Genetic Requirements for *E. coli* Extreme Acid Survival both Aerobically and Anaerobically

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Abstract

The gram-negative bacterium *Escherichia coli* is an intestinal microorganism that is affected greatly by extracellular environmental factors such as pH and oxygen availability. Prior research dealing with acid survival has focused mainly on aerobic conditions due to the difficulties associated with obtaining truly anoxic conditions. Through the use of a controlled atmosphere chamber (PLAS labs) we were able to examine the effects of certain genes (*cfa*, *gadC, rpoS, hypF*, and *fnr*) on anaerobic extreme acid survival. After much focused research on *cfa* in particular, it became apparent that whether the pH 2.0 exposure medium was autoclaved or filter sterilized, survival was affected. All previously stated strains were tested anaerobically and aerobically to determine how the exposure medium affected extreme acid survival. We found that under anaerobic conditions, all strains with the exception of *fnr* showed the effect of decreased survival when autoclaved media was used. The *cfa* mutant showed the largest effect of autoclaved vs. filtered media. The *gadC* and *rpoS* strains also showed an effect of autoclaved vs. filtered media anaerobically. These two strains (*gadC* and *rpoS*) did not survive aerobically, while the rest of the strains showed comparable survival whether autoclaved or filtered exposure medium was used.

Introduction

While *E. coli* grows within a range of external pH 4.5-9.0, it can survive at pH 2 for hours (Foster 2004). This is significant because the gastrointestinal (GI) tract is a very acidic environment, through which the bacterium must pass in order to reach the intestine. While acidity is a factor in how well *E. coli* can survive, oxygen concentration also plays a role, as there is a definite oxygen gradient in the GI tract from the proximal to the distal end (He 1999).

Survival in extreme acid for aerated cultures involves many different stress response systems, but in anaerobic cultures, it is not known whether these same factors/systems are involved. Cyclopropane fatty acids (CFAs) are a phospholipid component of many bacteria thought to relate to resistance of different *E. coli* strains to large decreases in pH (Chang 1999).

The GAD system is a decarboxylase/antiporter-dependent acid resistance-system and *gadC* has previously been shown to not be required for acid resistance anaerobically (Foster 2004 and Martinez, Riggins- not published).

The sigma factor RpoS regulates many gene products and acid resistance aerobically is thought to be dependent in some way on *rpoS*. Previous research suggested that this "requirement" could be overcome by anaerobic growth in moderate media (Small 1994).

HypF is an auxiliary protein that is involved in maturation for all of the hyrdogenases (Paschos 2002).

FNR is a major regulatory system that responds to low oxygen levels (Kang 2005).

Methods

Extreme-acid survival assays were carried out as reported previously (Noguchi 2010), with a few adjustments. All steps for any anoxic experiments were conducted in a controlled atmosphere chamber by PLAS Labs maintained at 37°C and containing a gas mixture of carbon dioxide (5%), hydrogen (10%), and nitrogen (balance). Overnight cultures were grown in LBK medium buffered to pH 5.5 with 100 mM MES for 16-18 hours. Aerobic cultures were grown in 2 mL of media in 16 mm metal cap tubes and rotated. Anoxic cultures were grown in 8 mL screw cap tubes and rotated end-over-end. Overnight cultures were then diluted 200-fold (aerobic) or 400-fold (anoxic) into LBK pH 2.0 and rotated for 2 hours. These were then diluted into minimal M63 pH 7 medium for a final dilution of either 1:4x10^5 (aerobic) or 1: 8x10^4 (anoxic) and then plated on LBK agar plates. For the control, overnight cultures were diluted and plated as above. All plates were then incubated overnight at 30°C and colonies were counted. Survival rates were calculated by comparing the exposed and non-exposed replicates for each strain and percent survival was calculated logarithmically. Each strain was tested at least 3 times by multiple people both aerobically and anaerobically.

Results

Aerobic acid survival

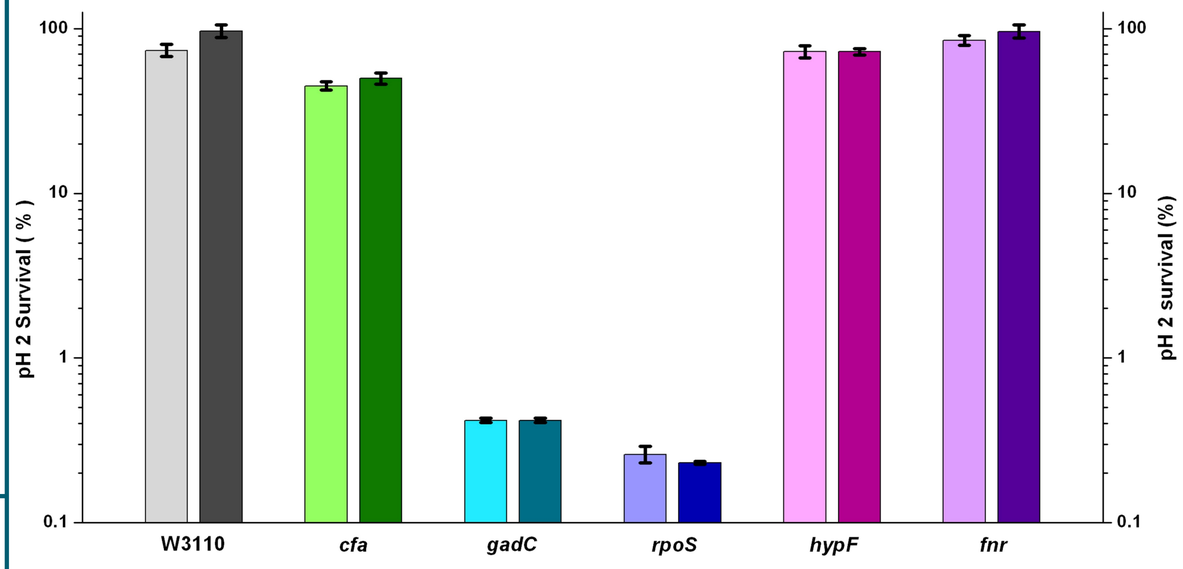

Figure 1: Acid survival of mutant strains under aerobic conditions. Strains lacking acid response genes (*cfa, gadC, rpoS, hypF,* and *fnr*) were cultured overnight and exposed to pH 2.0 for 2 hours before being diluted 1:400,000 under aerobic conditions. Dilutions were then plated and colonies were allowed to grow overnight at 30°C. The number of colonies present were then counted. These numbers were log transformed and a ratio of exposed-non exposed was obtained; a percentage was then calculated from that ratio. Light bars represent media that was autoclave sterilized and dark bars represent media that was filter sterilized. Error bars=SEM, n=6.

Anoxic (<0.3 ppm O_2) acid survival

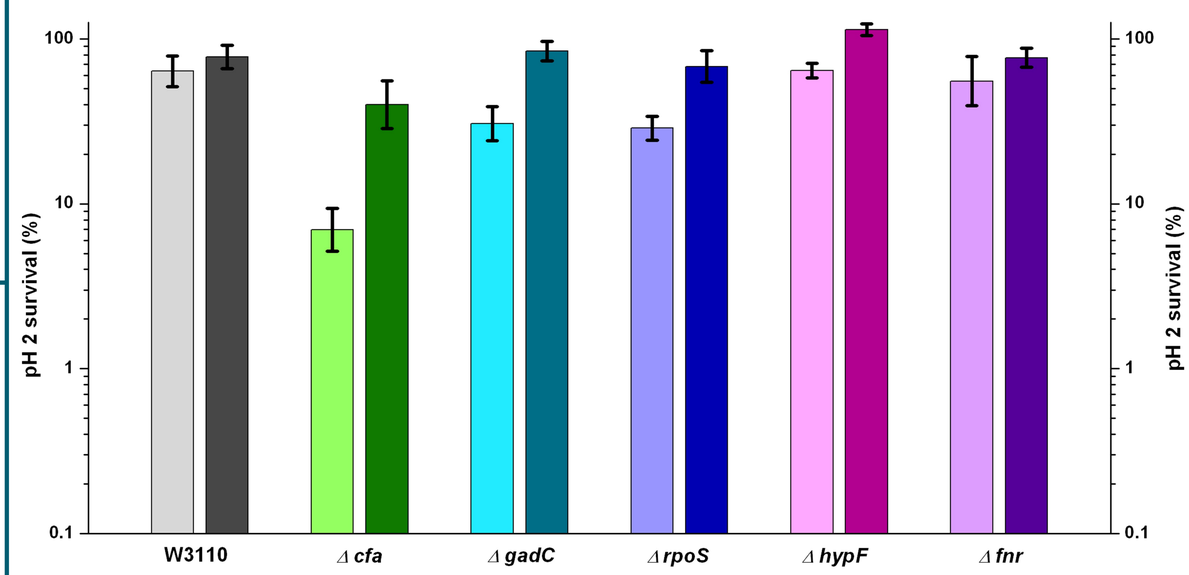

Figure 2: Acid survival of mutant strains under anoxic conditions. Strains lacking acid response genes (*cfa, gadC, rpoS, hypF,* and *fnr*) were cultured overnight and exposed to pH 2.0 for 2 hours before being diluted 1:80,000 under anoxic conditions. Dilutions were then plated and colonies were allowed to grow overnight at 30°C. The number of colonies present were then counted. These numbers were log transformed and a ratio of exposed-non exposed was obtained; a percentage was then calculated from that ratio. Light bars represent media that was autoclave sterilized and dark bars represent media that was filter sterilized. Error bars=SEM, n=6.

Acid survival of *cfa mutant*

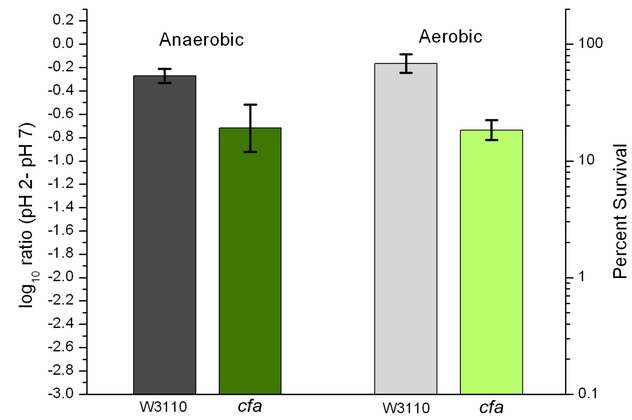

Figure 3: Acid survival of *cfa* mutant. Strains lacking the acid response gene *cfa* were cultured overnight and exposed to pH 2.0 for 2 hours before being diluted 1:400,000 and 1:80,000 under aerobic and anaerobic conditions, respectively. Dilutions were then plated and colonies were allowed to grow overnight at 30°C. The number of colonies present were then counted. These numbers were log transformed and a ratio of exposed-non exposed was obtained; a percentage was then calculated from that ratio. These experiments were done using minimal media. Error bars=SEM, n=6.

Conclusions

•Preliminary experiments done using *cfa* with minimal media indicated that *cfa* is needed for both aerobic and anaerobic acid survival. Further experiments showed that this requirement is not as large as we originally expected under aerobic conditions, although *cfa* is needed for anaerobic acid survival.

•With exposure to oxygen, the results obtained from autoclaved and filter sterilized exposure medium were comparable. Under aerobic conditions, *gadC* and *rpoS* are required.

•Under anaerobic conditions, all of the genes studied with the exception of *fnr* showed increased survival when the exposure medium was filtered rather than autoclaved. Many of the strains survived close to 100% with filtered media, showing that many of the genes previously thought to be required for acid resistance are not needed anaerobically (i.e. *gadC* and *rpoS*).

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