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# Analysis of SoxS in S. typhimurium by Transposon Mutagenesis

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# Analysis of SoxS in *S. typhimurium* by Transposon Mutagenesis

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## Abstract

Overexpression of *SoxS* shows significantly decreased growth, likely due to the costly mechanisms activated by this transcription factor. Members of *mar-sox-rob* regulon present in many enteric bacteria has been shown to regulate activities from antibiotic resistance to flagellar expression. This analysis attempts to restore normal growth in *SoxS* overexpression strains using transposon mutagenesis and later characterize the mutations that restored normal growth patterns.

## Introduction

*Salmonella* is a facultative anaerobe and facultative intracellular pathogen known to be a cause of foodborne gastrointestinal infection. More concerning is its ability to modulate its expression of pathogenic traits, such as host immune evasion and antibiotic resistance. One transcription factor that coordinates this response along with that to oxidative stress is *SoxS*. An interesting, previous observation of *SoxS* overexpression leading to inhibited growth warranted further study, but the exact cause remains unknown.

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## Objectives

- Creation of a strain that overexpresses SoxS
- Electroporation of a transposon-containing plasmid to the overexpression strain
- Enrichment for fastest growing strains through serially diluted culture
- Assay of enriched mutants for growth compared to original overexpression strain
- Reconstruction of fastest mutants using phage
- Sequencing of fastest mutants

## Methods

SoxS was ligated into a plasmid downstream of an arabinose-inducible promoter and electroporated into a *Salmonella* strain. Another plasmid containing a minitransposon was also electroporated into the same strain. The strains were then pooled into culture containing arabinose and dilute daily for 10 days. Individual mutants from these cultures were then isolated and their growth was measured against that of the original strain prior to mutagenesis. The strains growing faster than the original strain were then selected for further study.

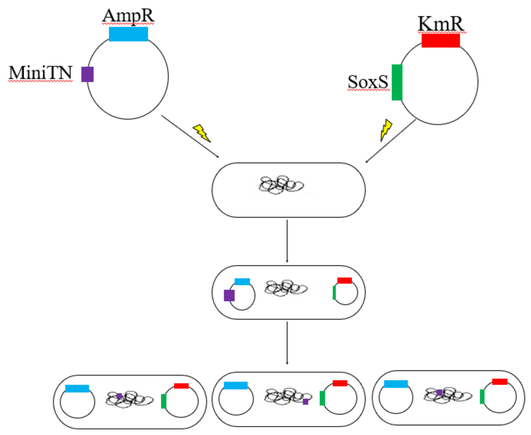


*Figure 1: Strain construction scheme: two plasmids were transformed in the wild-type. One with the minitransposon and the other containing the mutated, inducible SoxS gene. Once transformed, the minitransposon could then mutate other parts of the genome.*

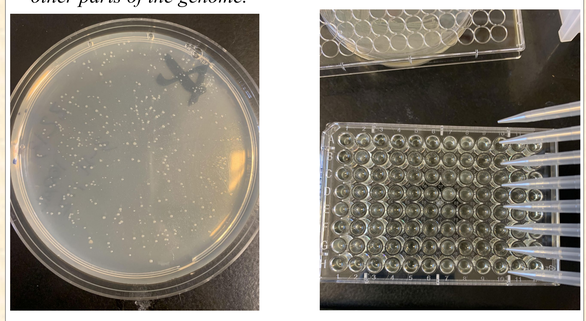

*Figure 2: Mutants yielded from electroporation (left) and microtiter plate utilized in growth assay (right)*

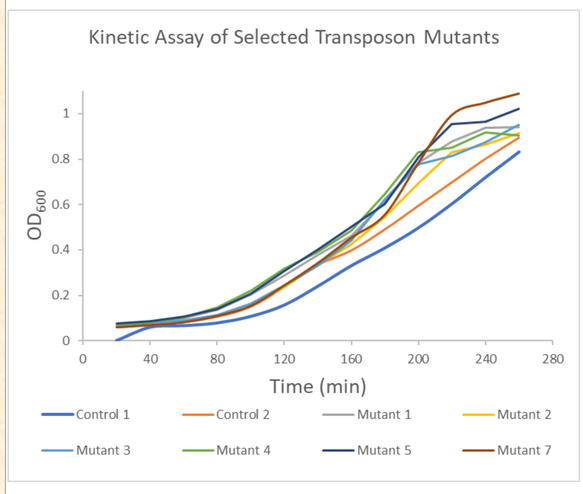


*Figure 3: Kinetic assay of individual mutants plotted against the overexpression strain (control). Growth measured by optical density at 600 nm.*

## Results

- Successful creation of the strain as described
- Performed enrichment of mutants selecting for increased rate of growth and isolated individual mutants
- Characterization of mutants for growth compared to SoxS overexpression strain ongoing

## Next Steps

- Characterization of the fastest growing mutants yielded from mutagenesis
- Reconstruction of the mutant genotype starting with SoxS overexpression strain using phage
- Sequencing of selected mutants

## References

Holden, E. R., & Webber, M. A. (2020). MarA, RamA, and SoxS as Mediators of the Stress Response: Survival at a Cost. *Frontiers in microbiology*, *11*, 828. https://doi.org/10.3389/fmicb.2020.00828

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