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Anaerobic Regulators Affect Anoxic Extreme-Acid Survival of E. coli

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Anaerobic Regulators Affect Anoxic Extreme-Acid Survival of *E. coli*

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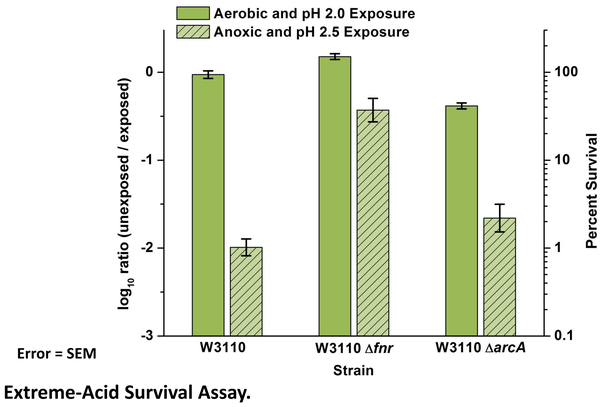
Abstract

E. coli survives extreme acid in the presence of oxygen, but its ability to survive in the absence of oxygen is unknown. Strains with null mutations for anaerobic regulators *fnr* or *arcA* were tested for extreme-acid survival phenotypes under anoxic conditions maintained by a Controlled Atmosphere Chamber (PlasLabs). Using qRT-PCR, differential expression of acid response genes was probed for under anoxic pH 5.5 conditions. The Δ*fnr* strain survived significantly better than the wild-type under anoxic extreme-acid challenge. Anoxic overnight growth and exposure sufficed to establish the *fnr*-dependent phenotype. qRT-PCR showed higher expression of vital acid resistance genes *cfa*, *gadB*, and *hdeA* in the Δ*fnr* strain. Thus, under anoxic conditions, FNR represses major acid resistance systems and negatively affects extreme-acid survival.

Introduction

E. coli uses several mechanisms to survive extreme acid in the human stomach (Richard 2003). These mechanisms have been studied mainly in the presence of oxygen. However, pockets of the upper digestive tract are anoxic and the effectiveness of acid survival without oxygen is unknown (Zilberstein 2007). In this study, it was hypothesized that FNR and ArcA, the regulators shifting metabolism to anaerobic mode (Levanon 2005), govern differential extreme-acid survival of E. coli in the presence or absence of oxygen. Because FNR and ArcA inhibit genes comprising the normal acid adaptation response, it is thought that *E. coli* must adopt alternate strategies to survive acid challenge when the regulators are active.

Δ*fnr* Mutation Enhances Extreme-Acid Survival Under Anoxia

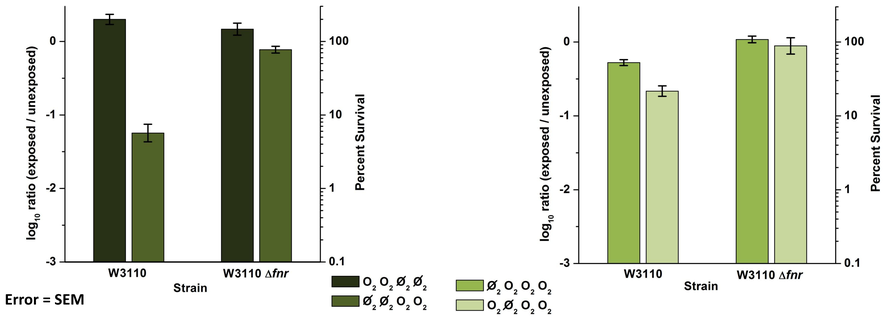


Extreme-Acid Survival Assay.
Extreme-Acid Survival Assays were performed as described previously with modifications (Hayes 2006).

Stage 1 - For each strain, overnight cultures were grown in LBK media buffered at pH 5.5 for 16-18 hours. Anoxic cultures were grown in 8 mL screw-cap tubes with end-over-end rotation. Aerobic cultures were grown in 2 mL amounts in 16 mL metal-cap tubes.
Stage 2 - Six technical replicates of the culture were diluted into pH 2.5 (anoxic) or 2 (aerobic) LBK media then incubated and rotated for 2 hours at 37 °C.
Stage 3 - After the incubation period, sequential dilutions were made for a total ratio of either 1:80,000 (anoxic) or 1:400,000 (aerobic) in pH 7 M63A medium. For control purposes, six replicates were subjected to the same dilution scheme except without extreme-acid exposure.
Stage 4 - 50 µL of each replicate was plated on LBK-agar and incubated overnight at 37 °C. The next day, the number of colonies on each plate was counted. The percent survival was estimated from the log ratios of the counts.

Anoxic stages were performed in the Controlled Atmosphere Chamber (Plas Labs). The chamber was initially purged of atmosphere via a vacuum pump and then a gas mixture of 5% CO_2, 10% H_2, and 85% N_2 was introduced. Any remaining oxygen was catalytically removed by a palladium canister. Experimental materials were introduced and removed from the main chamber by means of an intermediate chamber.

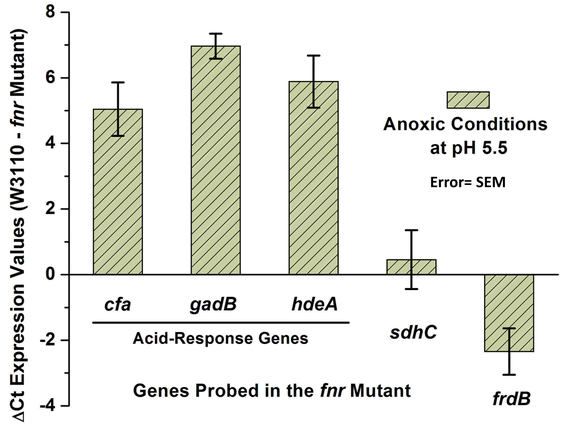
Growth and Exposure are the Key Stages for Establishing an *fnr*-dependent Extreme-Acid Survival Phenotype



Stage 1 – Growth – Stage 2 – Exposure – Stage 3 – Dilution – Stage 4 – Plating

O_2 = Aerated; $Ø_2$ = Anoxic (< 10 ppm of oxygen)

FNR Represses Transcription of Major Acid-Response Genes



Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR).
E. coli W3110 and JLS1115 (Δ*fnr*) cultures in pH 5.5 were grown to mid-late log phase within the Controlled Atmosphere Chamber (Plas Labs). Bacterial RNA was stabilized using the cold 10% phenol-ethanol stop solution as previously described (Hayes 2006) and isolated using the RNeasy Kit (Qiagen) followed by DNase treatment (Ambion).

Relative expression of each gene was quantified using the ABI Prism 7500 DNA analyzer (Applied Biosystems) as described previously (Hayes 2006). Targeted primer sequences were designed using Primer Express (Applied Biosystems) and obtained from Invitrogen. The SYBR Green PCR One-Step Protocol (Applied Biosystems) was used. Nucleic acid concentrations were as follows: 0.1 nM forward primer, 0.1 nM reverse primer, and **50 ng target RNA**. PCR cycling conditions were as follows: reverse transcription at 48°C for 30 min and 95°C for 10 min, 40 cycles of denaturation at 92°C for 15 s, and extension at 60°C for 1 min. The total RNA in each sample amplified was used as the basis to normalize individual gene expression profiles. Expression levels of the average of three technical replicates of each biological replicate of JLS1115 are presented relative to their expression in the wild-type control.

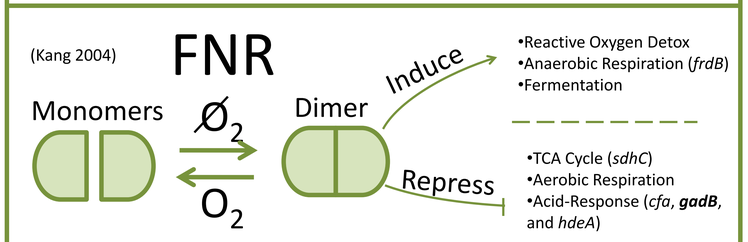
Conclusions

1. Δ*fnr* Mutation Enhances Extreme-Acid Survival Under Anoxia
When the assay is run under anoxic conditions we see that the survival of the wild-type and Δ*arcA* drops dramatically while that of Δ*fnr* is largely unaffected. This is the result we expect. FNR is the predominate regulator in conditions established by the anoxic chamber (< 10 ppm O_2). Δ*arcA* is more dominant under microaerobic conditions (Sawers 1999).

2. Growth and Exposure are the Key Stages for Establishing an *fnr*-dependent Extreme-Acid Survival Phenotype
It is not necessary to dilute or plate *E. coli* anoxically in order to observe the anoxic-enhanced extreme-acid survival phenotype. Indeed, dilution and plating shouldn't have an effect as they are simply steps that make it possible to observe the effects of a treatment that has already been imposed, that is, exposure to extreme pH.

3. FNR Represses Transcription of Major Acid-Response Genes
The genes *cfa*, *gadB*, and *hdeA* are important for an effective acid-survival response. By repressing these proteins under anoxia, FNR dramatically decreases the ability of *E. coli* to survive acid-shock.

Future Directions
One of our primary goals is to establish that a double mutant null for *fnr* and another acid-response protein that is repressed by FNR will lose its enhanced survival phenotype. We would also like to further inspect the differential expression between W3110 and Δ*fnr* for other *gad* genes.



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