Uncovering the Mechanisms of Transcriptional Regulation of ECA Biosynthesis in *E. coli* K-12

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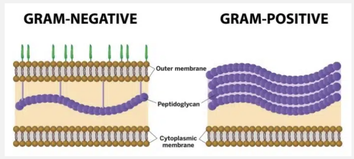
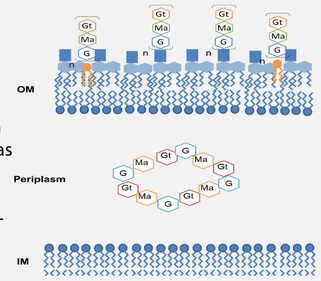
Background

Antibiotic resistance is a significant global threat with nearly 3 million antibiotic resistant infections per year in the US

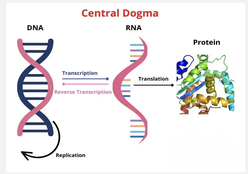
Resistance is especially prevalent among gram negative bacterial species, like *E. coli*



The gram negative cell envelope structure makes it particularly adept at resisting antibiotics



Enterbacterial Common Antigen (ECA):
- a highly conserved carbohydrate moiety in *Enterobacterales* outer membrane which has been shown to play an important role in regulating the membrane permeability barrier to protect bacterial cells from external stressors like antibiotics



Conducting biosynthetic studies of ECA's effect on outer membrane will help to identify targets for new antibiotics

Objectives

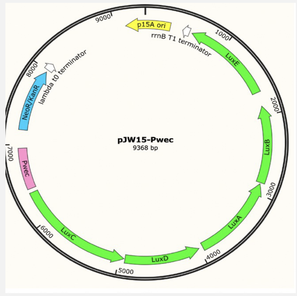
This work seeks to identify genes responsible for regulation of ECA at the transcriptional level and to determine the conditions in which ECA's activity on the permeability barrier is important.

Project Goals:
- Determine the role of potential regulators and stress responses on the *wec* operon expression
- Determine the effect of environmental conditions on *wec* operon expression

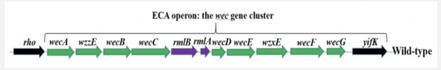
Methods

Category	Potential Regulators
Stress Response	OxyR, CspA, OmpR, RcsAB, Lrp, GlnG, GadX, Rob, MarA, SoxS, SlyA, PhoP, PhoB
Anaerobic growth	Fnr, ArcA, NarL
Lipid metabolism	GlpR, FadR
Carbohydrate metabolism	Mlc, NagC
Central metabolism	Crp, PdhR, Cra
Iron Homeostasis	Fur
Nucleotide metabolism	CytR
Amino acid metabolism	CysB, ArgR, GcvA, MetR, MetJ, ArgP
Nucleoid proteins	IhfAB, H-NS, Fis
Nitric oxide protection genes, motility, biofilms	NsrR
Motility	PdeL

Created a luminescent reporter plasmid containing the *wec* promoter sequence to measure transcriptional activity in strains deficient in the target potential regulator gene

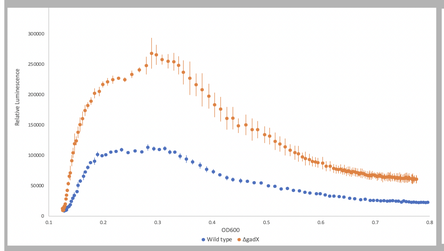


Compared sequence similarity between known binding sites of potential regulators and the *wec* promoter region

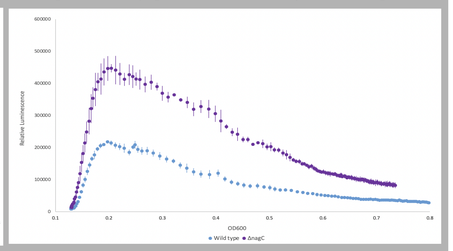


Potential Regulators with Changes in ECA Activity

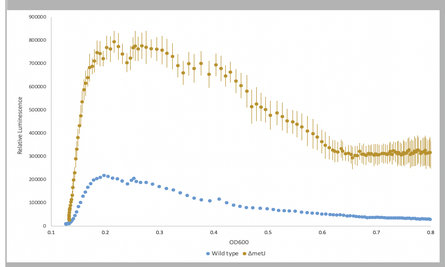
ΔgadX: Acid Resistance Regulator



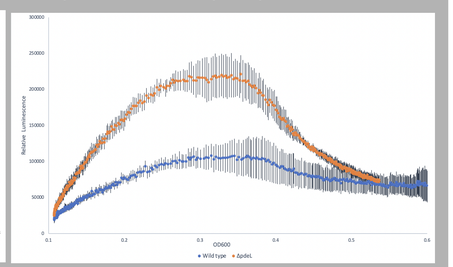
ΔnagC: N-acetylglucosamine Biosyntheis Regulator



ΔmetJ: Methionine Synthesis Repressor

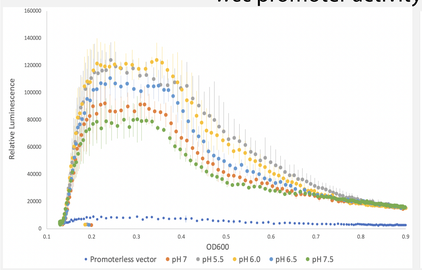


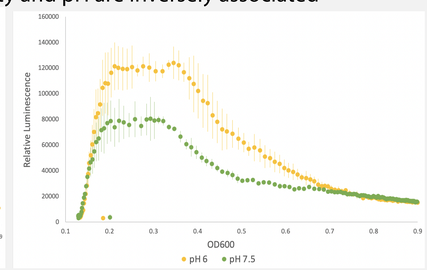
ΔpdeL: c-di-GMP phosphodiesterase



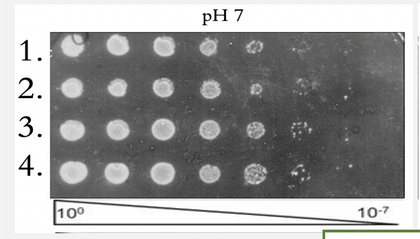
ECA Activity and Acid Stress

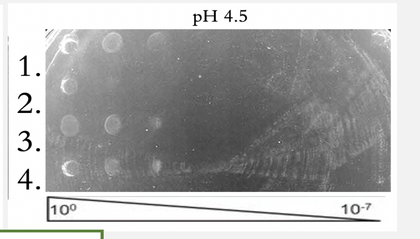
wec promoter activity and pH are inversely associated





Phosphoglycerol-associated ECA is protective against acidic conditions in minimal media





1. Wild type
2. ΔwecA (no ECA made)
3. ΔwaaL (ECA_{PG} & ECA_{CYC} only)
4. ΔwzzE (ECA_{PG} and ECA_{LPS} only)

Results and Next Steps

- pH level was found to influence expression of the *wec* operon, suggesting ECA may have a role in acid resistance
- Certain genes appear to have an affect on wec promoter activity; these genes will be further investigated to characterize their function in ECA biogenesis regulation
- Next steps include mutating putative regulator binding sites in *wec* operon and confirming direct binding through EMSA, and determining the mechanism of changes in regulation in response to environmental conditions

Acknowledgements

Committee Members:
Chair: Dr. Angela Mitchell
Dr. Beiyan Nan
Dr. Deborah Siegele
Dr. Anna Konovalova

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