Investigating aminoglycoside susceptibility in *Acinetobacter baumannii* and its relationship to oxidative stress

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Introduction

- *Acinetobacter baumannii* is a Gram-negative opportunistic pathogen that is one of the major causes of hospital-acquired infections in the current healthcare system (Moubareck and Halat 2020).
- *A. baumannii* is associated with high mortality due to many strains achieving multidrug resistance (Moubareck and Halat 2020).
- Previous work demonstrated that deletion of catalase genes *katE* and *katG* in *A. baumannii* increased the cells' resistance to aminoglycoside antibiotics; an increase in expression of the *adeAB* RND efflux pump was observed simultaneously (Kainth 2021).
- Gene knockout experiments deleting *katE*, *katG*, and *adeAB* genes in *A. baumannii* may help confirm a causal relationship between catalase gene deletion and *adeAB* efflux pump expression increase.
- Deletions were completed using either electroporation or conjugation methods to incorporate the non-replicative pMO130 plasmid backbone into the *A. baumannii* genome.
- The pMO130 plasmid is prepared by SOEing PCR and maintained in *E. coli* strains grown on LB plates containing gentamicin.
- Investigation of the effects of these deletions on aminoglycoside antibiotic susceptibility of A. baumannii strains was performed using minimum inhibitory concentration (MIC) tests.

Figure 1. Components of the pMO130 plasmid: (■) upstream region homologous to region upstream from gene of interest, (■) gentamicin resistance marker, (■) downstream region homologous to region downstream from gene of interest, (■) kanamycin resistance marker, and (■) *sacB* coding the SacB protein that metabolizes sucrose to produce a compound toxic to *A. baumannii* cells.

Objectives

- To generate *A. baumannii* strains with various combinations of *katE*, *katG*, and *adeAB* gene deletions.
- To use MIC tests to compare aminoglycoside antibiotic susceptibilities between *A. baumannii* strains.

Implications

- Confirming a causal relationship between catalase gene deletion and *adeAB* efflux pump expression increase may help to elucidate some of the mechanisms of antibiotic resistance in *A. baumannii.*
- This information may be applicable to the goal of creating new treatments for this dangerous pathogen.

Methods

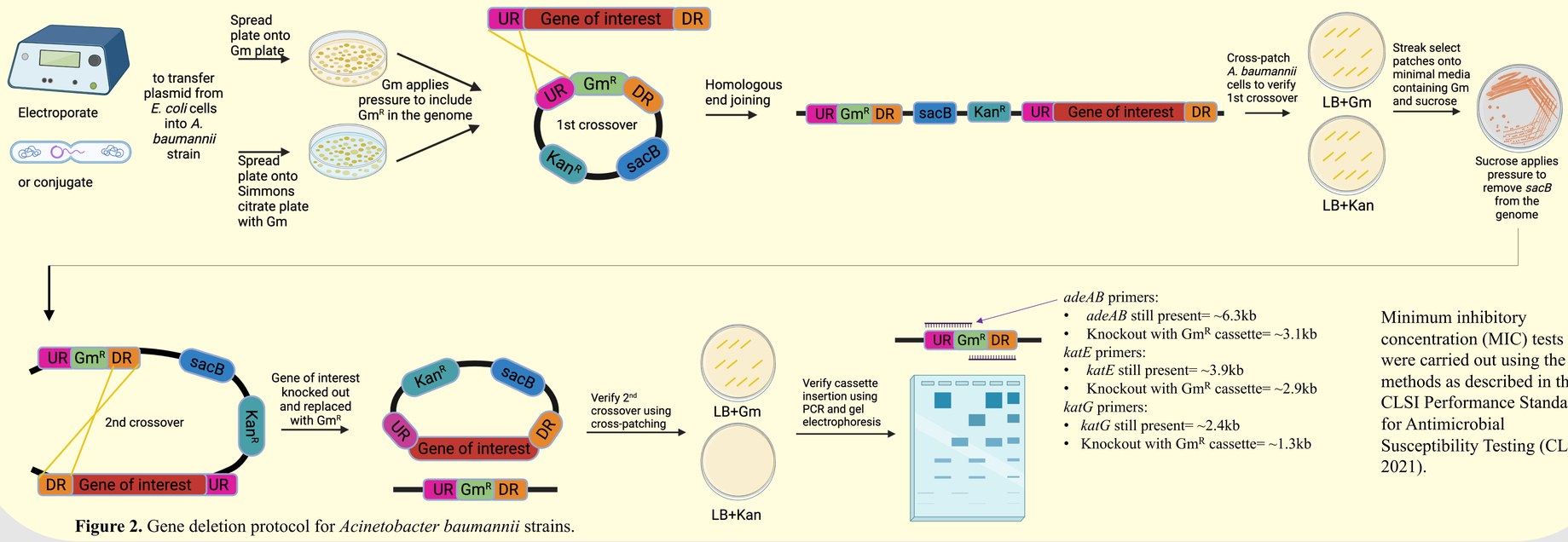

Figure 2. Gene deletion protocol for *Acinetobacter baumannii* strains.

Minimum inhibitory concentration (MIC) tests were carried out using the methods as described in the CLSI Performance Standards for Antimicrobial Susceptibility Testing (CLSI 2021).

Results

- Obtained 3 potential deletion strains that grew consistently on gentamicin but not kanamycin plates during the second crosspatch but was not able to screen these strains yet due to ineffective PCR primers.
- Completed MIC tests for ATCC17978 (wild type), AB155 (Δ*katG*), AB188 (Δ*katE*), and AB189 (Δ*katG*; Δ*katE*) strains in the presence of kanamycin in one condition and in the presence of kanamycin + 5mM Ascorbic acid (thought to remove reactive oxygen species) in the second condition.

Table 1. Minimum inhibitory concentration results for *A. baumannii* strains in the presence of the aminoglycoside antibiotic kanamycin (Kan) and ascorbic acid.

Trial	Strain	MIC with Kan	MIC with Kan + Ascorbic acid
1	ATCC17978	1	2
	AB188 (Δ*katE*)	1	2
2	ATCC17978	0.5	2
	AB188 (Δ*katE*)	0.5	8
3	ATCC17978	0.5	8
	AB155 (Δ*katG*)	0.5	2
	AB189 (Δ*katE; Δkat*G)	4	16
4	ATCC17978	0.5	8
	AB188 (Δ*katE*)	1	8
	AB155 (Δ*katG)*	1024	1
	AB189 (Δ*katE; Δkat*G)	1	4

Observed results of catalase deletion (Kainth 2021).
↓ Catalase = ↑ *adeAB* expression = ↓ Susceptibility to kanamycin = ↑ MIC

Expected results of ascorbic acid addition.
↑ Ascorbic acid = ↓ *adeAB* expression = ↑ Susceptibility to kanamycin = ↓ MIC

Figure 3. Observed and expected MIC results for knockout of catalase genes and addition of ascorbic acid.

- MIC results instead showed an increase in MIC as ascorbic acid was added.
- After a literature search some possible explanations for these results include interactions between the antibiotic and ascorbic acid or reactions between oxygen and ascorbic acid contributing to higher amounts of reactive oxygen species (Goswami et al. 2020; Richter and Loewen 1981).
- Planning a RT-qPCR experiment to see how *katE*, *katG*, and *adeAB* expression was affected in the wildtype strain with addition of 5 mM ascorbate.

Conclusion

- Potential deletion strains with ATCC17978:Δ*adeAB* Δ*katE*, ATCC17978:Δ*adeAB* Δ*katG* and ATCC17978:Δ*adeAB* Δ*katE* Δ*katG* genotypes generated
- MIC results for ATCC17978 in the presence of kanamycin and ascorbic acid led to an unexpected increase in MIC that may be explained by hypotheses in the literature
- RT-qPCR experiment designed to test some of these hypotheses and explore how ascorbic acid effects gene expression

Future Work

- Complete the generation of strains with various combinations of the described genes deleted
- Successfully screen these strains to confirm knockouts using PCR and gel electrophoresis
- Carry out pFLP technique on strains containing cassettes to excise the gentamicin resistance marker
- Test antibiotic susceptibility using MIC tests without resistance markers present
- Finish qPCR experiment and interpret results to better understand differences in my MIC results compared to the expected results

References

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