BACTERIAL METABOLIC RE-WIRING FUELS TRANSIENT RESISTANCE TO POSITIVELY CHARGED ANTIBIOTICS

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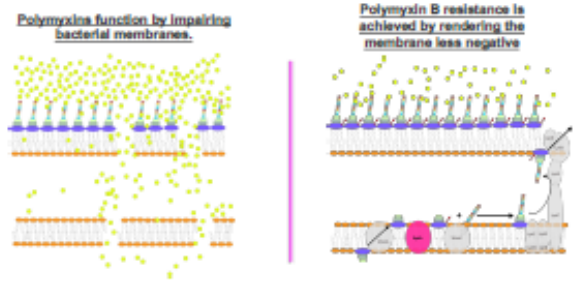
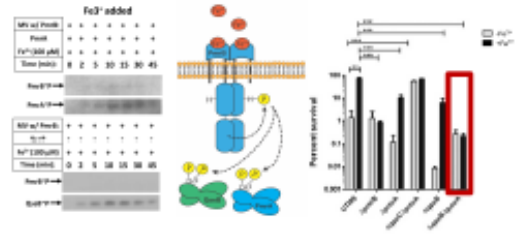
INTRODUCTION



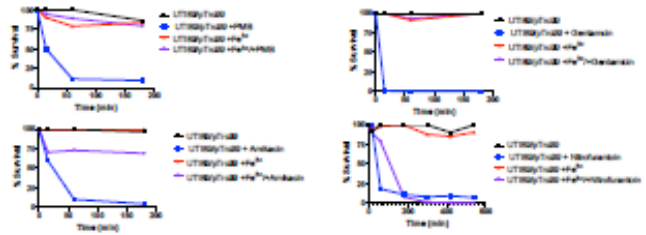
- Polymyxins are cationic antimicrobial peptides that are used in the clinic as a last resort antibiotic.
- Resistance to polymyxins are usually driven through LPS modifying genes, such as EptA/Mcr-1 which modify nascent LPS to increase the charge of the bacterial membrane. These systems can be encoded on mcr-1 plasmids.
- However, all *E. coli* encode a chromosomal Mcr-1 homologue: *pmrC*/EptA that is controlled by the PmrAB-QseBC systems.

BOTH PmrA AND QseB RESPONSE REGULATORS ARE NEEDED FOR MOUNTING TRANSIENT RESISTANCE TO POLYMYXIN B

Activation of PmrB by ferric iron leads to the phosphorylation of the response regulators PmrA and QseB.



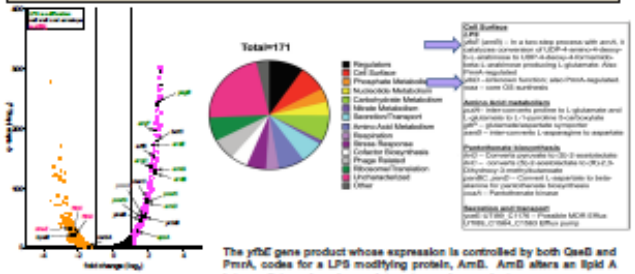
INDUCTION OF QseBC- PmrAB IN *E. coli* CONFERS RESISTANCE TO POSITIVELY CHARGED ANTIBIOTICS



- For survival assay approximately 1 X 10^8 CFUs/mL of bacteria from *E. coli* strain UTI89 were treated with polymyxin B (PMB), gentamicin, or amikacin for 180 minutes and nitrofurantoin for 480 minutes. Percent survival was calculated by comparison to the T = 0 time point.
- When the QseBC-PmrAB system was stimulated with ferric iron prior to and during an a challenge with positively charged antibiotics (polymyxin B, amikacin, and gentamicin), UTI89 resisted the antibiotic and had a percent survival over time similar to an untreated control.
- When challenged with nitrofurantoin, a neutral antibiotic, UTI89 did not have increased survival with the addition of ferric iron.

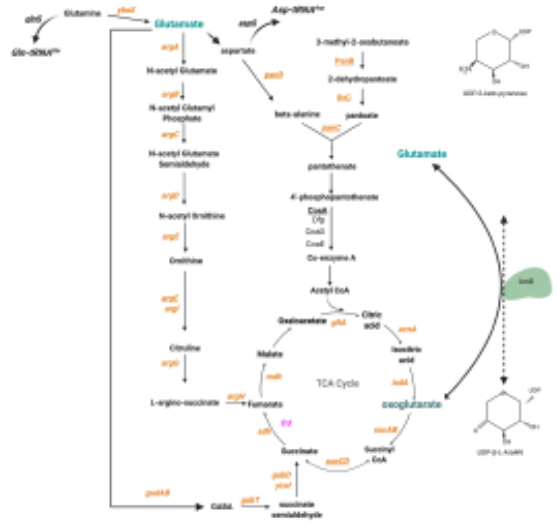
WHAT IS THE ROLE OF QseB IN MEDIATING RESISTANCE TO POSITIVELY CHARGED ANTIBIOTICS?

RNASeq AND chIP-ON-CHIP ANALYSES REVEAL THAT QseB CONTROLS METABOLIC PROCESSES ASSOCIATED WITH GLUTAMATE CATABOLISM



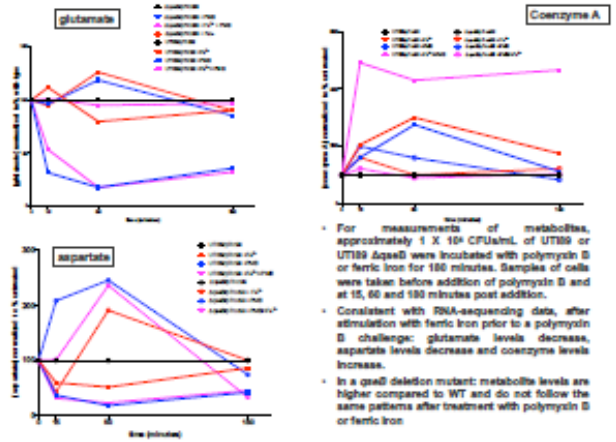
The *yfbE* gene product whose expression is controlled by both QseB and PmrA, codes for a LPS modifying protein, AmB. AmB alters an lipid A intermediate and produces glutamate as a by- product.

QseB CONTROLS A CENTRAL METABOLIC CIRCUIT



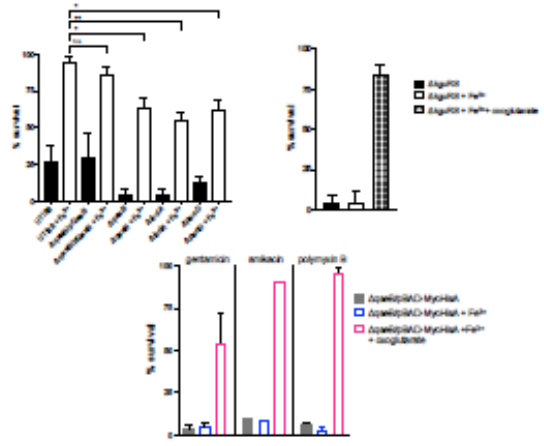
- 2-oxoglutarate and glutamate metabolism are connected and play critical roles in E. coli physiology.
- We propose that QseB controls the balance between the two metabolites as it pertains to their use and production during nascent LPS modification.
- If this is true, then we should be able to track changes in key metabolites in the UTI89, UTI89ΔqseB and complemented strains in response to PMB assault and following system activation with ferric iron.
- Addition of 2-oxoglutarate could rescue the qseB deletion mutant, while glutamate may potentiate its defect

METABOLIC ANALYSES PROBE GLUTAMATE, CoA AND ASPARTATE LEVELS IN RESPONSE TO POLYMYXIN ASSAULT IN THE PRESENCE OR ABSENCE OF QseB



- For measurements of metabolites, approximately 1 X 10^8 CFUs/mL of UTI89 or UTI89 ΔqseB were incubated with polymyxin B or ferric iron for 180 minutes. Samples of cells were taken before addition of polymyxin B and at 15, 60 and 180 minutes post addition.
- Consistent with RNA-sequencing data, after stimulation with ferric iron prior to a polymyxin B challenge: glutamate levels decrease, aspartate levels decrease and coenzyme levels increase.
- In a *qseB* deletion mutant: metabolite levels are higher compared to WT and do not follow the same patterns after treatment with polymyxin B or ferric iron

ADDITION OF 2-OXOGLUTARATE RESTORES RESISTANCE TO ANTIBIOTICS IN QseB DELETION MUTANT



- Addition of ferric iron before and during polymyxin B challenge increases survival of samples compared to untreated samples.
- Addition of oxoglutarate to ferric iron treated culture the media at the same time as addition of polymyxin B restores restores resistance to polymyxin B in qseB deletion mutant
- For survival assay approximately 1 X 10^8 CFUs/mL of bacteria from *E. coli* strain UTI89 were treated with polymyxin B (PMB), gentamicin, or amikacin for 60 minutes. Percent survival was calculated by comparison to control sample.

•NIH/NIAID R01 AI107652-01A1 and institutional funds to M.H. • NIH T
NSF Graduate Research Fellowship to E.J.B. • CMP/PMP mini-sabbatical awards to E.J.B.