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 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 mutations, have higher fitness compared to the ancestral strain when grown in acid. Each strain sustained one mutation in RNA polymerase (RNP), in either rpoC, rpoC or rpoD. To study the fitness contribution of these mutations, we competed the evolved isolates against strains with the ancestral RNP 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 strain and the evolved isolates, we conducted qRT-PCR using RNA isolated at acidic pH 5. Strains B11-1 and F9-2 showed lower arginine decarboxylase (adA) 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 E. coli has acid-inducible mechanisms that change the extracellular pH by producing cytoplasmic proton 

Methods
Experimental Evolution: Independent populations of E. coli originating from W3110-3B were continuously grown in moderate acid (pH 4.8, LB buffered with 100 mM HOMOPIPES) at 37°C. After 770 generations, the pH was lowered to 4.6. After 2000 generations, selected isolates were sequenced.

Transcriptions: Genes of interest were transduced into host strains using P1 Phage phage transduction. The region of interest was PCR-amplified and sequenced to identify outstanding recombinants and contaminants.

Competition Assays: Two strains, one containing a Δlac marker, were incubated with shaking in LB at 37°C, pH 4.6 for 24 hours. Before and after inoculation, samples were serially diluted into M3 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 (sorbic), pH 5 or pH 5.5 (anaerobic). Purified RNA was used as a template for qRT-PCR 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. The reaction conditions were 4°C for 30 min, 95°C for 10 min, 92°C for 40 cycles of 15 s denaturation, and 60°C for 1 min. Target fold change values were normalized to the total RNA in the reaction. Average CV values were calculated from 3 biological replicates run in triplicate.

Results

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

Figure 2: adA expression of pH 4.6 evolved strains, normalized to W3110-3B levels. RNA was isolated from cultures grown anaerobically at pH 5. Error bars are SEM.

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

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References