Genes involved in cell division in *Acinetobacter baumannii* are coregulated by UmuDAb and DdrR

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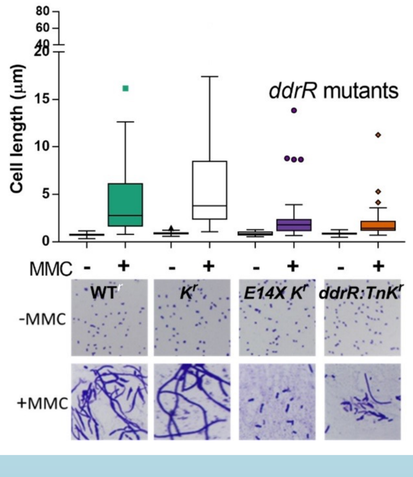
Introduction

In the presence of DNA damage, the multi-drug resistant bacteria *Acinetobacter baumannii* employs the proteins UmuDAb and DdrR to repress the expression of error-prone polymerases.



These coregulators may also affect the phenotypes of cell division and growth sensitivity after DNA damage. *ddrR* mutant cells do not form filaments after DNA damage.

Objectives

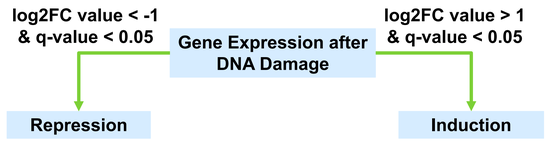
- Identify cell division genes that are dysregulated in *A. baumannii* ATCC 17978 after DNA damage
- Perform an *in silico* analysis of *A. baumannii* RNA-Seq transcriptome data in the wild-type (WT), *ddrR*-mutant (DR$^-$), and *umuDAb*- mutant (UD$^-$) strains, particularly in regard to cell division genes.
- Use RT-qPCR to validate the expression levels of cell division genes

Materials & Methods

RNA-Seq data analysis

To examine the expression requirements of the bacteria's cell division genes, we re-analyzed our previously acquired RNA-Seq data from wild-type, DR$^-$, and UD$^-$ strains[1,2].

1. We analyzed Cuffnorm- or HTSeq- normalized counts of the expression of 35 putative cell division genes (obtained from the KEGG database) using the Cuffdiff and DESeq2 pathways, respectively.
2. RNA-Seq data was sorted using Excel based on the following criteria:



RT-qPCR validation

To confirm the gene dysregulation suggested by the RNA-Seq data

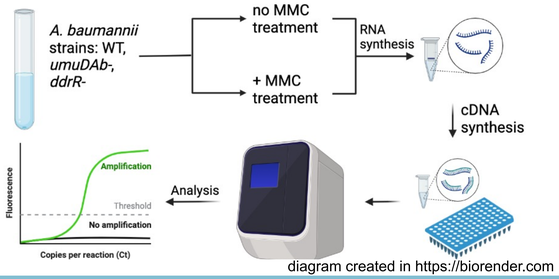
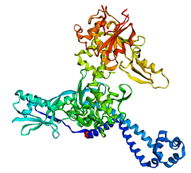


diagram created in https://biorender.com

Genes of Interest related to Cell Division

XerC and ParE function at a DNA damage-associated filamentation locus in *Escherichia coli*. XerC and Topoisomerase IV also interact with cell division protein FtsK.

parE (*A1S_3359*)



Crystal structure of catalytic core of *A. baumannii* TOPO IV (ParE-ParC fusion truncate). PDB: 2XKJ

- A topoisomerase IV subunit B: separates the physical linkage of two daughter chromosomal DNA at the end of replication
- Toxin of Type II *parE-parD* TA system

xerC (*A1S_2629*)

C-terminal domain of XerD recombinase in complex with gamma domain of FtsK. PDB: 5DCF

- A site-specific tyrosine recombinase
- Forms a cyclic heterotetrameric complex with XerD, which interacts via their C-terminal region

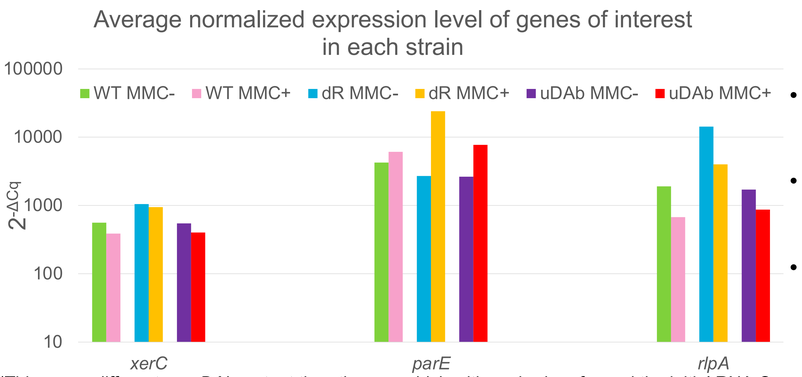
Results of RT-qPCR validation

Comparison of expression pattern of *A1S_3359* and *A1S_2629* in each strain based on the RNA-seq data results vs RT-qPCR data results.

	Summer Transcriptome Data Results			RT-qPCR Data Results		
Gene	17978 WT	*ddrR* null	*umuDAb* null	17978 WT	*ddrR* null	*umuDAb* null
***A1S_3359* (*parE*)**	↔	↑	↑	↑	↔	↔
***A1S_2629* (*xerC*)**	↓	↔	↔	↓	↔	↔

↑ = induction ↓ = repression ↔ = unchanged

Results of RNA-Seq analysis



*This was a different *umuDAb* mutant than the one which with we had performed the initial RNA-Seq

- Derepression of site-specific tyrosine recombinase *xerC* in both mutants
- Induction of topoisomerase IV/Type II toxin(/antitoxin) *parE* in both mutants
- Derepression of septal ring lytic transglycosylase *rlpA* in the *ddrR* mutant, but not the other strains

Conclusion

- Dysregulation of *parE* and *xerC* was observed in both UD$^-$ and DR$^-$ mutants through the results of RNA-Seq analysis and RT-qPCR
- RNA-Seq results of *parE* were not consistent with RT-qPCR results. However, a different trend was still observed in WT vs mutant strains
- To follow up on these results, we are constructing mutants with disruptions in these genes to observe their resulting cell division and growth phenotypes. These results may also help test whether DdrR and/or UmuDAb regulate these genes.

References

1. **Hare JM, Ferrell JC, Witkowski TA, Grice AN**. 2014. Prophage induction and differential RecA and UmuDAb transcriptome regulation in the DNA damage responses of *Acinetobacter baumannii* and *Acinetobacter Baylyi*. PLoS ONE **9**.

2. **Peterson MA, Grice AN, Hare JM**. 2020. A Corepressor participates in LexA-independent regulation of error-prone polymerases in *Acinetobacter*. Microbiology **166**:212–226.

Acknowledgements

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