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The Effect of Non-Standard Laboratory Conditions on the Acid Resistance of Escherichia coli

M. Michael Harden

The Effect of Non-Standard Laboratory Conditions on the Acid Resistance of *Escherichia coli*

M. Michael Harden ’14 and Joan L. Slonczewski
Department of Biology, Kenyon College, Summer Science 2012

Abstract

The arginine-dependent acid resistance system (AR3) of *E. coli* protects the cell from acid stress by consuming protons via the decarboxylation of arginine. We investigated this system by exposing an *adiA* mutant, which lacks the central arginine decarboxylase enzyme, to extreme acid challenges under different conditions. The survival assays demonstrated that AR3 is induced aerobically in the presence of extracellular arginine in Brain-Heart Infusion media, and that the system is not necessary for survival under anaerobic conditions. We then predicted that *E. coli*’s ability to resist an acid challenge was dependent upon additional media conditions, including the presence of reactive oxygen species (ROS). To test this, *E. coli* was exposed to more extreme acid challenges in autoclaved and filtered media under aerobic and anaerobic conditions, ranging from pH 1.2 to pH 2. We observed that *E. coli* is capable of surviving more extreme acid challenges when exposed in filtered media, which contains fewer ROS than autoclaved media. Finally, we resolved to identify more genes involved in acid resistance by establishing an adaptive laboratory evolution procedure. This protocol required a pH and dilution scheme at which *E. coli* would be challenged sufficiently, but would still grow at a reasonable pace. We monitored *E. coli* growth over a range of pH values and with varying dilutions, and found that a pH of 4.8 with a 1:4000 daily dilution scheme was optimal.

Introduction

***E. coli* and acid resistance**

• Various acid stress responses are essential for survival during passage through the stomach, a constantly changing enviroment (1, 2)
• Traditional laboratory conditions neglect the intricacies of bacterial acid resistance

Arginine-dependent acid resistance system (AR3)

• Method by which *E. coli* can survive acid stress; arginine decarboxylase enzyme (*adiA*) replaces the carboxyl group on arginine with a proton
• The product is exchanged for new arginine via a transporter (1)
• This project explores AR3 under various experimental condition, including the presence of oxygen, and growth in complex media

Reactive Oxygen Species (ROS) and acid resistance

• Autoclaving tryptone-based growth media produces ROS (3)
• This project explores the effect of ROS on *E. coli* acid resistance by exposing strains to high ROS or low ROS during (an)aerobic extreme acid challenge

Adaptive Laboratory Evolution (ALE)

• ALE protocol, defined here as continuously growing *E. coli* strains under moderately acidic conditions, is aimed at identifying spontaneous mutations that increase acid resistance (4)
• This project aims to design an ALE protocol by establishing ideal *E. coli* growth conditions (dilution scheme and pH challenge)

Methods

Acid Survival Assay: Acid-adapted overnight cultures of *E. coli* strain W3110 (wild type) or JLS1209 (*ΔadiA*::kan) were grown to stationary phase in LBK or brain-heart Infusion media (BHI) buffered with 100 mM MES to pH 5.5. Overnight cultures were diluted 1:200 (aerobic conditions) or 1:400 (anaerobic conditions) in either acidic minimal media broth (M63) supplemented with 3 mM arginine (for AR3 experiments), or acidic LBK (for extreme acid experiments), and incubated for 2 hours at 37 °C. Exposed cells were then serially diluted to a final dilution of 1:400,000 (aerobic conditions) or 1:80,000 (anaerobic conditions) in pH 7.0 M63, and plated onto LBK agar. Overnight cultures were also diluted 1:200 or 1:400 in M63 pH 7.0 without being challenged, serially diluted in the same way as the exposure treatments, and plated onto LBK agar. The colony counts of both treatments were compared to determine the percent survival (5). Anaerobic experiments were carried out in a controlled atmosphere chamber containing an anoxic mixture of hydrogen, carbon dioxide, and nitrogen. All media used during anaerobic experiments was equilibrated to the anoxic conditions for at least 24 hours prior to the experiment (6).

Adaptive Laboratory Evolution Procedure: An overnight culture of W3110 was prepared as described above. 200 µL of LBK buffered with 100 mM HOMOPIPES was aliquoted into wells on a 96-well plate, and each well inoculated with 2 µL of overnight culture. The well plate was placed in a spectramax reader for 22 hours, which maintained the cultures at 37 °C, and measured the OD 600 at 15 minute intervals. At the end of the scanning protocol, each culture would be diluted (to varying final dilutions) into fresh media in a new well plate, and the scanning procedure would be repeated.

Results

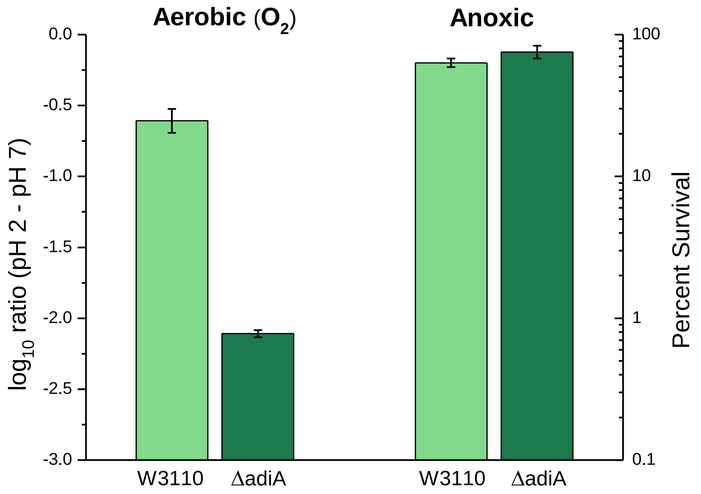


Figure 1. AR3 induction is oxygen dependent. Strains were grown overnight in BHI buffered to pH 5.5 and exposed in pH 2 M63 for 2 hours in the presence of arginine under aerobic or anaerobic conditions. Error bars = SEM, n=6.

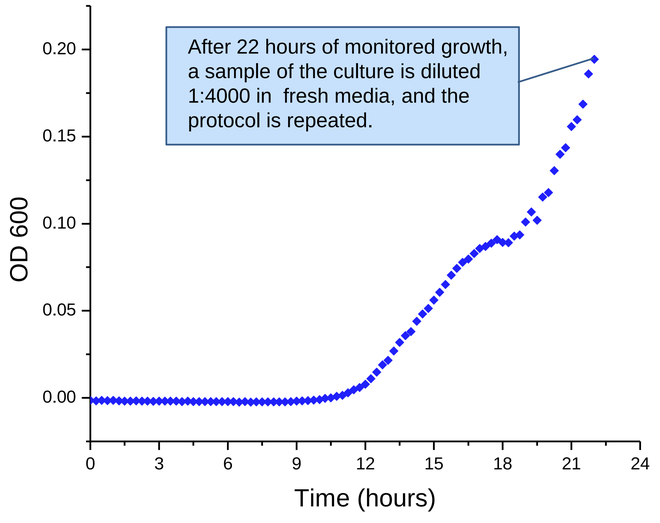
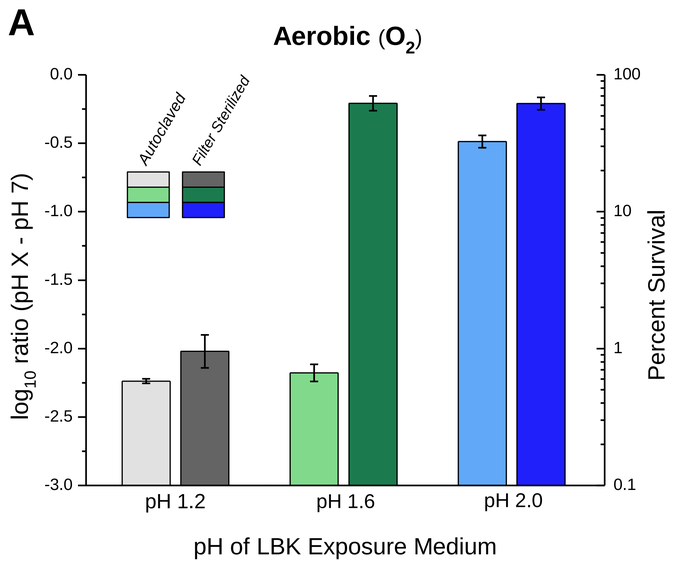


Figure 2. 1:4000 daily dilution and pH 4.8 are optimal conditions for ALE growth. *E. coli* strains were grown and monitored at varying pH challenges for 22 hours and re-diluted daily using varying dilution schemes.



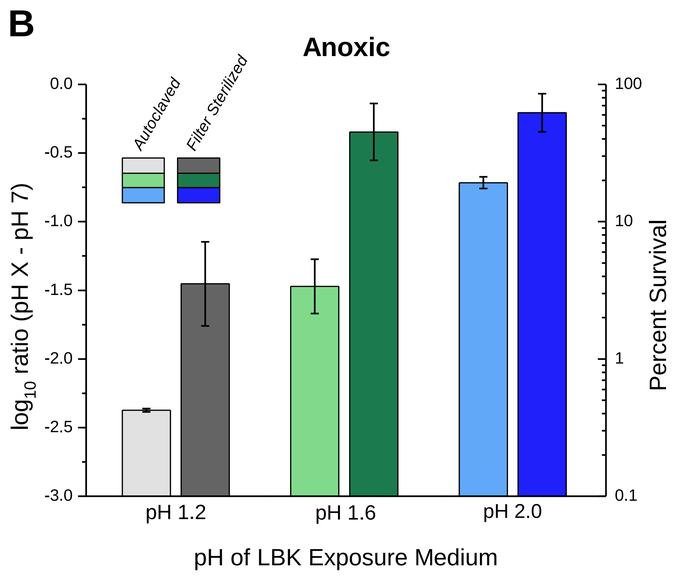


Figure 3. Filter sterilizing exposure media allows *E. coli* to survive in more extreme acid. Strains were grown overnight in LBK buffered to pH 5.5 and exposed for 2 hours at varying pHs in high-ROS autoclaved LBK or low-ROS filter-sterilized LBK. Exposure was done under both aerobic conditions (A) and anoxic conditions (B). Error bars = SEM, n=6.

Conclusions

AR3 is only induced when *E. coli* is cultured in BHI and exposed under aerobic conditions:

• AR3 is not induced in *E. coli* when the bacteria are cultured overnight in LBK buffered to pH 5.5, and no survival following a pH 2 acid challenge is observed under those conditions. However, AR3 is induced when cultured in BHI buffered to pH 5.5, which means that AR3 is active under these complex media conditions (data not shown).
• When *E. coli* is cultured in BHI media buffered to pH 5.5, AR3 is induced in the presence of oxygen, but is not induced under anaerobic conditions (Figure 1). Thus, *E. coli* must have alternative means of resisting acid stress under anaerobic conditions.

ROS-rich autoclaved exposure media prevents *E. coli* from surviving more extreme acid challenges:

• *E. coli* is able to survive under more acidic conditions when exposed in filter sterilized LBK than when exposed in ROS-rich autoclaved LBK. This result is consistent in both the presence and absence of oxygen (Figure 3).

Optimal ALE conditions are a pH challenge of 4.8 with a 1:4000 daily dilution:

• A continuous pH challenge of 4.8 coupled with a 1:4000 daily dilution scheme is the optimal set of conditions for an ALE protocol which sufficiently challenges *E. coli*, but simultaneously allows for a reasonable growth rate (Figure 2).

Acknowledgements

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