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RNA polymerase mutations and decarboxylase activity in acid-evolved strains of Escherichia coli

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RNA polymerase mutations and decarboxylase activity in acid-evolved strains of *Escherichia coli*

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Abstract

Escherichia coli can grow in mild acid (pH 4.6-6.5) and survive extreme acid (pH 1.5-3), allowing colonization of the human gut. To study how *E. coli* adapts to acidic conditions at the low end of its growth range, we previously conducted a 2000-generation laboratory evolution project of *E. coli* at pH 4.6 buffered with 100 mM HOMOPIPES and 40 mM malate (Harden et al ., 2015). The evolved isolates, each of which acquired several selective mutations, have higher fitness compared to the ancestral strain when competed in acid. Each strain sustained one mutation in RNA polymerase (RNAP), in either *rpoB, rpoC* or *rpoD*. To study the fitness contribution of these mutations, we competed the evolved isolates against strains with the ancestral RNAP allele. One of the evolved isolates, B11-1 showed a loss in fitness after the replacement of its evolved *rpoC* with the ancestral sequence. Additionally, the majority of the evolved isolates lost lysine decarboxylase (*cadA*) activity, suggesting that overexpression of this acid-resistance mechanism and others may be energetically unfavorable in a buffered system. To examine the differences in decarboxylase gene expression between the ancestral stain and the evolved isolates, we conducted qRT-PCR using RNA isolated at anaerobic pH 5. Strains B11-1 and F9-2 showed lower arginine decarboxylase (*adiA*) expression than the ancestral strain.

Introduction

Pathogenicity of *Escherichia coli* depends on its ability to contend with acidic pH of the human gut.[1]

- *E. coli* maintains internal pH homeostasis in moderate acid and can survive extreme acid for several hours.[2,3]

E. coli has acid-inducible mechanisms that change the extracellular pH by consuming cytoplasmic protons.

- *E. coli* produces decarboxylases for substrates such as lysine (*cadA*) and arginine (*adiA*).[4,5]
- Maximum *cadA* expression occurs at pH 5.5, and *adiA* peaks at pH 5.0.[6]
- Both decarboxylase systems are most highly expressed under anaerobic conditions.

One way to study how *E. coli* adapts to stressful conditions is through long-term laboratory evolution. Recently, a low-pH (4.6-4.8) evolution project was conducted, with cultures continuously grown in a buffered system for 2000 generations.[7]

- Evolved isolates have an acid-specific competitive advantage over the ancestral strain
- The majority of the isolates lost CadA activity
- One strain has a missense mutation in *adiY*, the regulator of *adiA*.

Each isolate sustained one mutation in RNA polymerase in either *rpoB, rpoC* or *rpoD*.

- We examined the role of the RNAP mutations by competing each evolved isolate with a strain in which the evolved RNAP allele had been replaced with the ancestral sequence.

The acid-evolved *E. coli* may have altered acid-resistance gene expression

- We conducted qRT-PCR to compare decarboxylase expression between the wild type and ancestral strains at acidic pH levels.

Methods

Experimental Evolution: Independent populations of *E. coli* originating from W3110-D8 were continuously grown in moderate acid (pH 4.8, LBK_{mal} buffered with 100 mM HOMOPIPES) at 37 ºC. After 770 generations, the pH was lowered to 4.6. After 2000 generations, selected isolates were sequenced.

Transductions: Genes of interest were transduced into host strains using P1vir phage transduction. The region of interest was PCR-amplified and sequenced to identify cotransductants and recombinants.

Competition Assays: Two strains, one containing a *Δlac* marker, were incubated with shaking in LBK_{mal}, pH 4.6 for 24 hours. Before and after incubation, samples were serially diluted into M63 minimal media and plated. The ratio of blue and white colonies was used to determine the relative fitness of competing strains.

RNA isolation: RNA was isolated, using an ice-cold solution of ethanol with 10% phenol, from cultures grown in media buffered at pH 4.6 (aerobic), pH 5 or pH 5.5 (anaerobic). RNA was purified using an RNeasy kit (Qiagen) with DNase treatment.

qRT-PCR: Primer sequences were designed using Primer Express and supplied by Invitrogen. Gene expression was quantified using SYBR Green PCR One-Step protocol. Cycling conditions were 48 ºC for 30 min, 95 ºC for 10 min, 92 ºC for 40 cycles of 15 s denaturation, and 60 ºC for 1 min. Target gene expression was normalized to the total RNA in the reaction. Average C_t values were calculated from 3 biological replicates run in triplicate.

Results

Replacement of B11-1 *rpoC* mutation with ancestral sequence leads to a fitness decrease.

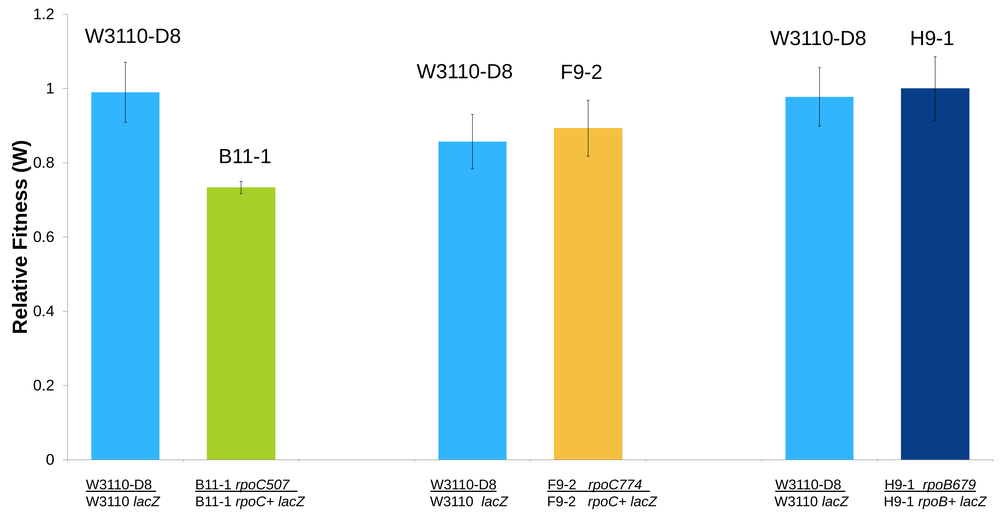

Figure 1: Relative fitness values of *Δlac* evolved strains B11-1, F9-2, and H9-1 against counterpart strain transduced to have the ancestral RNAP sequence, compared to W3110-D8 competed against W3110*Δlac*. W value of lower than 1 indicates that absence of RNAP mutation is associated with a fitness decrease. Error bars are SEM.

Compared to the parent strain, acid-evolved isolates underexpress *adiA* at anaerobic pH 5.

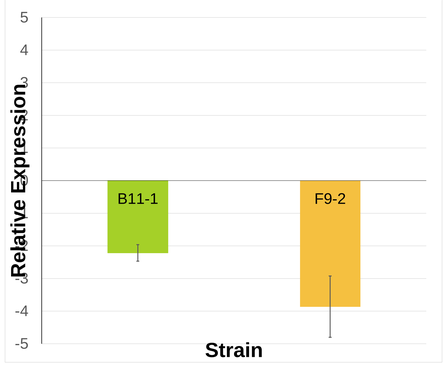

Figure 2: *adiA* expression of pH 4.6 evolved strains, normalized to W3110-D8 levels. RNA was isolated from cultures grown anaerobically at pH 5. Error bars are SEM.

Strain Profiles

Well Position	B11-1	F9-2	H9-1
CadA Activity	Negative	Positive	Negative
Gad Activity	Positive	Negative	Positive
adiY mutation	Present	Not present	Not present
RNAP mutation	V507L	I774S	A679V
Mutation function	*rpoC*	*rpoC*	*rpoB*

Table 1: Selected mutations observed in three isolates of pH 4.6 experimental evolution. Sequences were compared to the *E. coli* K-12 reference genome and mutations determined using a Breseq computational pipeline.

Conclusions

RNA Polymerase Mutations and Fitness

In one of the evolved populations, B11-1, replacing the *rpoC* mutation with the ancestral sequence results in a strain with lower acid fitness.

There was no difference observed in fitness of F9-2 or H9-1 upon replacement of the RNAP mutation.

- RNAP mutations likely occurred early in experimental evolution.
- These mutations on their own may be less important for overall fitness in the 2000-generation isolates.
- RNAP mutations could have been maintained because they increased the evolvability of the strain.

Competing the ancestral strain with and without the RNAP mutations could give a more accurate representation of the evolutionary benefit of these mutations.

Decarboxylase Activity

The evolved isolates B11-1 and F9-2 have lower arginine decarboxylase expression than the parent strain under conditions of peak expression for the enzyme (anaerobic, pH 5).

- The production of decarboxylases may be energetically inefficient under the experimental conditions.
- If medium and periplasm are strongly buffered, decarboxylase activity could be wasting amino acids. Decarboxylated products would be exported, but pH would not change.
- B11-1 has a mutation in the regulator of adiA, which may serve to lower expression.

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References

1. Evans, DF, Pye, G, Bramley, R., Clark, AG, Dyson, TJ, and Hardcastle, JD. 1988. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut, 29: 1035-1041..
2. Slonczewski, JL, Rosen, BP, Alger, JR, and Macnab, RM. 1981. pH homeostasis in *Escherichia coli*: Measurement by 31P nuclear magnetic resonance of methylphosphonate and phosphate. Proceedings of the National Academy of Sciences of the , 78: 6271-6275.
3. Lin, J, Lee, IS, Frey, J, Slonczewski, JL, & Foster, JW. (1995). Comparative analysis of extreme acid survival in *Salmonella typhimurium, Shigella flexneri,* and *Escherichia coli.* Journal of Bacteriology, 177: 4097-4104.
4. Meng SY, Bennett G. 1992. Nucleotide sequence of the *Escherichia coli* cad operon: a system for neutralization of low extracellular pH. Journal of Bacteriology. 174:2659-2669.
5. Stim-Herndon KP, Flores TM, and Bennett GN. 1996. Molecular characterization of adiY, a regulatory gene which affects expression of the biodegradative acid-induced arginine decarboxylase gene (adiA) of *Escherichia coli*. Microbiology 142:1311-1320.
6. Kanjee, U, Gutsche, I, Ramachandran, S, and Houry, WA. 2011. The enzymatic activities of the Escherichia coli basic aliphatic amino acid decarboxylases exhibit a pH zone of inhibition. Biochemistry. 50: 9388-9398.
7. Harden, MM, He, A, Creamer, K, Clark, MW, Hamdallah, I, Martinez, KA, Kresslein, RL, Bush, SP, Slonczewski, JL. 2015. Acid-adapted strains of *Escherichia coli* K-12 obtained by experimental evolution. Appl Environ Microbiol, 81: 1932-1941.