Laboratory Evolution of Acid-Adapted *Escherichia coli* Strains

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Abstract

Escherichia coli are normally able to grow at an external pH range of 5 to 9 [2]. In this experiment, we investigated whether *E. coli* were able to grow below pH 5. We grew twenty-four replicates of *E. coli* in moderately acidic media with malate, a carbon source which consumes acid. These populations grew and adapted in pH 4.8 media for 730 generations in pH 4.8 since September 17, 2012 before being lowered on January 7, 2013 to pH 4.6 for 770 generations. We predicted that the evolved strains would have a higher growth rate than the parental strain in moderate acid. The growth curves showed that the evolved strains had adapted specifically to the media they were grown in. Evolved strains showed faster growth in acid with malate than parental strains. Without the presence of malate, the evolved strains showed varied growth in comparison to the parental. An Enterotube test battery revealed the loss of lysine decarboxylase in wells A7, H9, and A11, which is known to be beneficial in combating acid stress in bacteria. As we continue to grow the evolved strains in moderate acid, we anticipate more acid resistant development.

Introduction

The gram negative bacterium, *Escherichia coli*, is able to grow in an external pH range of 5 to 9 [1]. *E. coli* have an intricate regulatory network, which allows for survival in acidic environments [2]. One of these regulatory networks is the utilization of lysine decarboxylase (CadA). CadA is an enzyme which converts the amino acid, lysine, into cadavarine [3]. In this process, CO_2 is removed from lysine and replaced with a proton.

By evolving a standard strain of *E. coli* to maintain growth in low pH, it would be easier to identify what genes were utilized to maintain acid resistance. Preliminary experiments have shown that *E. coli* are capable of evolving traits in response to their environment [1].

In these series of experiments, we anticipated the *E. coli* populations grown in moderate acid for 1500 generations would show increased growth in low pH in comparison to the parental *E. coli* strain.

Methods

Laboratory Evolution
Since September 17, 2012, an overnight culture of W3110 was diluted 1:4000 into LBK, 100mM HOMOPIPES 6g/L D/L malate, pH 4.8. *E. coli* growth was monitored with a spectrophotometer, set at 37°C to measure the absorbance for twenty-two hours at OD_{450} in intervals of fifteen minutes daily [5]. Within two hours of reading completion, cultures from the plate were diluted 1:4000 into fresh medium in a new sterile well plate. Over the summer, the medium was changed to LBK, 100mM HOMOPIPES 10g/l D/L malate, pH 4.6, and dilutions increased to 1:100.

Growth Curves
Overnight cultures were grown in LBK, pH 7.0 at 37°C, rotating. Overnights were diluted 1:400 into 20ml of buffered LBK with and without malate. The dilutions were shaken in aerobic conditions in the water shaker, set at 37°C. Cultures were read at OD_{600}. Each strain had three replicates. Growth rates and mean doubling rate was determined from linear growth.

Lysine Decarboxylase Test
Isolated colonies were transferred into a 96-well plate, each well containing 200µl of lysine decarboxylase broth, pH 6.5. The well plate incubated anaerobically at 37°C overnight. Color change from purple to yellow indicates that the pH is <5.2; lysine decarboxylase is not present.

Results

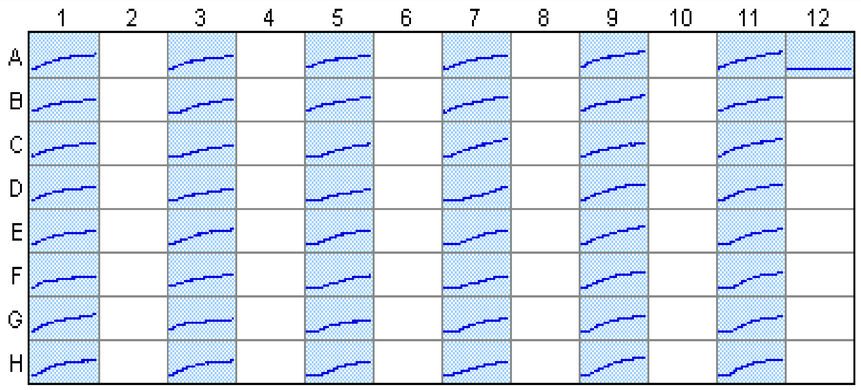

Figure 1: The growth curves of evolved strain populations. Each well contains a population of strains grown in moderate pH for over 1500 generations. Populations from column 7, 8, and 9 were bifurcated from columns 1, 3, and 5 respectively on January 7, 2013 in pH 4.6 media, while column 1, 3, and 5 were continued on pH 4.8 media until May 17, 2013.

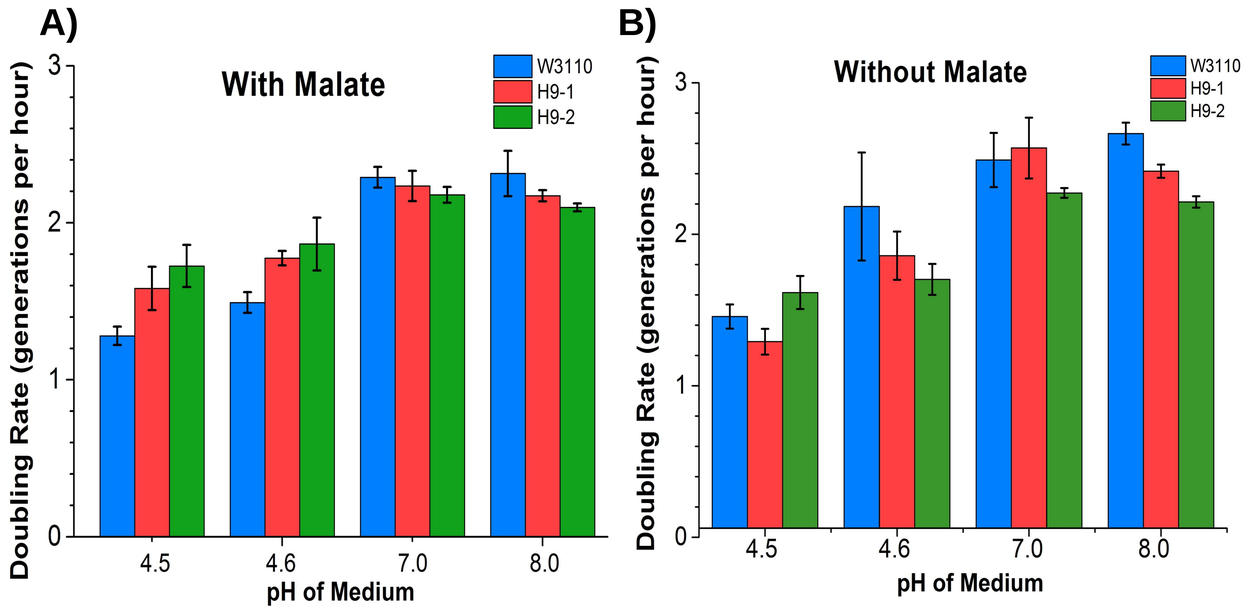

Figure 2: Evolved strains show higher growth rates than W3110 in moderate acid with malate. A) Doubling rate of evolved isolates and parental strain in presence of malate B) Doubling rate without malate present. Error bars = SEM. GLM, P_{pH} and $P_{pH*malate} < 0.05$ for all three groups. $P_{malate} < 0.05$ for W3110, but $P_{malate} > 0.05$ for evolved isolates.

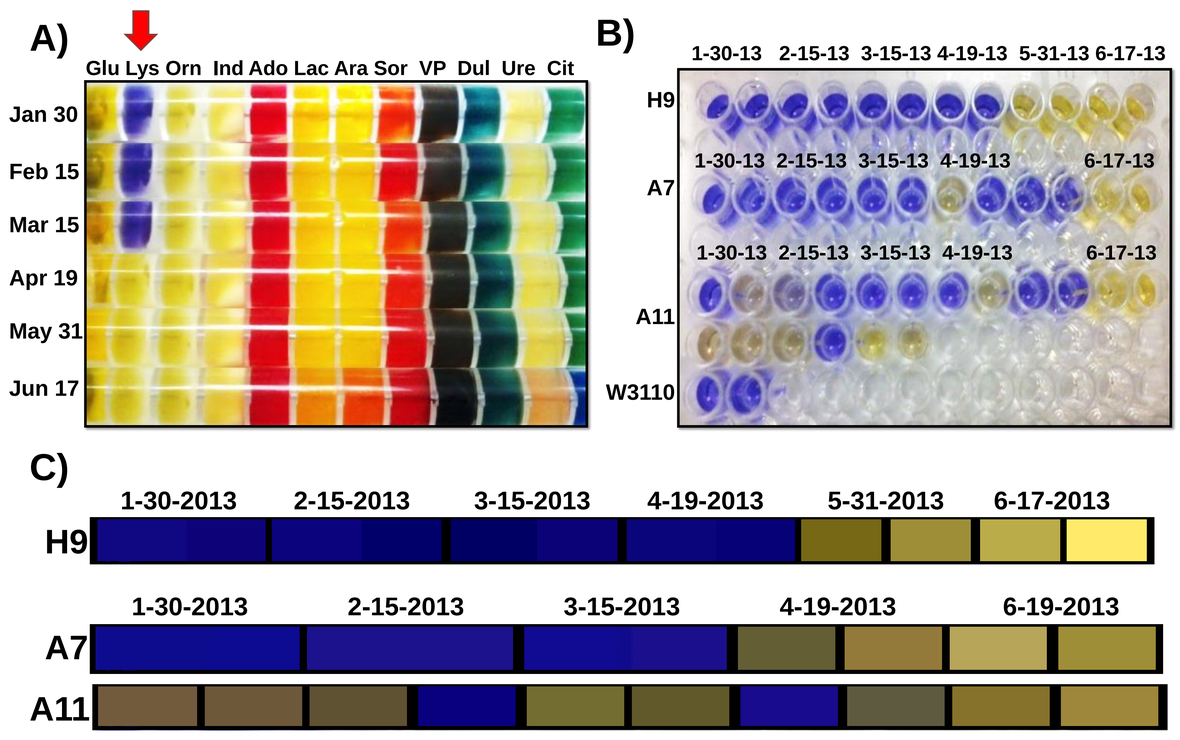

Figure 3: Evolved strains lost function of lysine decarboxylase (CadA). A) Enterotube tests showed that isolates in H9 population had lost CadA by mid-April. Purple indicates that lysine decarboxylase is present. Yellow represents lack of a functional lysine decarboxylase. B/C) Using the lysine decarboxyase broth test, we estimated CadA loss appeared in H9 population during late-May, in A7 population around mid-April, and in A11, in late-January.

Conclusions

Evolved strains are able to utilize the presence of malate to grow in moderate acid.

- The evolved strains consistently had a higher growth rate in pH 4.5 and 4.6 than the parental strain (W3110) only in the presence of malate (Figure 2)
- Without malate, the evolved strains do not consistently show faster growth rates than the parental strain

Acid-adapted strains showed slightly slower growth than parent at high pH.

- As evolved strains have been grown in a moderate acid, it is possible that the ability to grow in moderately basic environments was reduced or lost in order to upregulate the acid growth

Evolved strains lost lysine decarboxylase.

- Although lysine decarboxylase has been a known enzyme used in bacteria to regulate acid stress, the evolved strains have lost this expression (Figure 3)
- Populations such as A11 show loss of lysine decarboxylase prior to a change in environment from pH 4.8 to 4.6
- The loss of lysine decarboxylase could be an adaptation to accommodate moderate acid growth, which could be detrimental to its survival in extreme acids

As we continue growing our acid-adapted strains in moderate acid, we anticipate the populations to further develop its acid resistance. We will track the progress of this development through fitness competition assays with parental strains in moderately acidic environments. In addition, we plan to determine at what time period each population began to lose lysine decarboxylase and how it has affected their growth and survival in various pH. In the near future, we foresee submitting isolates from the populations as well as the parental strain to be sequenced for genomic analysis for comparison.

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