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Finding gene candidates that interact with MarA to control hilA expression in Salmonella enterica

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Finding gene candidates that interact with MarA to control *hilA* expression in *Salmonella enterica*

UMSL

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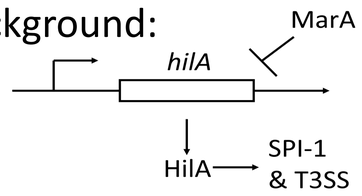
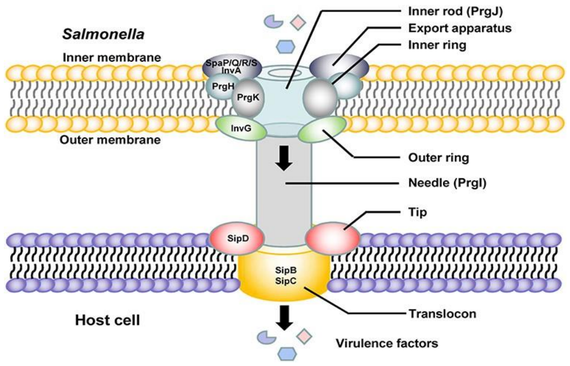
Intro/Background:

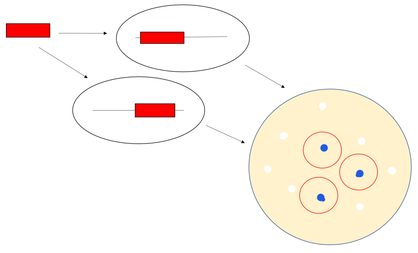


hilA expression is necessary for SPI-1 and T3SS activation in *S. enterica*. MarA is a transcription factor that inhibits the expression of *hilA.* MarA inhibits *hilA* expression indirectly, but associated genes are unknown.

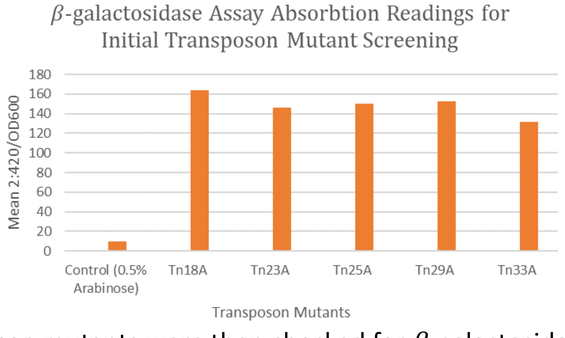


Lou, L., Zhang, P., Piao, R., & Wang, Y. (2019). Salmonella pathogenicity island 1 (SPI-1) and its complex regulatory network. *Frontiers in cellular and infection microbiology*, *9*, 270.

Methods:

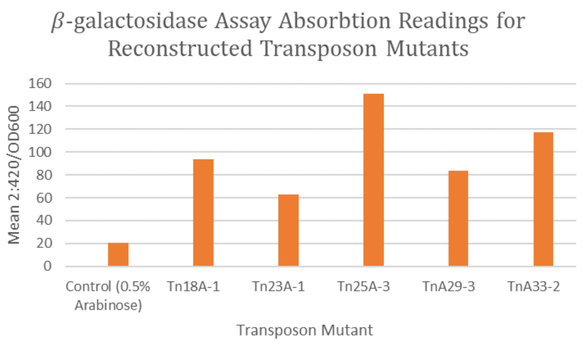


Transposon mutagenesis was used to find candidate genes that may work with MarA to repress *hilA* expression. The insertion of the transposon was random and mutants were screened and chosen using L-agar plates containing X-gal*.

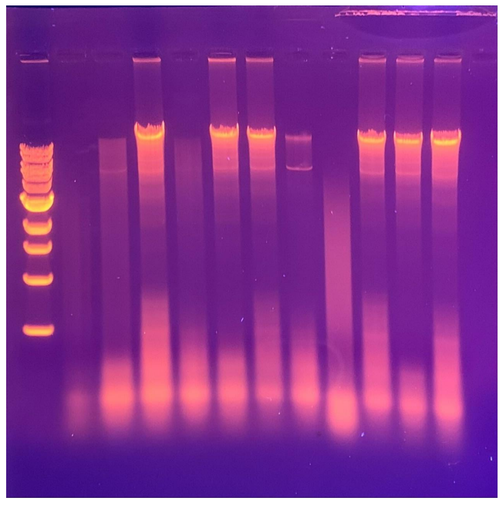


Chosen mutants were then checked for β-galactosidase activity* to ensure that repression of *hilA* was relieved via interruption of the MarA-associated gene by the inserted transposon. *hilA* expression can be observed through this assay by putting the gene under control of the *lacZ* promoter.

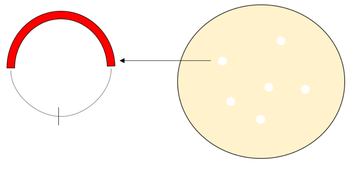
*work done by Alexandra King



Once the mutants were confirmed to have significantly increased *hilA* expression, they were reconstructed via transductions using p22 phage. These reconstructed mutants were then checked for β-galactosidase activity again to confirm the effects of the mutation.

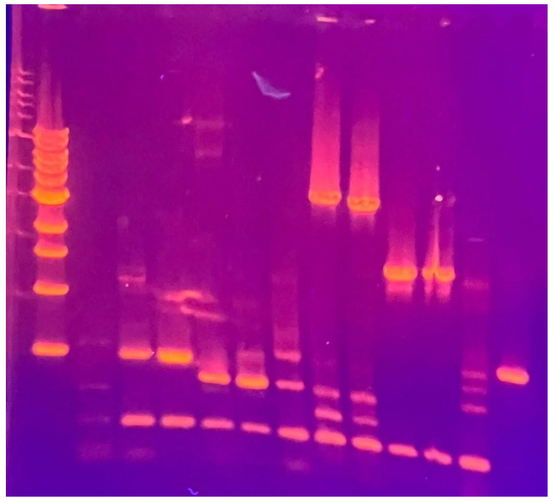


Chromosomal DNA was then isolated from the confirmed mutants, digested with the restriction endonuclease BamHI and ligated. The cut sites used cut around the transposon, allowed self-ligation, and included sequence from the *S. enterica* genome. This is done by cutting just outside of the transposon to include part of the *Salmonella* genome that was interrupted. This is crucial to identifying the gene(s).



The ligated plasmids were then electroporated into *E. coli*. Only cells with plasmids including the transposon were able to grow on the plates including kanamycin.

The colonies were re-streaked onto L-agar plates containing kanamycin to confirm antibiotic resistance and, therefore, presence of the transposon. The transposon used and inserted to create the mutants included a gene for antibiotic resistance to kanamycin to allow for easy screening and selection



The plasmids were then isolated from the *E. coli* colonies that grew. Above is a photo of PCR products from the minipreps ran on an agarose gel. The primers used for this PCR amplified the portion of the plasmid containing part of the *S. enterica* genome (candidate gene).

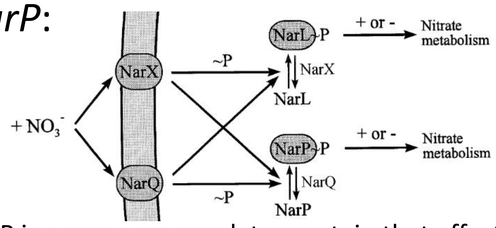
Transposon Mutant	Sequence	Gene Candidate
pKH1/Tn25A-3	5'...ACCTGGGACAGGCGATCAATTANCTGAACGATANAAAGGATACAACAACCCGTTGTGAGAGAAAACACANTACTCCCTGAAG...3'	*narP*
pKH2/Tn25A-3	5'...TGCACTAATTAGGGTAAAACATANGCGCTTGACAATGTGGCAAATCTGGACGATTTCCGCGCTGAAAACAATAATCATTCT...3'	*narP*
pKH6/Tn29A-3	5'...GATGGTTGAGATGTGAATAAGAGACAGGNCNTGNCAGGGCGCTGGCGTGGGCACAGCGCTGTATGAGAGGGTATCGCCTGCC...3'	*nfi*
pKH7/Tn29A-3	5'...TGCCTCAACTTTCCAATTCAACATTCCATCATGATACTACACCAGATTCATTTGCGTCTGGACCGGTTGGATAGCTGGCTGAACGTT...3'	*nfi*
pKH8/Tn33A-2	5'...TGATGGTTGAGATGTGTATAAGAGACAGCCCTNGACCTGGTCGTACATAGCGAAAATTATCTATTACCTTGTTAGCGACATGCGT...3'	*csgA*
pKH9/Tn33A-2	5'...ATTATCTATTACCTTGTTAACGACATTCGTTTTTTGTTAACGCGNCTATACGATGAAAATCATGTCCGTGGAAACATTTTTAATAA...3'	*csgA*
pKH11/Tn33A-2	5'...GCGAAAATTATCTATTACCTTGTTAGCGACATGCGTTTTTTGTTAACGCGTTCTTACTATGAAGAGTATGTCCGTGGAAACATTTTT...3'	*csgA*

The minipreps, along with the primers mentioned above, were then mailed to the Danforth Center to be sequenced. These sequencing products were compared to sequences in the National Center for Biotechnology Information database. The most intriguing MarA-associated gene candidates are mentioned here: *narP*, *nfi*, and *csgA*. It is worth noting that the duplicate gene candidates come from the same mutants. This allows us to see that the techniques used up to this point don't damage or mutate the DNA.

Analysis & Future Work:

It is very important and helpful to know what the gene candidates are and their functions for future work on this ongoing project. Knowing the functions and activity of these genes can lead us to the answer of how they may interact with MarA and how drugs can be made to target the gene directly or indirectly to combat the major problem of increasing antibiotic resistance in bacteria.

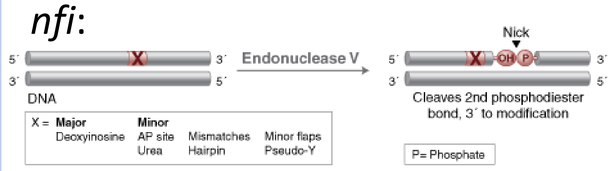
narP:



NarP is a response regulator protein that affects expression of nitrate and nitrite catabolic genes in the context of aerobic electron transport (Unden et al., 1997).

Unden, G., & Bongaerts, J. (1997). Alternative respiratory pathways of Escherichia coli: energetics and transcriptional regulation in response to electron acceptors. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1320(3), 217-234.

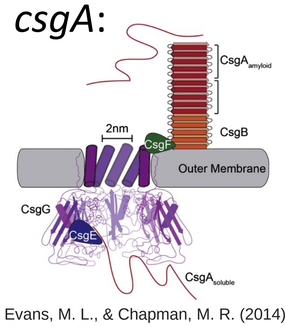
nfi:



nfi is a gene that encodes for endonuclease V. Endonuclease V most commonly cleaves DNA at points where there is a deoxyinosine for repair.

Endonuclease V. New England BioLabs Inc. https://www.neb.com/products/m0305-endonuclease-v#Citations%20&%20Technical%20Literature

csgA:



csgA is an important subunit protein that is apart of the curli protein complex. The main function of the curli complex is biofilm formation.

Evans, M. L., & Chapman, M. R. (2014). Curli biogenesis: order out of disorder. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1843*(8), 1551-1558.

The next steps to take in this project include mutating these gene candidates and seeing how it affects *S. enterica*, *marA*, *hilA*, and other variables related to *Salmonella* pathogenesis. The methods explained here will continue to be repeated, as well, to find more gene candidates until the picture is complete.