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The Effects of high pH on acid adapted Escherichia coli

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The Effects of high pH on acid adapted *Escherichia coli*

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Abstract

Escherichia coli possesses the ability to grow under a wide range of pH stresses. *E. coli* can grow in moderate acid (pH 4.5 – 5.0) and can grow in moderate base (pH 9.0). After 2,000 generations of growth in moderately acidic buffered complex media (pH 4.6), twenty-four *E. coli* populations independently evolved to acquire a fitness advantage when co-cultured with the ancestral D8 W3110 strain in buffered pH 4.6 medium. Each of the sequenced evolved strains possessed at a least one mutation to RNApolymerase. Of the evolved strains, four (JLSE0079, JLSE0083, JLSE0091, and JLSE0137) were co-cultured with the ancestral D8 W3110 strain in moderately basic buffered pH 9.0 medium to assess the effects of newly acquired mutations beneficial in moderate acid. The mutations in evolved strains JLSE0091 and JLSE0137 were only slightly antagonistic, whereas mutations in evolved strains JLSE0079 and JLSE0083 caused a more significant reduction of fitness in moderate base. Furthermore, an adaptive laboratory evolution (ALE) experiment was established by growing and re-diluted twenty-four independent D-8 W3110 *E. coli* populations in buffered moderately basic medium (pH 9.0). After ~600 generations of growth, we have seen a significant improvement in growth and have adapted our *E. coli* strains to survive in pH 9.2 media.

Introduction

- *Escherichia coli* must survive exposure to acidic stresses as low as pH 1.5 in the stomach and exposure to basic stresses exceeding pH 10 at the pancreatic duct.
- During growth, E. coli’s cytoplasmic pH is normally maintained within the range of pH 7.4 to 7.9, yet it has the ability to grow within the range of pH 4.5 to 9.0.
- After 2,000 generations of growth in buffered complex medium (pH 4.6), the evolved strains have developed a fitness advantage over the ancestral wild-type strain when co-cultured in moderate acid.
- Each evolved strain has at least one missense mutation in RNApolymerase which affects numerous genes.
- This study aims to compete the ancestral strain with four evolved strains (JLSE0079, JLSE0083, JLSE0091, and JLSE0137) in buffered high pH media (pH 9.0) in order to assess any fitness effects, under alkaline stress, of newly acquired mutations beneficial in moderate acid.
- We began an adaptive laboratory evolution (ALE) experiment to assess how *E. coli* grows under alkaline conditions (pH 9.0).

Methods

Competition Assays

Both the ancestral *E. coli* wild-type D8 W3110 strain and an acid adapted strain (JLSE 0079, JLSE 0083, JLSE 0091, and JLSE 0137) were co-cultured in buffered pH 9.0 LBK media to determine the fitness of the evolved strains under alkaline stresses. The lac- ancestral wildtype and lac+ evolved strains were cultured for 24 hours and rediluted (1:100) in new media and allowed to overnight. The competition cultures were prepared by adding equal volumes of ancestral and evolved culture into buffered pH 9.0 media. The competition medium was then diluted (1:400,000) in M63 minimal glucose media and plated on X-Gal (4 µL/mL) plates. The remainder of the competition media was shaken at 37°C with aeration in water bath for 24 hours. The cultures were then diluted (1:400,000) in M63 minimal glucose media and plated on X-Gal (4 µL/mL) plates. The colony counts of the plates before and after the dilutions were used to determine the fitness of both the ancestral strain and the evolved strain. The strain with the greatest growth rate was determined to outcompete the other strain. Each competition assay was replicated on 6 plates.

High pH (pH 9.0) Adaptive Laboratory Evolution (ALE)

Each day, twenty-four *E. coli* D8 wild-type strains are independently cultured in 200 µL of LBK pH 9.2 buffered with 150 mM AMPSO on a 96-well plate. After the cultures on the 96-well plate are allowed to grow in a spectramax reader at 37°C for 22 hours, a 1:100 dilution is taken from the previous culture day’s cultures and placed into fresh media. The old plated are stored in a -80°C freezer after glycerol buffered to pH 7.5 is added to each individual culture. The spectramax measures the OD 450 at 15 minute intervals.

Results

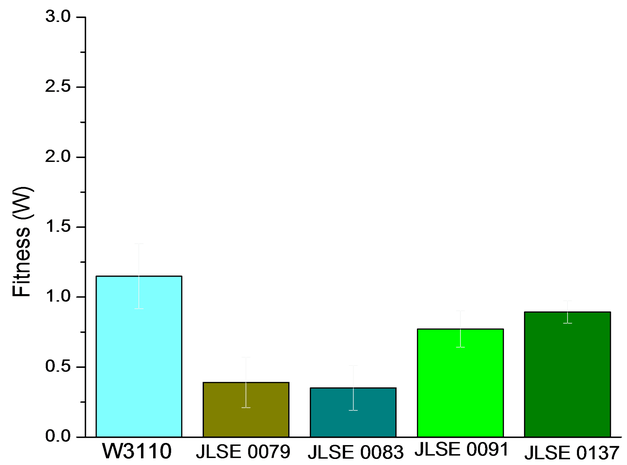

Figure 1: Fitness of four of the 2000th generation isolates relative to the ancestral wild-type strain when co-cultured for 24 hours in buffered pH 9.0 LBK medium.

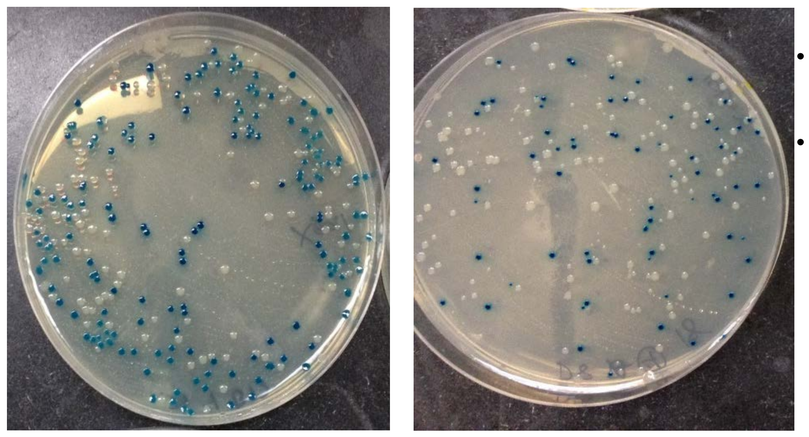

Figure 2: *lac+* JLSE0079 v. *lac-* W3110 D8. LBK X-gal plate co-cultured with a *lac-* wild-type strain and a *lac+* JLSE0079 strain after exposure a 24 hour exposure to basic medium (pH9.0). *lac-* wild-type colonies are white and a *lac+* JLSE 0079 colonies are blue.

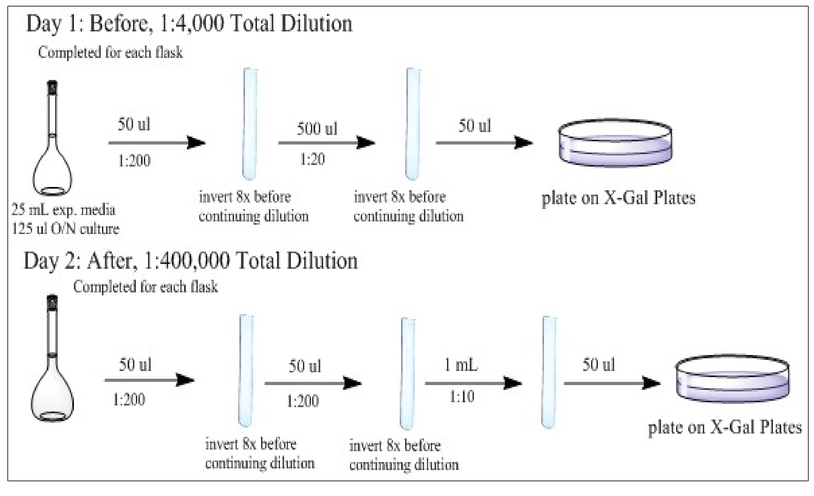

Figure 3: Procedure used to conduct the competition assays.

Conclusions

- **Moderate to heavy reduction in fitness of the 2000th generation isolates relative to the ancestral wild-type strain when co-cultured for 24 hours in buffered pH 9.0 LBK media.**
  - Strains JLSE0079 and JLSE0083 demonstrated a significant reduction of fitness when co-cultured with the ancestral wild-type strain in buffered pH 9.0 media for 24 hours.
  - JLSE0091 and JLSE0137 demonstrated a moderate reduction of fitness when co-cultured with the ancestral wild-type strain in buffered pH 9.0 media for 24 hours.
- **High variability in colony size and colony counts were exhibited under alkaline stress conditions.**
  - Colony sizes, colony counts, and ultimately fitness were not as consistent as they were when co-cultured under moderate acidic stress (the evolved condition).
  - This high variability has also been demonstrated in other experiments that observe effects of observed in beneficial mutation in *Escherichia coli*.
- ***E. coli* strains evolved increased growth rate after ~600 generation in buffered pH 9.0 medium**
- ***E. coli* strains have evolved to grow well at pH 9.2**

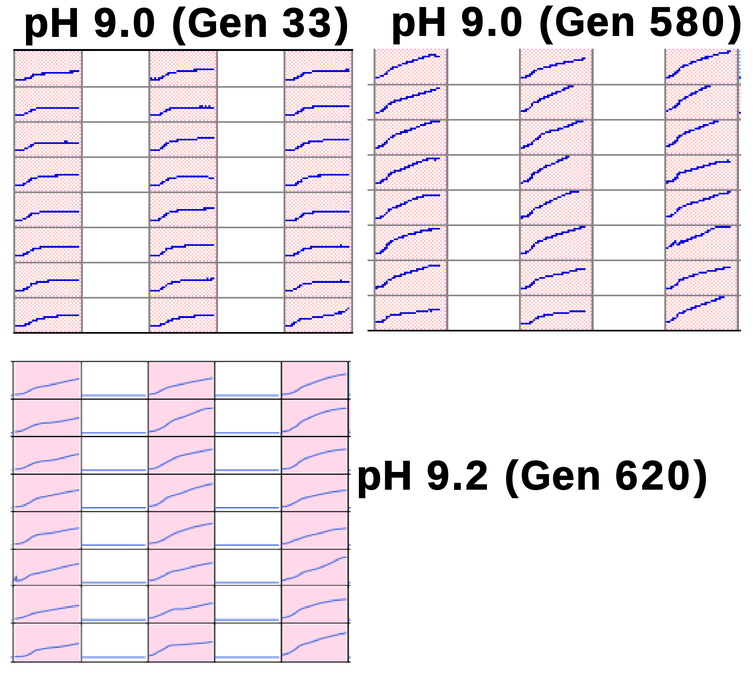

Figure 4: Grow curves of ALE cultures on a 96-well plate after 22 hours of growth in buffered LBK media.

Acknowledgements

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