



Antibiotic Resistance: The Mechanisms of Efflux Pumps

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Background

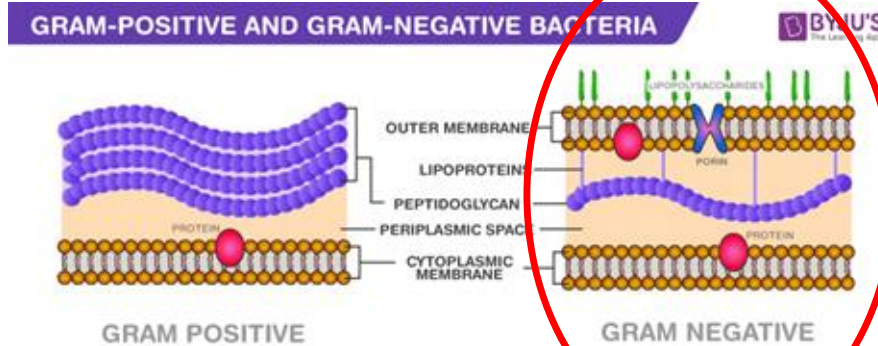


Fig. 1: Structure of Gram-Positive and Gram-Negative Bacteria

*RND: Resistance-Nodulation-Division

RND

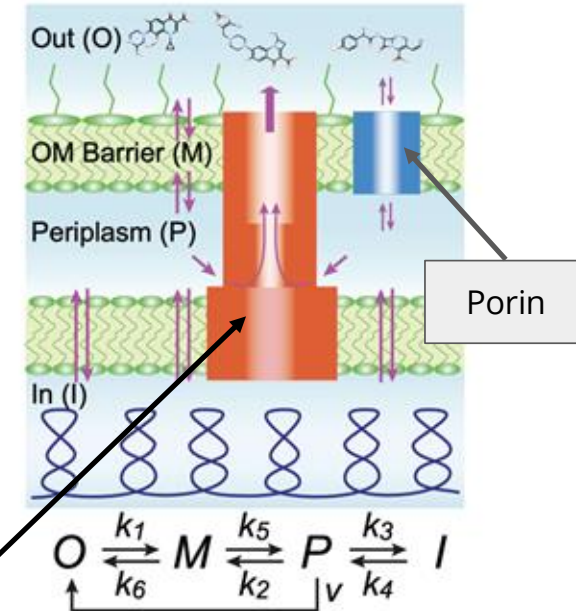
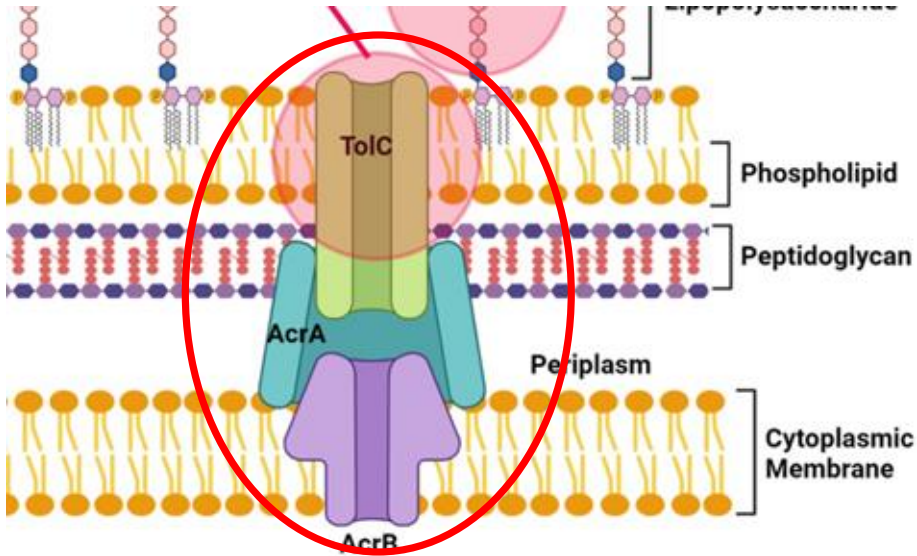


Fig. 2: Kinetic model of Gram-negative bacteria

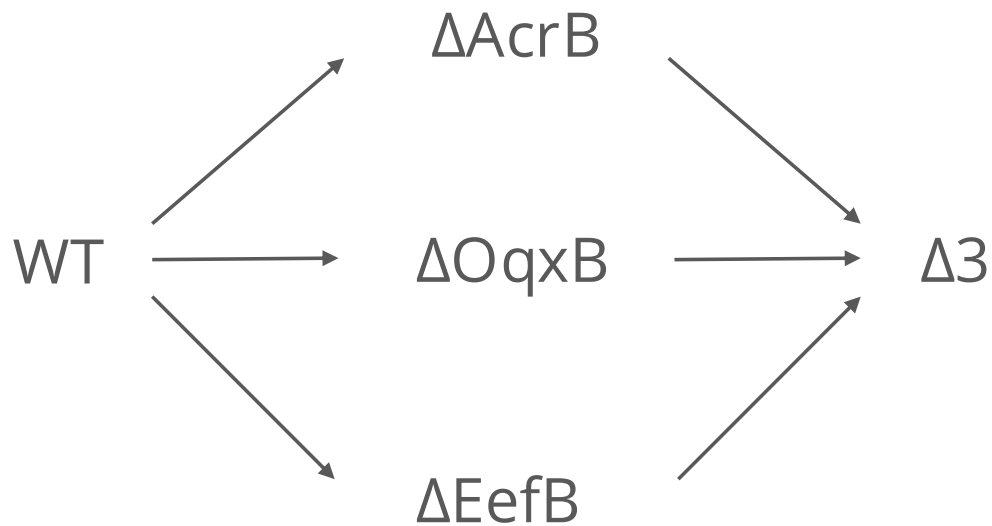


Background



- **AcrB** pumps toxic compounds
- **AcrA** connects both proteins
- **TolC** creates channel to outer membrane

Fig 3: Structure of AcrAB-TolC in Klebsiella Pneumoniae

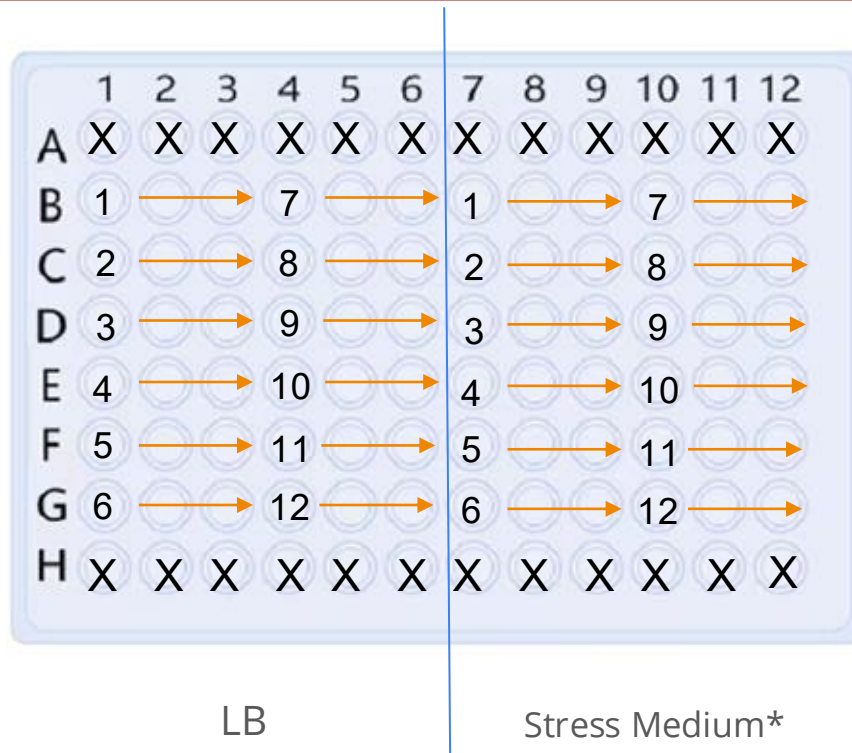




Stress Test

1. WT
2. Δ AcrB
3. Δ EefB
4. Δ EefBC
5. Δ OqxB
6. Δ AcrB Δ OqxB
7. Δ AcrB Δ EefB
8. Δ AcrB Δ EefBC

9. 1.4.2.1
 10. 2.3.3.1
 11. 1.4.3.1
 12. 2.3.1.1
- } Δ 3

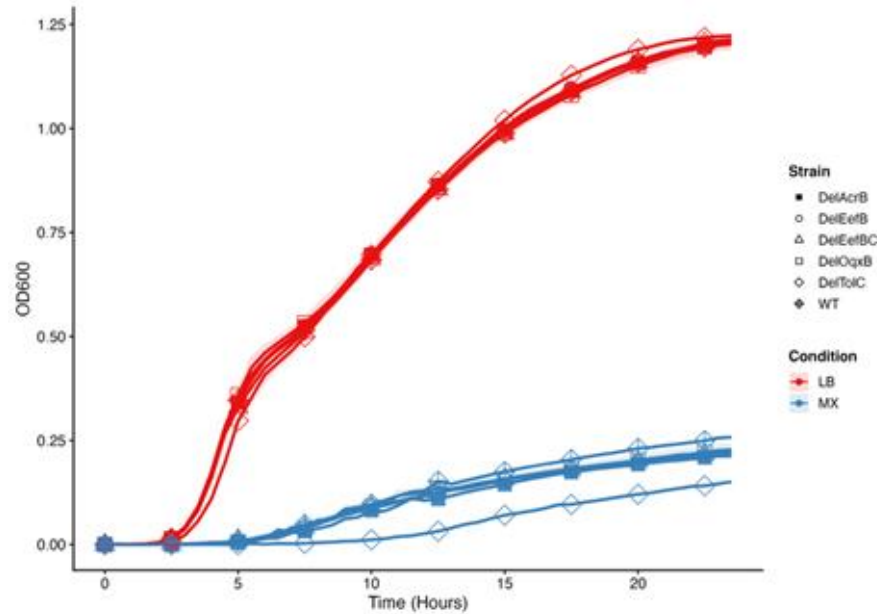


Stress conditions:

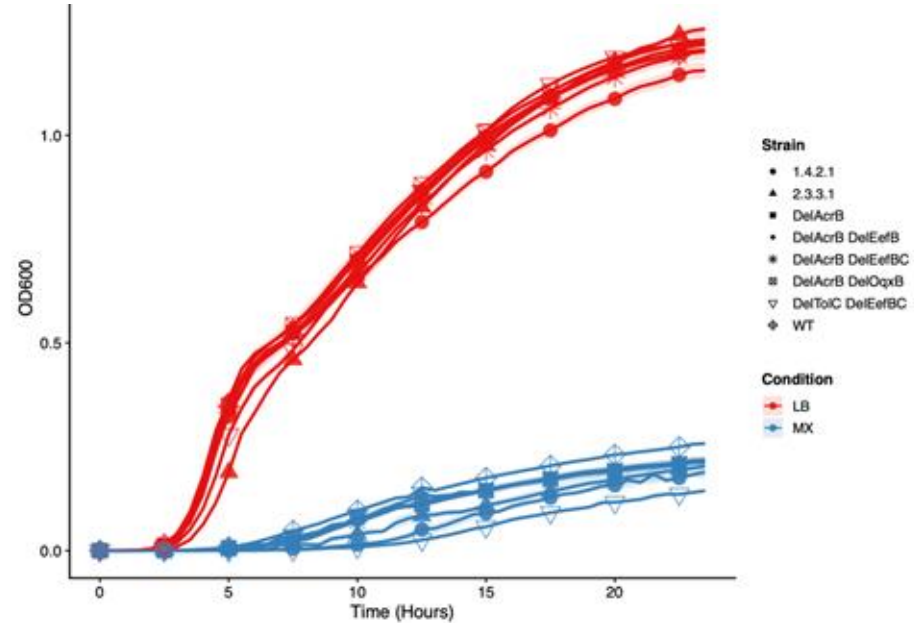
- MX
- 0.5% Bile
- 0.5 M NaCl
- Low Iron
- pH 4.6



Growth Curves: LB v. MX



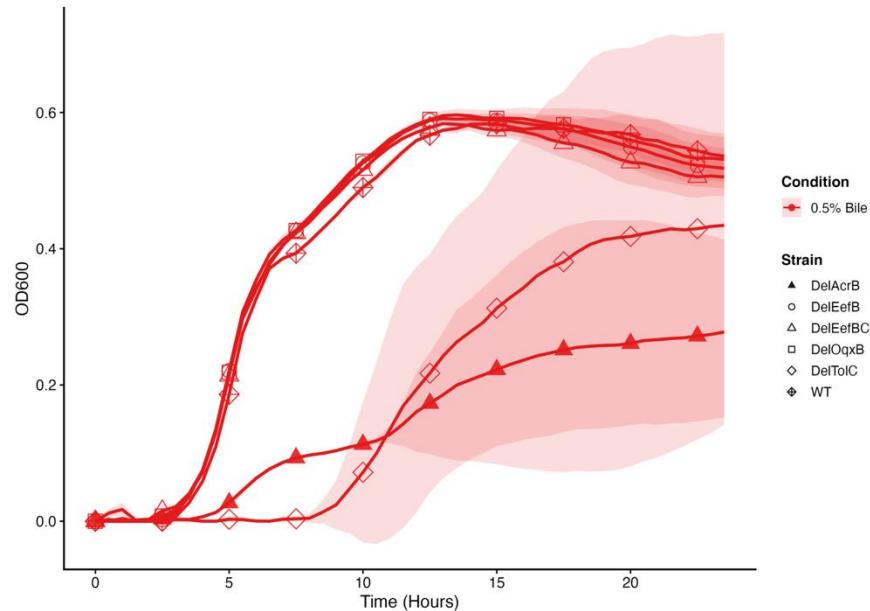
Single Mutants



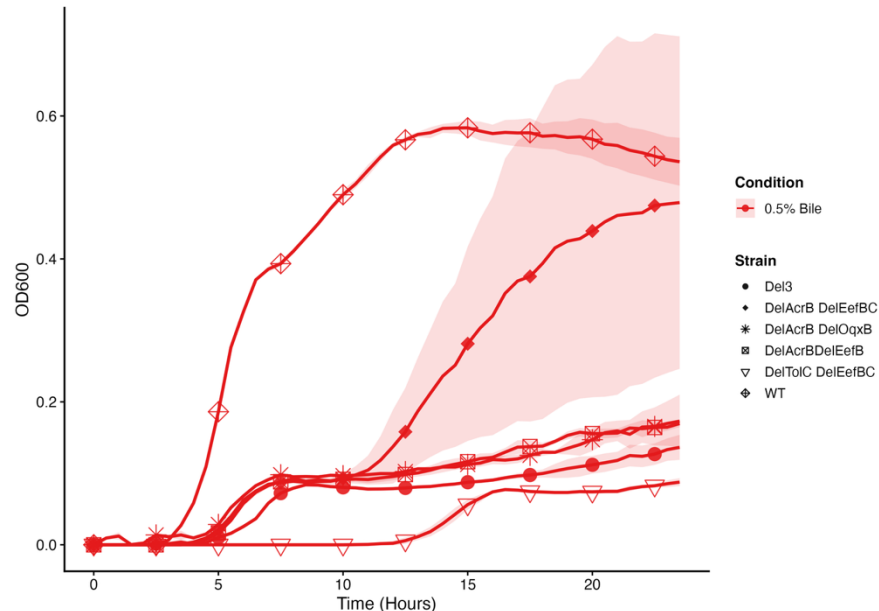
2+ Mutants



Growth Curves: 0.5% Bile Salt



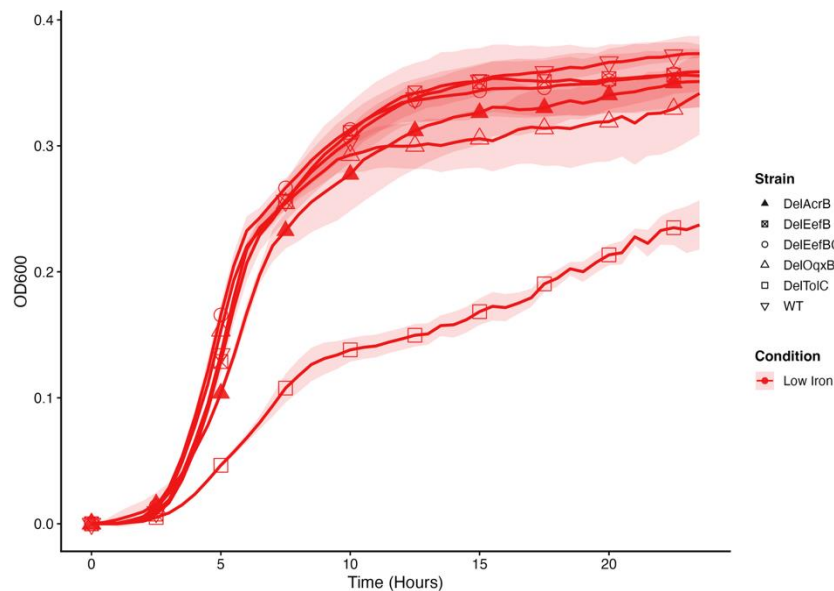
Single Mutants



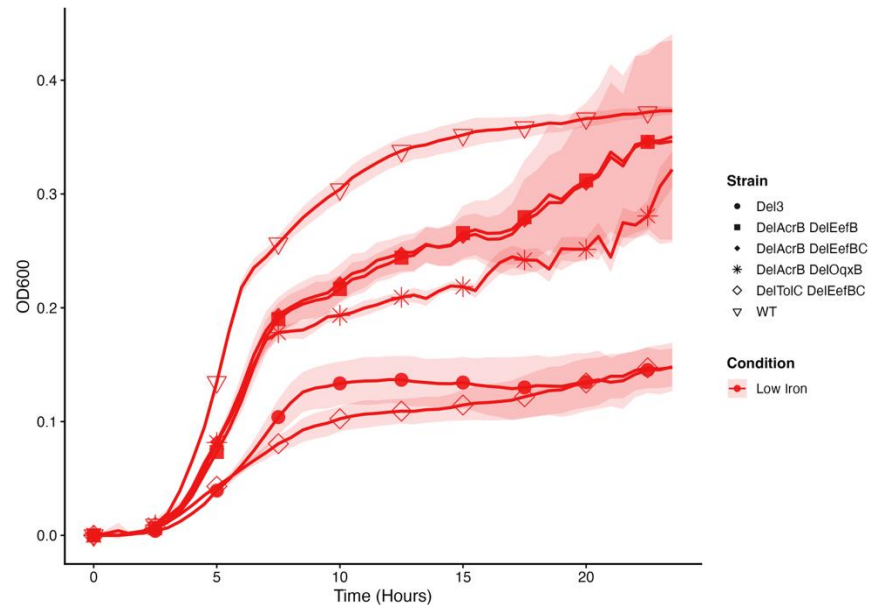
2+ Mutants



Growth Curves: Low Iron



Single Mutants



2+ Mutants

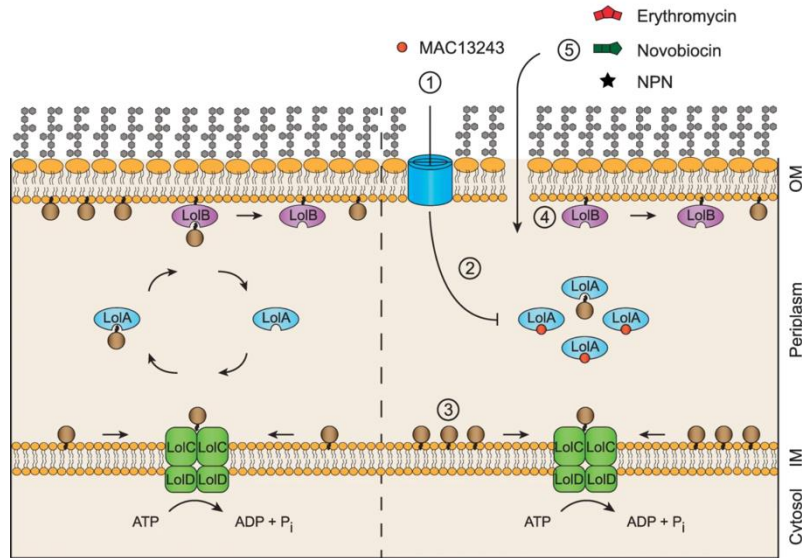


Heat Map

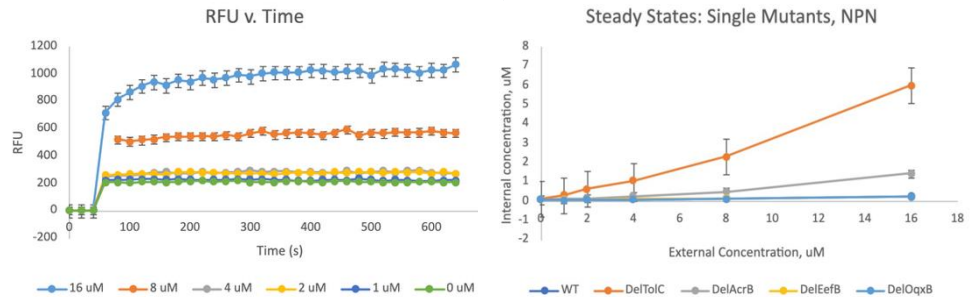
	LB	MX	0.5% Bile	0.5 M NaCl	Low Iron	pH 4.6
WT	15.95 ± 0.93	2.82 ± 0.02	9.70 ± 0.06	12.80 ± 0.82	6.16 ± 0.15	10.61 ± 0.16
ΔTolC	16.32 ± 0.42	1.16 ± 0.01	3.23 ± 4.24	12.47 ± 0.31	3.05 ± 0.15	12.63 ± 0.50
ΔAcrB	16.34 ± 0.44	2.32 ± 0.01	4.05 ± 1.55	12.81 ± 0.58	5.65 ± 0.34	13.38 ± 0.11
ΔEefB	16.14 ± 0.46	2.46 ± 0.01	9.74 ± 0.12	12.57 ± 0.76	6.07 ± 0.44	13.47 ± 0.05
ΔOqxB	16.33 ± 0.48	2.54 ± 0.16	9.94 ± 0.11	12.56 ± 0.87	5.63 ± 0.38	13.38 ± 0.12
ΔEefBC	16.08 ± 0.51	2.52 ± 0.09	9.60 ± 0.08	12.53 ± 1.11	6.15 ± 0.34	13.38 ± 0.04
ΔTolCΔEefBC	15.39 ± 2.21	1.07 ± 0.03	6.23 ± 0.35	10.56 ± 2.17	2.10 ± 0.23	7.79 ± 5.62
ΔAcrBΔEefB	16.47 ± 0.35	2.36 ± 0.07	4.46 ± 3.29	13.02 ± 0.60	4.72 ± 0.53	12.81 ± 0.47
ΔAcrBΔEefBC	16.03 ± 0.41	2.29 ± 0.10	6.08 ± 1.90	12.61 ± 0.37	4.76 ± 0.44	12.30 ± 0.55
ΔAcrBΔOqxB	16.70 ± 0.43	2.32 ± 0.10	4.47 ± 3.24	12.69 ± 0.64	4.09 ± 0.14	13.14 ± 0.13
Δ3	14.97 ± 1.30	1.61 ± 0.12	2.17 ± 0.70	11.48 ± 0.74	3.43 ± 0.40	11.69 ± 0.38



Outer Membrane Permeability Assay



Two probes: NPN and HT

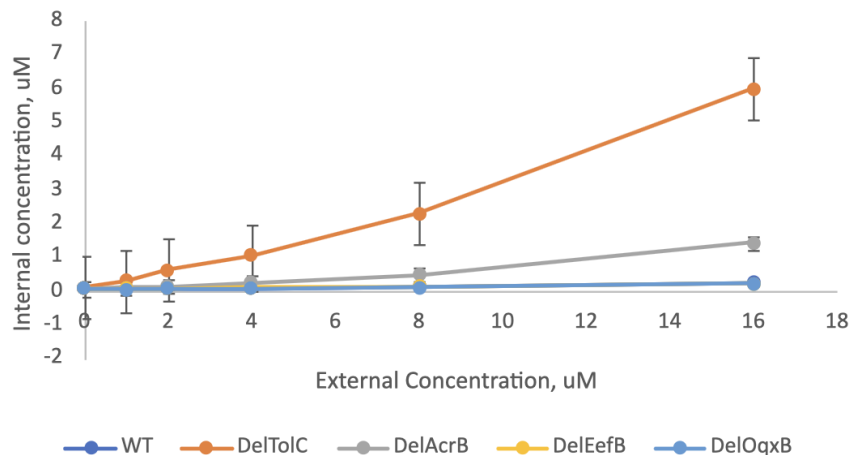


$$F = A1 + A2[1 - \exp(-kt)]$$

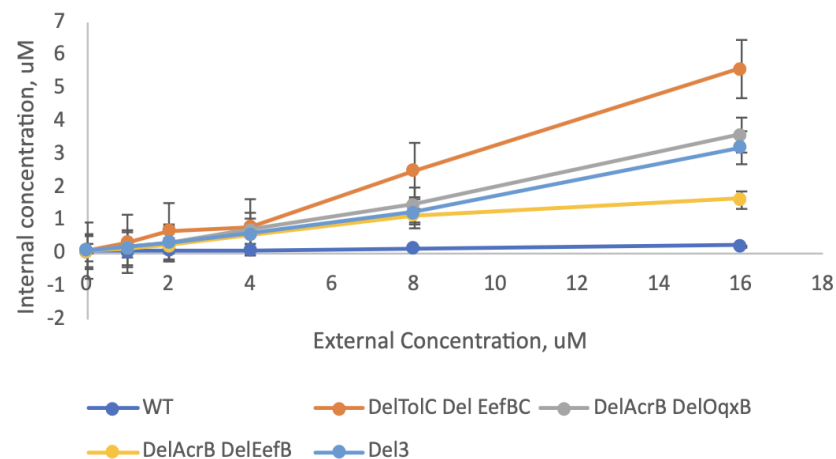


Uptake Curves: NPN

Steady States: Single Mutants, NPN



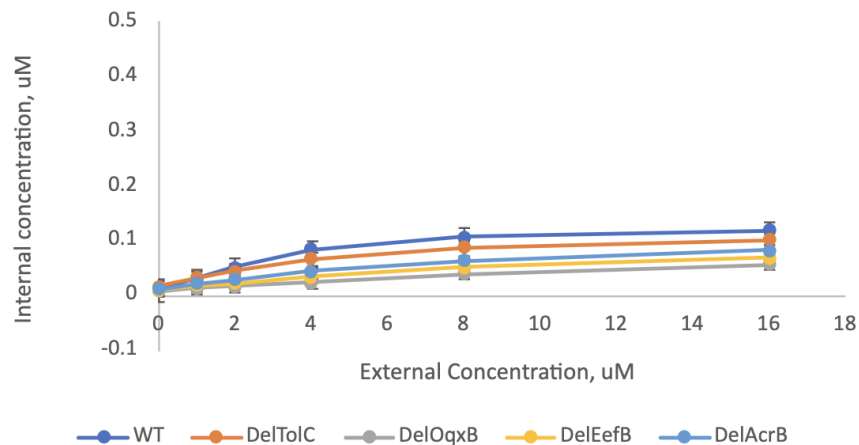
Steady State: NPN, Double Mutants



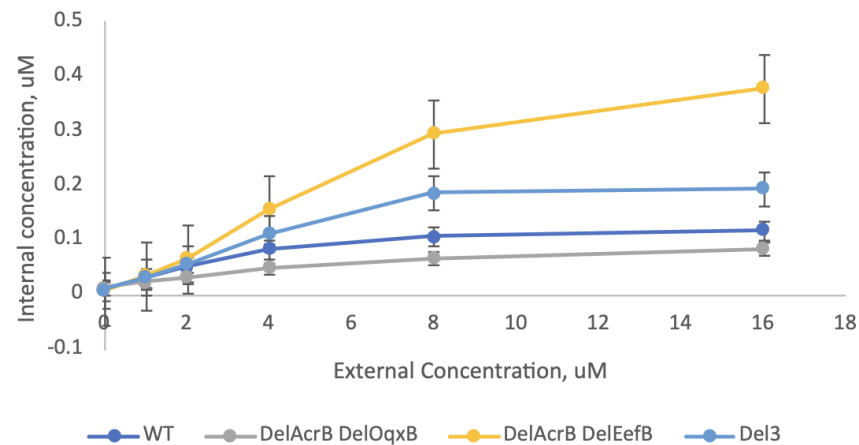


Uptake Curves: HT

Steady State: HT, Single Mutants



Steady State: HT, Double Mutants





Conclusions

- Deletion of **multiple** efflux pumps had greater effect than deleting **single** pumps
- Growth under infection related conditions varied by mutant
- TolC deletion resulted in the greatest NPN accumulation
- $\Delta\text{AcrB}\Delta\text{EefB}$ resulted in the greatest HT accumulation

Future directions:

- Determine which efflux pumps contribute most to antibiotic resistance
- Test additional antibiotics and membrane probes
- Investigate whether observed phenotypes translate to increased susceptibility



Questions?