Mutation of potential binding sites for lipopolysaccharide on OmpF and how they affect colicin N toxicity.

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Aims

❖ Mutate 10 OmpF residues (See Figure 2.) and find out if they are lipopolysaccharide binding sites.

❖Determine whether the binding of lipopolysaccharide to OmpF is required for colicin N to interact with OmpF and kill cells.

Introduction

❖ Colicin N is toxic to certain *E. coli* strains and is produced by specific *E. coli* (Jacob and Wollman, 1952).

❖ Colicin N binds to lipopolysaccharide, which is abundant on the cell membrane. Lipopolysaccharide in turn is bound to a trimeric membrane protein known as OmpF (Baboolal et al., 2008).

❖ *E. coli* that do not express OmpF or homologues of OmpF will not be killed by colicin N (Evans, Cooper and Lakey, 1996).

Methods

❖ Quikchange mutagenesis:

• Polymerase Chain Reaction using primers with non-wild-type sequences incorporated into pMS119 plasmids were used to mutate potential LPS binding sites on OmpF from positively charged residues to uncharged glutamate (Q) residues (see figure 2.). DPN1 was added to the PCR product to denature the methylated wild-type template.

❖ Spot Test:

• BZB1107 *E. coli* cells were transformed because they do not express porins without plasmids. BE3000 cells were used as a control because they naturally express wild type ompF.

• 20 µl of *E. coli* cells (see figure 4.) were plated with 1 ml of LB, 1 ml of sloppy agar, and 100 µg/ml of ampicillin and/or 30 µg/ml kanamycin if the cells were not sensitive to them to form a lawn. A set of BZB1107 cells with plasmids were also induced with 1mM of IPTG.

• For each plate, 2 µl of colicin N were 'spotted' in concentrations of: 3 mg/ml, 0.3 mg/ml, 0.03 mg/ml, 0.003 mg/ml, and the buffer alone as a control.

❖ Gel running:

• WT OmpF, and two OmpF mutants (K209Q and K210Q) and (K160Q, K209Q, and K210Q) were purified and two gels run with the products of the purification (see figures 6. and 7.).

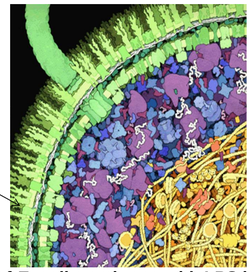

Figure 1. Diagram of *E. coli* membrane with LPS bound to OmpF
('Molecular Art' by David S. Goodsell http://www.scripps.edu/pub/goodsell/)

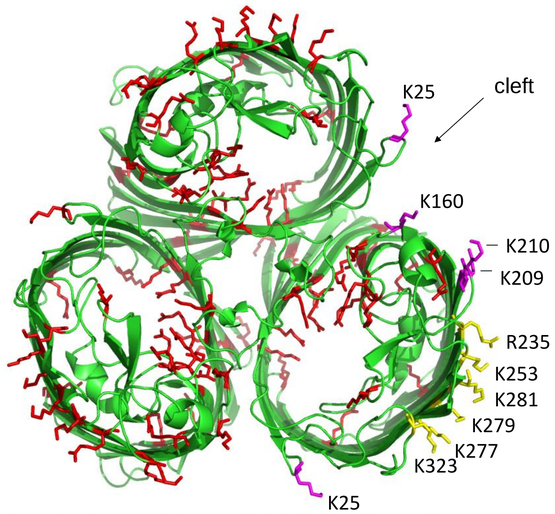

Figure 2. Diagram of OmpF with labelled residues of interest.
Yellow residues: failed mutations
Purple residues: successful mutations
The first letter of the residue name denotes the wild type residue.
The number is the location of the residue in the sequence of OmpF.

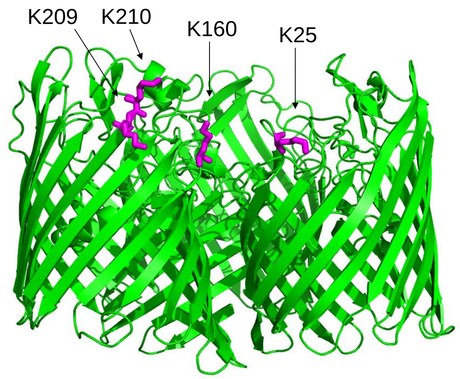

Figure 3. Horizontal view of OmpF facing a cleft, with residues near the cleft highlighted in purple.

Cell:	OmpF Type:	Control:
BE3000	Wild Type	Positive
BZB1107	Wild Type	Positive
BZB1107	None	Negative
BZB1107	K209Q, K210Q	N/A
BZB1107	K160Q, K209Q, K210Q	N/A

Figure 4. Table showing cells used for spot test.

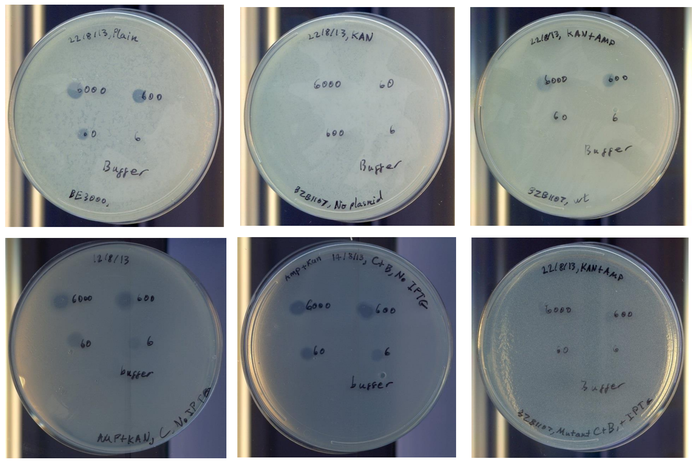

Figure 5. Plates from spot test assay.
B: K160Q, C: K209Q and K210Q.
(The plate on the bottom right was induced with IPTG)

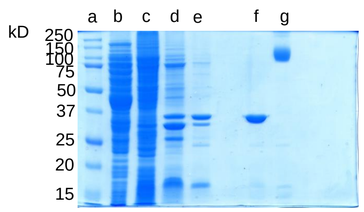

Figure 6. Wild Type OmpF purification products.

Figure 6.	
a	Marker
b	Soluble Fraction
c	Supernatant Wash I
d	Supernatant Wash II
e	Supernatant Wash III
f	Boiled OmpF Extract
g	Unboiled OmpF Extract

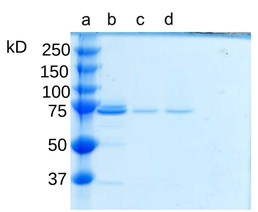

Figure 7. Comparison of all OmpFs.
Left to right: wild type, double mutant, and triple mutant Loaded in 20 µl.

Figure 7.	
a	Marker
b	Wild Type OmpF
c	Double mutant OmpF
d	Triple mutant OmpF

Results

❖ Spot Test:

• The uninduced cells with plasmids were killed by colicin N.

• The cells induced with IPTG seemed to have partial resistance to colicin N.

• Fewer induced cells grew on the plates.

❖Gel Running:

• The boiled OmpF had a higher band than the unboiled OmpF.

• The unboiled wild type OmpF had a 'ladder' trailing the band, which was not present on any other band.

Conclusion

❖ Spot Test:

• The inducing process causes cells to focus their resources on the expression of OmpF rather than division, leading to less division in induced cells.

•The BZB1107 cells which lacked plasmids were not killed by colicin N, where as the BE3000 cells, which naturally expressed OmpF, were killed. This confirms that OmpF is required for colicin N to lyse cells.

• 'Leaky' plasmids cause cells to express OmpF without being induced. They could be the cause for the uninduced cells being killed by colicin N.

❖ Gel Running:

• When boiled the OmpF denatures into a monomer, hence the lower band signifying a lower molecular weight to that of the unboiled OmpF .

• The 'ladder' is the differing amounts of LPS bound to OmpF, which confirms that LPS only binds to wild type OmpF. Thus, the mutations we induced prevent LPS from binding to OmpF.

Future Work

❖ A western blot should be carried out to confirm that LPS does not bind to the mutant OmpF.

❖ A plate reading assay should be carried out to find the minimum inhibitory concentration of colicin N for the cells.

❖ A potassium efflux assay could also be used to support the findings of the plate reading assay.

❖ To check that the primers were functional, we could pair one primer of interest with a primer that we know works and run a PCR with them.

References

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2)Jacob, F., L. Siminovitch, and E. Wollman (1952), 'Sur la biosynthèse d'une colicine et sur son mode d'action', Ann. Inst. Pasteur (Paris,, 83, pp. 295-315.

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Acknowledgements

I'd like to thank Professor Lakey for allowing me to work during the summer to do this project in his laboratory, and the members of his lab, especially Wanatchaporn Arunmanee, for being very helpful and patient with me during the project, as well as making me feel welcome during the project. I'd also like to thank Newcastle University for supporting this project by providing an internal grant.