Elucidation of Gene Regulation That Allow *Salmonella enterica* to Survive in Low Magnesium Conditions

Fiona Lindsay-Olivera [1], Brooke Burris [2], and Dr. James Slauch [2]

Parkland College, Champaign, Illinois[1]

Department of Microbiology, College of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign[2]

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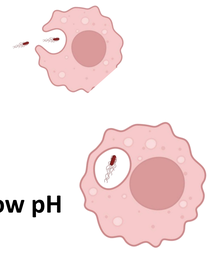
Microbial Interactions Create Research Opportunities for Community College Students

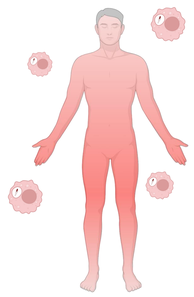
Background

- The increased virulence of *Salmonella enterica* in extraintestinal infections has been attributed to its ability survive and replicate in macrophage phagosome.

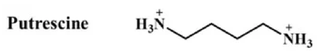


Salmonella can adapt to low pH and low Mg2+ conditions encountered in the phagosome.[1]

- To counteract the low magnesium conditions in the macrophage phagosome and ensure viability, intracellular polyamine (PA) production is critical.[1]



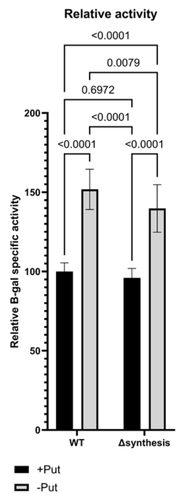
- Salmonella can synthesize putrescine in addition to other polyamines.



Project Aim

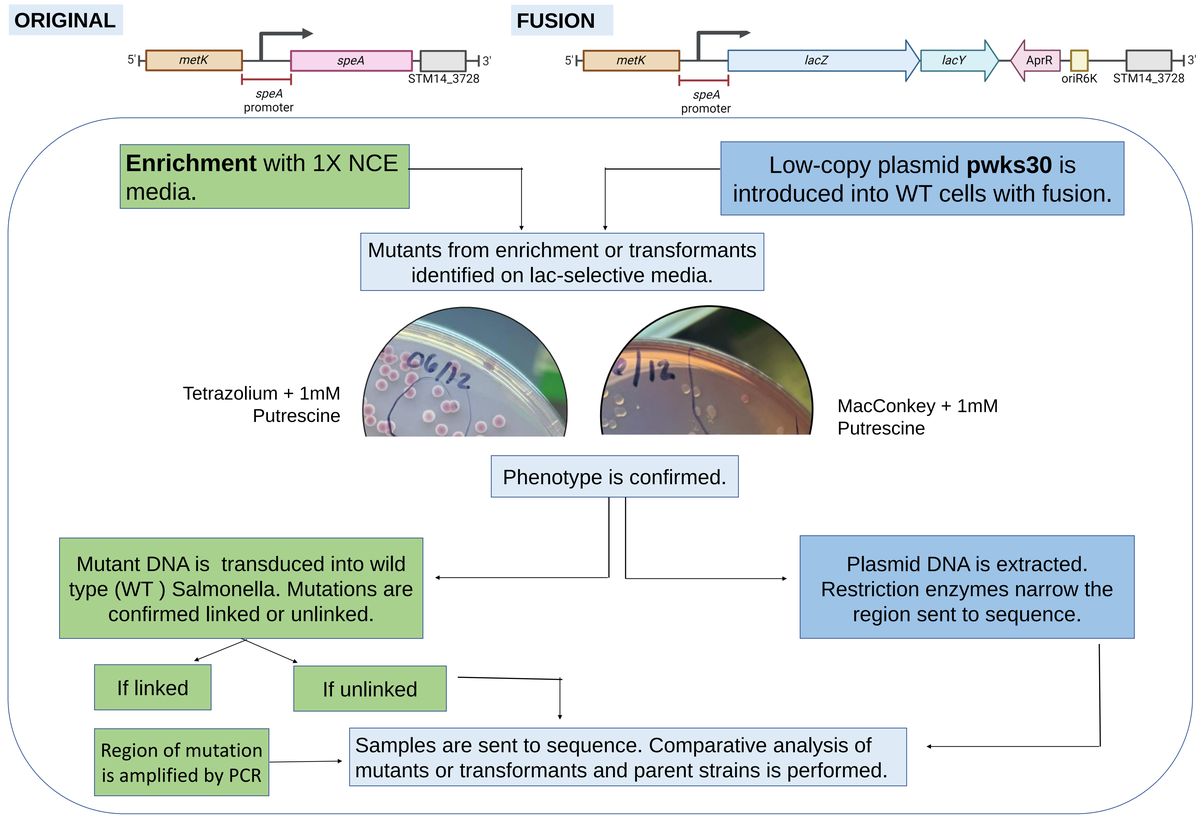
Putrescine production has been proven indispensable to the adaptation of Salmonella enterica to low Mg2+ conditions.[1]

- The *speA* gene is the first committed step to Putrescine synthesis. It has been shown to me upregulated in the absence of putrescine. **Its regulation is unknown.**



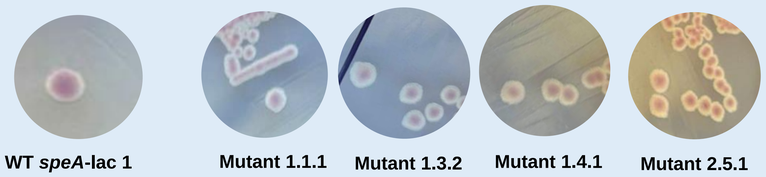
Graph 1. Results from β-galactosidase assays on Δ*speA*-lacZY in WT and Δsynth (Δ speA, Δ speB, ΔspeC, ΔspeF, ΔspeDE Δ speG ΔcadA, ΔldcC) backgrounds. The β-galactosidase activity units are defined as (mmol of ortho-nitrophenol formed per minute) 10^6/(OD600 mL of cell suspension) and are presented as the mean ± SD, *n*=9. Statistical analyses were performed using an unpaired t test.

Using the *speA*-lacZY fusion to elucidate regulation of *speA:*

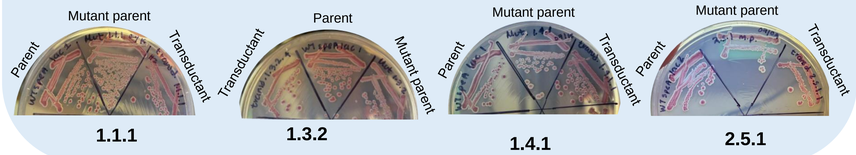


Results

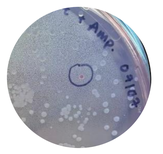
Four mutants were found from a seven-day enrichment.



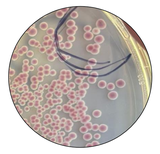
Transductant phenotypes indicate unlinked regulation of *speA*.



About 150 transformants with mutant phenotype where selected and plasmid DNA was extracted.



Transformants in MacConkey +1mM Putrescine



Transformants in Tetrazolium +1mM Putrescine

Conclusions

The absence of the mutant phenotype in the transductants lead us to believe that the mutation is not linked to the fusions, therefore **the regulation of the *speA* gene is not in the fusion but somewhere else in the genome.** The significant increased frequency of mutations in plates with putrescine may also rule out regulation by a repressor.

Future Work

- Genomic DNA extraction of four mutants.
- Comparative genomics on sequenced mutants to identify the location of the gene and/ or genes regulating *speA* activity.
- Plasmid DNA extraction from transformants and remove duplicates from sample pool.
- Amplify genome fragment in plasmid and sequence for regulatory elements.

References

1. Iwadate, Y.; Golubeva, Y. A.; Slauch, J. M. Cation Homeostasis: Coordinate Regulation of Polyamine and Magnesium Levels in Salmonella. mBio 2023, 14 (1), e02698-22. DOI: 10.1128/mbio.02698-22.
2. Iwadate Y, Ramezanifard R, Golubeva YA, Fenlon LA, Slauch JM. Pa eA (YtfL) protects from cadaverine and putrescine stress in *Salmonella* Typhimurium and *E. coli*. *Mol Microbiol*. 2021; 115: 1379–1394. https://doi.org/10.1111/mmi.14686

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