Base Resistance in *Escherichia coli*
Potential Stress Resistance Genes and Regulators

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Abstract

Enteric neutrophiles such as *Escherichia coli* must survive exposure to extremely acidic and basic conditions during passage through the human digestive system. While the genes responsible for survival in extremely acidic conditions are fairly well understood (1), very little is known about the genes that contribute to base resistance. This project examines the roles in base resistance of several genes identified as potential base resistance factors by a previous study: *aceF*, *arcB*, *gadC*, *hdeA*, *hdeB*, *hlpA*, *degP*, *rpoS*, *surA*, and *ygbT*.

To accomplish this task, we set out to determine the ideal conditions for conducting high-pH survival assays. Since some acid resistance systems require the presence of amino acid supplements in the growth medium, we hypothesized that survival rates would be significantly higher in Luria-Bertani broth (LBK) supplemented with amino acids than in unsupplemented M63 minimal media. We found that the high-pH survival rate of the wild-type strain was optimal (near 100%) with pH 9.8 LBK media, while the *rpoS* knockout strain, which is a known indicator of pH stress, did not survive these conditions.

Across three replicates of survival experiments, two genes presented a consistent decreased high-pH survival rate in their respective knockout strains relative to the wild-type: *surA* and *aceF*. The absence of another gene, *hlpA*, appeared to have a significant impact on base survival in at least one experimental replicate. The other genes tested in this project were not necessary for optimal high pH survival.

Introduction

Escherichia coli faces many challenges before reaching the green pastures of the large intestine. While many studies have been done examining the mechanisms and regulators of acid resistance in *Escherichia coli*, very little is known about the mechanisms and regulators of base resistance despite the medically relevant basic secretions of the gallbladder and pancreas The objective of this project is to evaluate the importance of a handful of genes that were previously identified as potential contributors to base resistance in *Escherichia coli*. As such, this project focused on:

- **Preparing strains for experiments**: Our lab uses a standard wild-type background (W3110) for all of our experiments, making it easier to compare knockout strain phenotypes. Knockout mutations for each gene in this study have to be transferred into this background by P1-phage transduction. Successful transformation is then confirmed by testing for a knockout-associated antibiotic resistance allele.
- **Establishing Ideal Testing Conditions**: Under what conditions is the organism's survival "appropriately" challenged? To determine the ideal testing conditions, a range of media type and exposure pH conditions are tested in initial survival experiments.
- **Conducting Survival Assays**: By comparing the survival rate of strains missing each gene to the survival rate of the wild-type under highly basic conditions, we can evaluate the relative importance of each gene for base resistance.

Gene List

aceF Encodes dihydrolipoamide acetyltransferase, a major component of the pyruvate dehydrogenase complex.
arcB Encodes the sensor kinase component of the ArcAB regulator system, detects changes in respiration and redox state
degP Encodes a periplasmic chaperone that prevents protein damage during heat shock.
Fnr A major regulator of gene expression under anoxic conditions.
gadC Encodes a glutamate-γ butyric acid antiporter, known to play an important role in acid resistance.
hdeA, hdeB Encode two similar periplasmic protein chaperones that have a known role in acid resistance.
hlpA Encodes a periplasmic protein chaperone that supports the chaperone surA.
surA Encodes a periplasmic protein chaperone that escorts outer membrane proteins to the bamC assembly point.
ygbT Encodes a protein of unknown function which is thought to contribute to base resistance.

Methods

- **Preparation of strains**: Knockout strains featuring kanamycin resistance cassettes were ordered from the Keio collection. The knockout, kanamycin-resistant genotype was transferred into the W3110 background by P1 phage transduction. An initial round of infection of Keio strain produced virus containing bacterial genomic DNA. Subsequent infection of the W3110 wild-type cells resulted in insertion of the kanamycin resistance cassette into the gene of interest, effectively "breaking" the gene. Transformed bacteria were treated with a chelating sodium citrate Luria broth solution to halt the viral reproductive cycle. Finally, samples were streaked on kanamycin-supplemented agar plates to isolate successfully transduced colonies.
- **Establishing ideal testing conditions**: The process of identifying ideal testing conditions was split into two major components: determining the optimal type of media for the exposure treatment and finding the appropriate pH for the exposure treatment. Two media types were considered: nutrient-rich LBK and nutrient poor M63. Next, pH values ranging from 9.80 – 10.00 were selected for testing (based on earlier work by lab alumnus Jackson Cabo). Media and pH combinations were tested through wild-type survival assays and high-pH survival rates were compared to survival in neutral control conditions.
- **Aerobic survival assays**: Initially, sample colonies from each strain were grown overnight in slightly basic LB media to stationary phase. This overnight treatment activated the transcription of any base-resistance genes present in the strain. Subsequently, to determine the high-pH stress survival rate for each strain, the number of colonies that appeared on agar plates after a two-hour exposure period was compared to the number of colonies on a control plate utilizing the same dilution factor. Log 10-transformed ratios of exposure colonies to control colonies for each knockout were graphed and compared to the wild-type survival rate.

Results - Figures

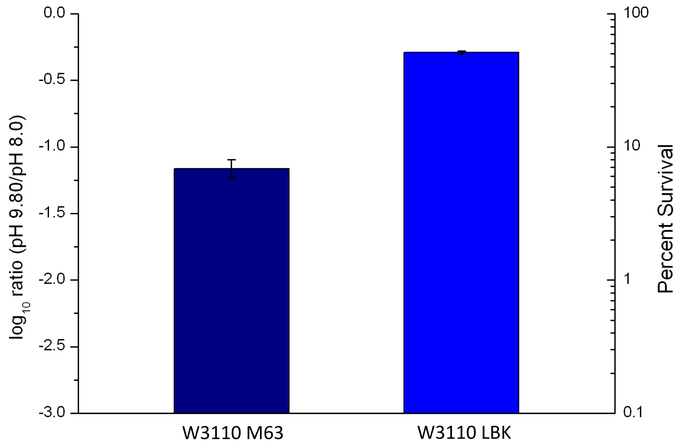

Figure 1. Base exposure survival is higher with LBK exposure media than M63 exposure media. Error bars = SEM. Two-sample t-test, t = 11.32, p = 0.000.

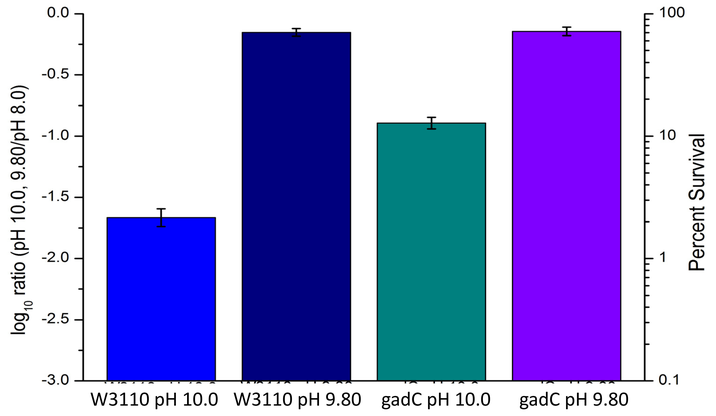

Figure 2. Survival is significantly higher with an exposure pH of 9.80 than with an exposure pH of 10.00. Error bars = SEM. Two-sample t-test$_{W3110,\ gadC}$, t = 19.80, 11.38, p = 0.000, 0.000.

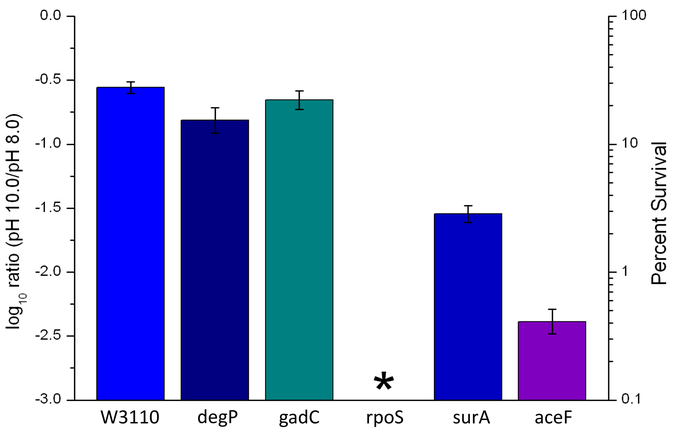

Figure 3. *rpoS*, *surA*, and *aceF* knockouts have significantly decreased survival after base exposure relative to the wild-type. While degP had significantly decreased high-pH survival as well, further replicates indicate that this result was atypical. Error bars = SEM. Two-sample t-test$_{surA,\ aceF}$, t = 12.27, 17.91, p = 0.000, 0.000.

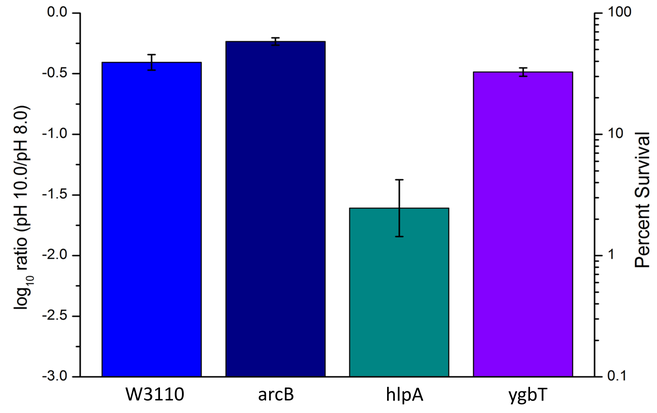

Figure 4. *hlpA* knockout shows significantly decreased base exposure survival relative to the wild-type strain. While this result was seen in other replicates, it was not consistently significant. Error bars = SEM. Two-sample t-test$_{hlpA}$, t = 4.72, p = 0.005.

Conclusions

Determination of Optimal Testing Conditions

- High-pH survival was significantly better in the LBK medium exposure condition than in the M63 exposure condition. In addition, high-pH survival is significantly improved by reducing the pH of the exposure treatment by as little as 0.2 pH units.

Base Exposure Survival Assays

- *surA, aceF*, and *hlpA* knockouts displayed decreased base stress survival relative to the wild-type. These results indicate that the protein products of these genes make important contributions to base resistance.

Interpretation of Results

- Since it encodes a periplasmic chaperone (4), *surA* likely increases base resistance by protecting base-sensitive proteins crossing the periplasm from damage. This in turn helps prevent the aggregation of misfolded proteins into large, difficult to degrade knots.
- Similarly, *hlpA* encodes a periplasmic chaperone (4) protein that likely protects base-sensitive proteins during base exposure through a similar mechanism.
- The potential role of *aceF* in base resistance is less obvious. Since it encodes part of the pyruvate dehydrogenase complex (5), the activity of aceF has no clear link to base resistance. *aceF* may indirectly provide base resistance by affecting the rate of consumption of protons in the cytoplasm.

References

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