Abstract

E. coli are Gram-negative neutrophilic bacteria that endure a wide variety of pH stresses on their way to colonize the gastrointestinal tract. Strains of E. coli that evolved in acidic conditions for 2,000 generations were tested in a variety of ways to better understand their fitness advantage over wild type E. coli in pH 4.8.

Wild type E. coli uses Lysine and Arginine decarboxylase systems to consume H+ and release CO₂, in order to decrease the acidity of their environment. Lysine and Arginine decarboxylase systems are used to determine whether their acid regulation mechanisms remained functional after the evolution. These assays revealed that long term exposure to acid resulted in a loss in these systems.

DNA-Seq analysis was used to compare gene expression of evolved isolates and wild type. Surprisingly, this analysis showed a general down-regulation of acid stress response genes and an up-regulation of catabolism genes. We believe that inability to decrease the acidity of the media resulted in constitutive expression of acid response genes, causing useful energy to be wasted attempting to regulate a buffered environment.

Fluorescent microscopy revealed that internal pH of both wild type and evolved strains was equivalently lower in acidic conditions. Cell morphology was also only dependent on the conditions of the media present, causing filamentation of both wild type and acid-evolved strains in acidic media.

Introduction

E. coli must survive pH as low as 2 as it passes through the stomach on its way to colonize the human gastrointestinal tract (1), and can grow in pH as low as 4.6 (2). Although it can grow in these harsh conditions, E. coli is a neutrophil and must use acid resistance mechanisms such as proton motor force to maintain a neutral internal pH (3).

Previously, wild type E. coli was evolved in buffered media at pH 4.8 for 2000 generations and fitness was increased over time. Amino acid decarboxylase systems are used to help neutralize internal pH through the integration of a cytoplasmic proton with an amino acid (4). We previously reported that the acid-evolved strains showed progressive loss of lysine decarboxylase activity (4).

Additionally, mutations in the RNA polymerase subunits are involved the expression of stress dependent genes (5). Growth of E. coli under midly acidic conditions (pH 6.0) has been shown to cause old pole cells to grow more slowly than under neutral conditions (6).

Methods

Microscopy:

Fluorescence microscopy was conducted at pH 4.8 and 7.0 on the wild type (W3110) and one acid evolved clone (B11-1) with a GFP plasmid introduced. Cells were cultured in Luria-Bertani (LB) media and imaged on an Olympus BX61W1-FV5 microscope. The cells received continuous access to nutrients in a media perusal chamber (biophotons).

RNASeq:

Arvind Bhagat of United States Department of Agriculture Research Service conducted the RNA sequencing for these strains. Strand reads were then analyzed with the R-package DESeq to determine the relative expression of various mRNA sequences. Fold change expression was then cropped by a fold change up or down along with a q-value <0.001.

Decarboxylase Assays:

In a 96 well plate, E. coli was cultured in 200 μl Moeller decarboxylase broth (6 g L-lysine or L-arginine, 1 g glucose, 3 g L-glutamine, and 15 mM sodium thioglycolate) adjusted to pH 6.8 and 5.5 respectively) by picking single colonies from growth on agar plates. The plates were sealed for anaerobiosis and incubated at 37°C. After 24 hours of growth the plate was visually inspected for changes in media color as a result of changing pH. The plate was also read in a SpectraMax Plus384 microplate reader (Molecular Devices). The ratio of absorbance between 570-590 nm and 400-450 nm to create a ratio of yellow to purple absorbance. The higher the ratio, the greater the activity of the lysine and arginine decarboxylase systems.

Depressed Internal pH and Filaments in Acid Stress

Depressed pH in strains B11-1 and W3110 is evident in images A and B. This indicates that the acid evolved strain is better adapted to low pH conditions than the wild type strain.

Identification of Acid Resistant Genes

Table 1: Top 20 upregulated genes in strains F11, B11, and F9 as compared to the ancestral transcriptome. Genes encoding catabolic pathways, sugar alcohol pathways, glycerol degradation, and anaerobic respiration tend to be upregulated (yellow). Also upregulated are genes that fight oxidative stress (green).

Table 2: Top 20 downregulated genes in strains F11, B11, and F9 as compared to the ancestral transcriptome. Genes involved in acid stress are downregulated (green). Deletion of the gad regulon in F11 and F9 (shown in table 1) causes the apparent downregulation of gad regulon genes due to their loss through mutation.

Figure 1: Depressed internal pH and filamentation in acid stress. Figure 2: Fluorescent images of E. coli cells in pH 4.8 appear more blue, confirming the lower internal pH as a result of stressful media. Using the calibration from a standard curve, pH can be calculated from the ratio of two fluorescence intensities. Ratios show that green correlates to neutral pH and blue correlates to acidic pH.

Figure 3: Differences in internal pH between B11-1 and W3110 at pH 7.0 and 4.8 media observed through fluorescence microscopy. Cells maintain lower internal pH when exposed to the pH 4.8 media. Error bars=95% CI.

Conclusions

Acid-evolved strains upregulate catabolism and downregulate acid stress genes compared to the ancestor.

In F11 and F9, deletion of the gad regulon causes these genes to appear highly downregulated.

Catabolism genes upregulated in the acid-evolved strains include glycerol catabolism, sugar alcohol breakdown, and anaerobic respiration.

Fluorescence Microscopy:

B11-1 does not significantly show a difference in its ability to maintain a higher internal pH when compared to the ancestor.

Wild type E. coli at pH 4.8 begin to filament and have increased cell length compared to cells in the neutral condition.

Both B11-1 and the ancestor maintain a similarly lower internal pH in the acidic condition, and do not differ in the regulation of internal pH.

Lysine and Arginine Decarboxylase Activity

Acid-evolved E. coli tend to select against the acid resistance mechanisms of amino acid decarboxylases when exposed to low pH for many generations.

F9-2 maintains its amino acid decarboxylase activity after 2,000 generations in acid.

Reversion of the RNA polymerase of B11-1 to the wild type polymerase revealed a restoration in arginine decarboxylase activity, although not to wild type levels.

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References