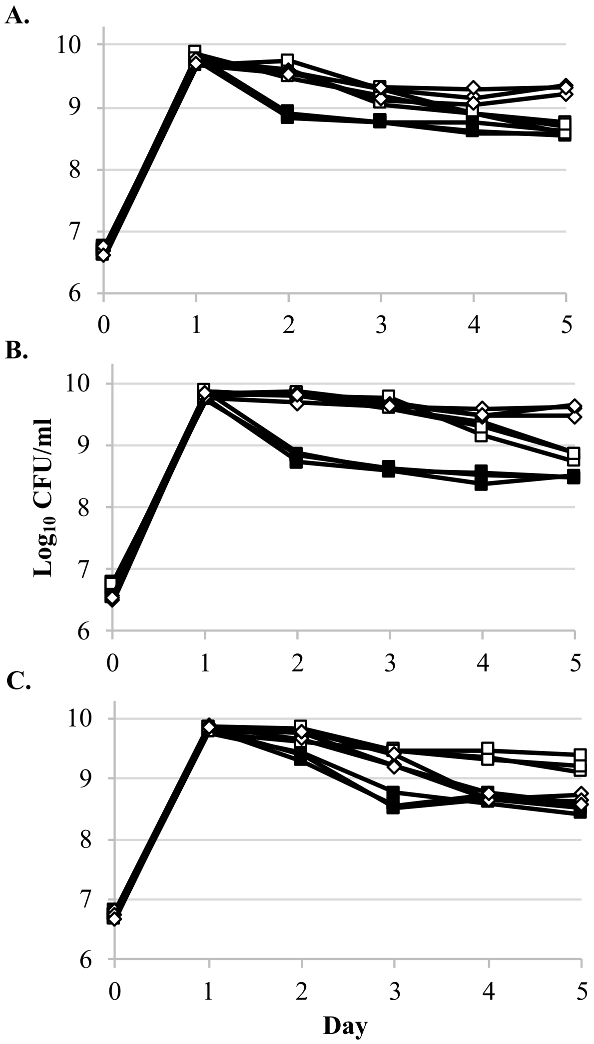


Figure S2 | Single gene null strains corresponding to mutations in glycolate degradation pathway genes have altered delayed death phenotype. The RNA-sequencing analysis (Table 3; Table S1; Table S2) showed significant upregulation of the *glxR*, *glcD*, and *gcl* genes. Here, we restructured the data from Figure 7 to make each panel correspond to the growth physiology of one of the null strains made. Untreated cultures (closed squares), 5 µl (~17.6 mM) ethanol (open diamonds), and ~17.6 mM 1-butanol (open squares). (A) GlxR null populations; (B) GlcD null populations; (C) Gcl null populations.

REFERENCES

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