Control of Acid and Antibiotic Resistance Systems by an RNAP Alpha Subunit Mutation in Benzoate Evolved *Escherichia coli*

Jeremy P. Moore ‘19 and Joan L. Slonczewski
Department of Biology, Kenyon College, Summer Science 2016

Abstract

From the long-term evolution of *Escherichia coli K12* in the presence of benzoate conducted by the Slonczewski laboratory, 6 clades of *Escherichia coli* have been isolated that have adapted to high benzoate concentrations. One strain, A1-1, shows increased benzoate fitness but lowered antibiotic resistance that may be due to mutations in some organic acid resistance regulators and *rpoA* (the RNA-polymerase alpha subunit). The mutant *rpoA* allele of A1-1 was reverted to wild-type through linked transduction of a kanamycin resistance marker at the nearby gene *yhdN*. The arginine decarboxylase (*adiAYC*) system, an acid resistance operon which had been previously non-functional, was re-activated in the *rpoA*+ construct. Also, the *rpoA*+ construct’s chloramphenicol resistance was restored to near wild-type levels although it showed lower fitness than its parent evolved strain in high benzoate concentration. Using real-time PCR, we were unable to demonstrate a reversion to wild-type expression of *marA* (a positive regulator of the *marRAB* operon which is involved in antibiotic resistance). It is possible the *rpoA* mutation is affecting antibiotic resistance through a non-*mar* pathway.

Introduction

- Multiple antibiotic resistance phenotype can be induced in *Escherichia coli* through interactions between salicylate and the *marRAB* operon (Cohen *et al*, 1993).
- *rpoA* encodes the alpha subunit of RNA polymerase. The alpha subunit comprises an N-terminal domain responsible for holoenzyme assembly, and a C-terminal domain that recognizes upstream promoter elements (A and T rich sequences upstream of the -35 box in many promoters) and trans-acting transcription factors. Mobility of the linker region between domains allows for more interactions with transcription factors (Jeon *et al*, 1997).
- *E. coli* were evolved for nearly 2000 generations in the presence of benzoate by the Slonczewski laboratory. Strains resulting from this experiment have increased fitness in 20 mM benzoate but lowered resistance to antibiotics (Creamer *et al.,* Submitted 2016).

Methods

- **Construction of A1-1 rpoA+:** An *rpoA* amino acid substitution in A1-1 was reverted to wild type by transducing a kanamycin resistance marker at the linked gene *yhdN* using P1 phage. The KAN marker was removed using flp recombinase.
- **Reversion confirmation:** A 260 bp region flanking the *rpoA* mutation was PCR amplified and sequenced in four successful *yhdN* deletants.
- **Decarboxylase assays:** Colonies were inoculated into 96-well plate wells with media containing L-lysine or L-arginine at pH 6.5 or 5.5 respectively. Bromocresol purple dye was used to indicate pH and would turn purple at higher pH. Ratio of yellow to purple light absorption was measured in a a spectramax and used to quantify relative expression of a decarboxylase.
- **Growth curves:** A 200 uL aliquot of growth media was innoculated with 1 µL of *E. coli*, and OD 600 values were read every 15 minutes for 22 hours. Growth curves were read kinetically in sterile 96-well plates in a spectramax spectrophotometer.
- **Real-time PCR:** Used to quantify expression of *marA.* Primers were designed using NCBI primer blast. Reverse transcriptase was included in the well plate to generate cDNAs during the run.

Differential expression in A1-1

a)

Gene	Fold Change	Gene	Fold Change
dctR	0.002131228	hycA	0.17795137
gadC	0.006733433	ydaQ	0.183489743
gadB	0.007873799	ariR	0.188513686
gadA	0.008890645	hyaE	0.193284721
yhiM	0.06701275	adiA	0.196650307
yedX	0.085374518	kil	0.199570393
hycB	0.107882543	hycG	0.202794396
narH_1	0.111546737	hycH	0.203290928
hydN	0.112093895	pgaA	0.206035606
narZ_1	0.124931379	ydfZ	0.207076698
ymgC	0.127105742	ydaF	0.210457156
narJ	0.132465655	rnb	0.211190579
hycF	0.136410613	ymgG	0.217166896
appC	0.139141219	hyaF	0.223486361
hycE	0.141465879	adiC	0.233761879
narI_1	0.147421179	entC	0.234452497
appB	0.167562221	fimC	0.241894449
hycC	0.174800141	ybdZ	0.247721725
hycD_2	0.174800201	hyaD	0.248542488
yjbE	0.175402202	hyaB	0.249493229

b)

Gene	Fold Change	Gene	Fold Change
glcE	62.03339958	dppC	10.10375764
lldD	42.07253895	sucD	9.596032888
yjcH	36.51142487	ecnB	9.335953933
astA	27.96533487	srlA	8.441457888
astC	27.44118925	sucC	8.408508456
lldR	26.03956976	dppB	8.293810087
garP	24.85133531	hyi	7.815990674
dppF	24.64102026	glcA	7.289504166
glcG	21.56761034	ugpE	7.044705292
dppD	18.25577217	fadM	6.72995272
astE	17.60388195	ysgA	6.687996073
putA	16.14467411	paaC	6.658284966
glcB	15.45336076	srlE	6.595790569
actP	14.47337805	aceA	6.452795864
garL	14.39860462	paaA	6.42910395
astD	13.83955859	garR	6.259980538
glcF	12.72580365	sucB	6.256475792
astB	12.46950811	osmY	6.190567027
lldP	11.98272403	dppA	5.864057223
yhjV	10.87174904	yjiY	5.776829254

Table 2. Most upregulated (**a**) and downregulated (**b**) genes in A1-1 compared to the wild-type in benzoate as determined by RNAseq. Genes that had been deleted entirely in the strain and calculated genes have not been included in the table.

A1-1 Mutations

Mutation	Annotation	Gene	Description
A→C	F63V (TTC→GTC)	folD ←	bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase
(C)8→9	intergenic (-85/+615)	elbA ← / ← ycgX	hypothetical protein/hypothetical protein
Δ4 :: insH (+) +4 bp	coding (79-82/267 nt)	ariR →	Biofilm repressor protein
C→A	E1459* (GAG→TAG)	yfhM ←	α2 macroglublin
C→A	P190P (CCG→CCT)	fucA ←	L-fuculose-1-phosphate aldolase
C→T	G373S (GGC→AGC)	rpoB ←	RNA polymerase subunit beta
A→G	N107S (AAC→AGC)	cpxA →	sensory histidine kinase in two-component regulatory system with CpxR
G→A	R320H (CGT→CAT)	bcsB →	regulator of cellulose synthase, cyclic di-GMP binding
Δ10,738 bp	insH-mediated	[gadW]–slp	[gadW], mdtF, mdtE, gadE, hdeD, hdeA, hdeB, yhiD, yhiF, slp
Δ204 bp	insH-mediated	slp ← / → insH	outer membrane lipoprotein/IS5 transposase and trans-activator
A→C	K271Q (AAA→CAA)	rpoA →	RNA polymerase subunit alpha

Table 1. List of mutations present in the A1-1 strain of the benzoate evolution experiment. Mutations were called using the breseq computational pipeline.

rpoA Point Mutation Lowers Antibiotic Resistance and *adiAYC* Expression

rpoA+ Growth Under Benzoate and Antibiotic Stress

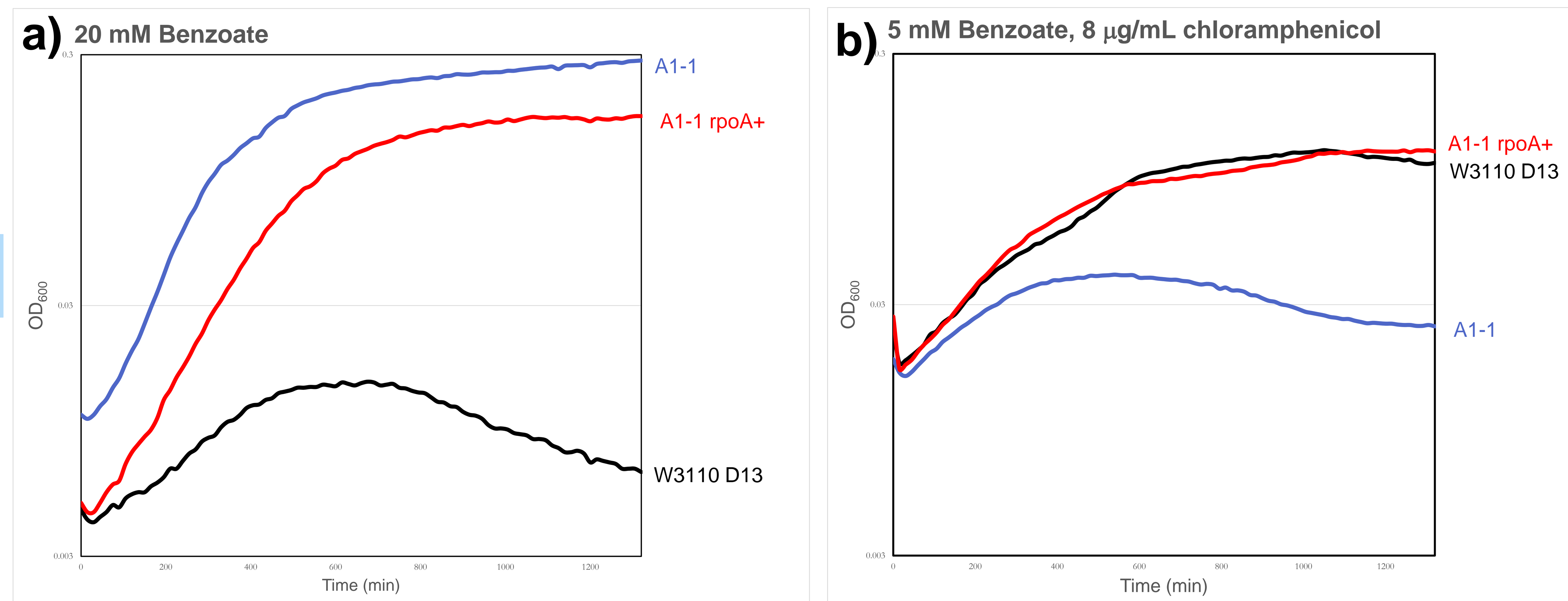

Figure 1. Representative growth curves of W3110 D13 (wild), A1-1, and A1-1 rpoA+. Representative curves were chosen based on their fit to statistical tests run on endpoint growth values. **a)** The rpoA revertant had slightly lower fitness in benzoate conditions, but was still far more successful than the wild-type (TukeyHSD, $p < 0.05$, $n = 24$). **b)** The rpoA reversion appears to have restored A1-1’s chloramphenicol resistance as the rpoA revertant had similar fitness in antibiotics as the wild-type (TukeyHSD, $p > 0.05$, $n = 24$). Statistical difference was determined using endpoint OD’s.

Decarboxylase Activity

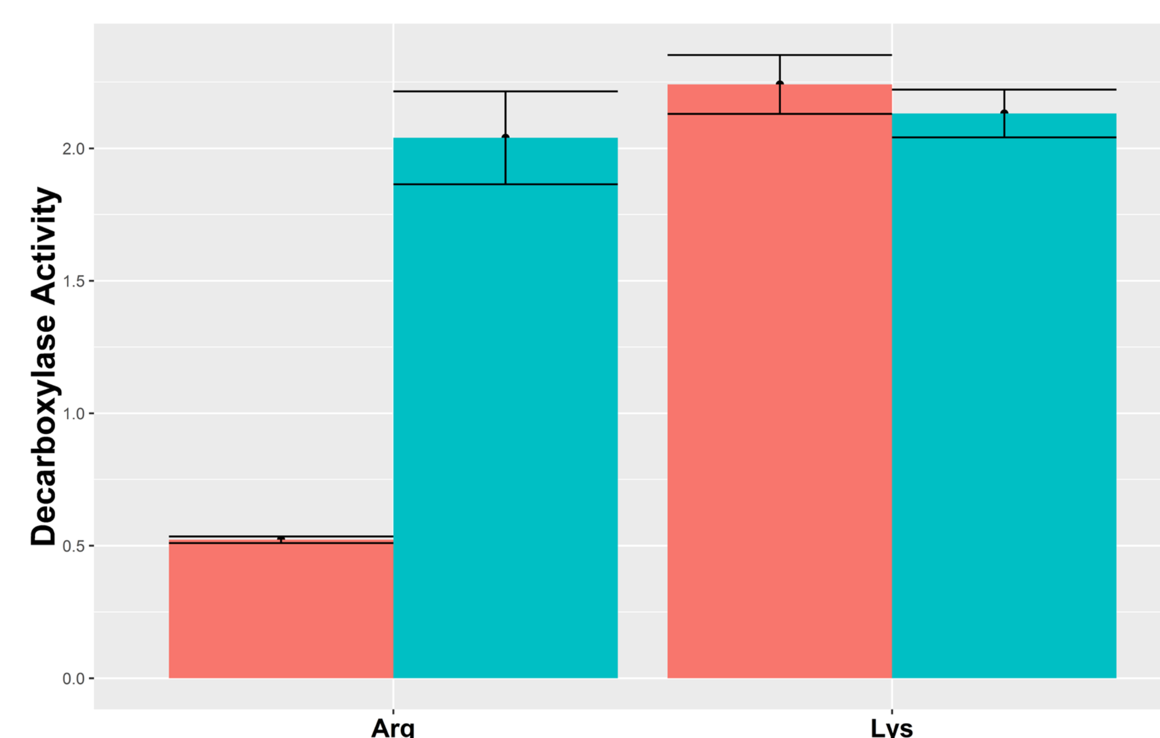

Figure 2. Activity of arginine and lysine decarboxylase systems in the benzoate evolved A1-1 strain and A1-1 a wild-type reverted *rpoA* locus. While the lysine decarboxylase appeared unaffected by the mutation, the rpoA mutation in A1-1 appeared to inhibit arginine decarboxylase activity. Bars = Mean Activity, Error bars = SEM

marA Expression

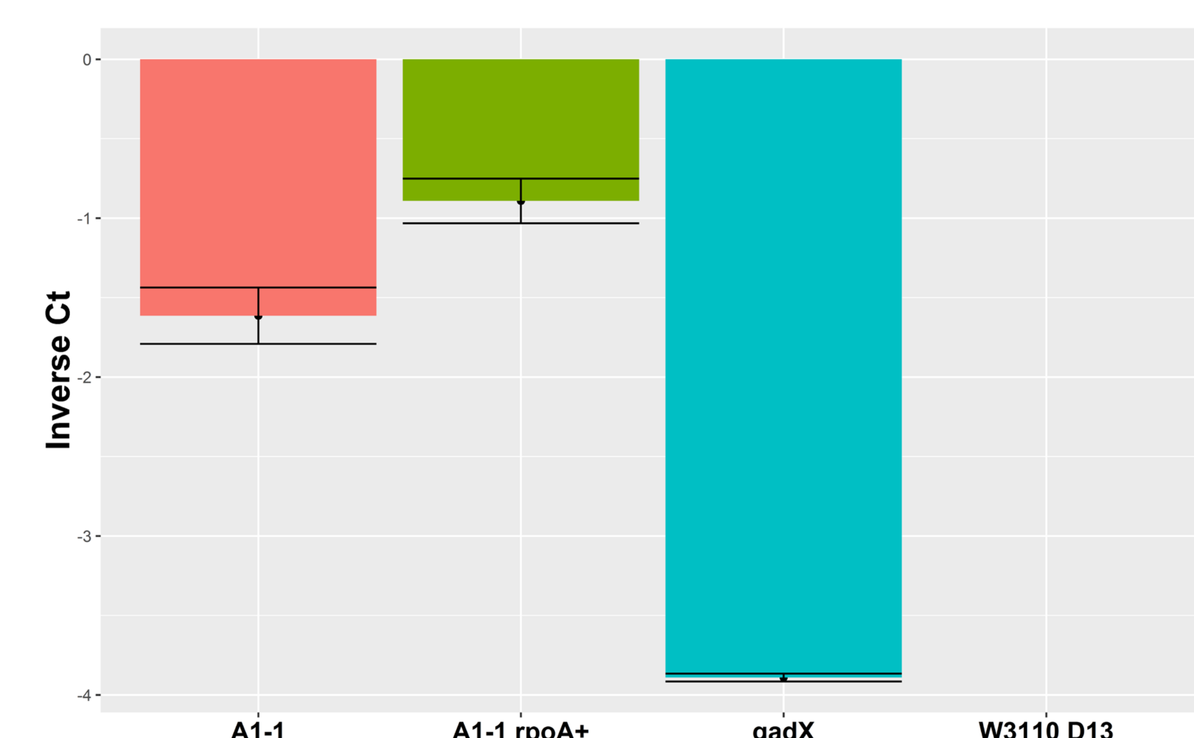

Figure 3. *marA* expression normalized to WT (W3110 D13). A *gadX* deletant appears to have reduced marA expression, but it is still unclear whether or not the rpoA mutation is the ultimate source of A1-1’s reduced chloramphenicol resistance. Bars = inverse log2(Ct), Error bars = SEM.

Conclusions

- *rpoA* mutation in A1-1 increases benzoate fitness by downregulating acid fitness genes, and reduces antibiotic resistance
  - Lowered expression of genes constitutively expressed under benzoate stress is common in our strains
  - Reduces energy stress
- Could also have effects on the *gad* regulon which was deleted in A1-1
  - Effects would be masked by a loss of *gad* late in the evolution
- Still unsure what causes increased benzoate fitness in A1-1
  - Possibly involves the deletion of *ymgB,* a known biofilm repressor that is activated by benzoate (Attila *et al.*, 2009; Kannan *et al.*, 2008)
- Biofilm formation could form a physical barrier against benzoate.
  - Will investigate biofilm formation in benzoate evolved strains with 96-well plate crystal violet assay and microscopy techniques

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References

1. Cohen SP, Levy SB, Foulds J, Rosner JL. 1993. Salicylate induction of antibiotic resistance in *Escherichia coli*: activation of the *mar* operon and a *mar*-independent pathway. Journal of Bacteriology 175(24): 7856 – 7862.
2. Jeon YH, Yamazaki T, Otomo T, Ishihama A, Kyogoku Y. 1997. Flexible linker in the RNA polymerase alpha subunit facilitates the independent motion of the C-terminal activator of the contact domain. J. Mol. Biol. 267: 953 – 962.
3. Attila c, Ueda A, Wood TK. 2009. 5-Flourouracil reduces biofilm formation in *Escherichia coli* K-12 through global regulator AriR as an antivirulence compound. Appl. Micriobiol Biotechnol 82: 525 – 533.
4. Kannan G, Wilks JC, Fitzgerald DM, Jones BD, BonDurant SS, Slonczewski JL. 2008. Rapid acid treatment of *Escherichia coli:* transcriptomic response and recovery. BMC Microbiology 8:37.