Investigating the effect of Colicin N T domain on *E.coli* mutants

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Introduction

Bacteria have the ability to produce toxins called colicins to compete with each other for space and nutrients, this study uses colicin N (ColN) which kills *Escherichia coli* (*E.coli*). ColN is a pore forming colicin which causes potassium efflux from the bacteria and leads to cell death. ColN requires *E.coli* proteins OmpF and TolA to function[1].

Colicins have a modular design of 3 domains (figure 1):
•Translocation (T) domain – amino terminal
•Receptor-binding (R) domain
•Cytotoxic (C) domain – carboxyl terminal

Figure 1: Representation of ColN domains.

Unpublished data from the Lakey lab has shown that isolated Colicin N T domain (ColN-T) is toxic to cells but is less active than the full length protein.

This study investigates ColN-T, which includes amino acids 1-90 of the protein, and has a PelB leader sequence to target it to the periplasm[2].

Aims

1. To purify the full length ColN T domain
2. How does Colicin N T domain overexpression in the periplasm effect *E. coli*?
3. Does over expression of the ColN-T protect from ColN?

Purifying Colicin N T domain

ColN-T(amino acids 1-90) was transformed into BL21AI E.coli cells. ColN-T production was induced using 0.2% arabinose. Nickel affinity purification was used to purify ColN-T by its 6-His Tag. A western blot was then carried out to confirm the purified protein was ColN-T (figure 2).

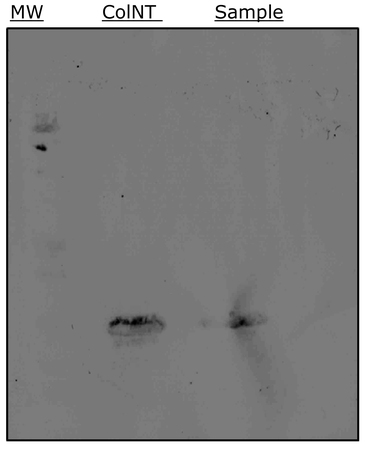


Figure 2: The Western blot confirming the presence of Colicin N T domain.
MW=molecular weight markers,
ColNT=pure ColNT stock solution
Sample=the protein purified in this experiment,

The PelB leader sequence when attached to ColN-T gives the protein a Mr of 12.2kDa, and when the PelB is cleaved on the way to the periplasm ColN-T alone is 9.9kDa. Therefore 2 bands should be seen on the gel following the whole cell pellet preparation. The two forms of ColN-T have shown up on the western blot as a smudge showing the purification was successful (figure 2).

How does Colicin N T domain effect E.coli?

The MC1000 strain of E.coli was transformed with a pBAD33 plasmid containing the gene for ColN-T1-90 and the PelB leader sequence which targets to the periplasm. ColN-T is induced with 0.2% arabinose and repressed with 0.2% glucose. The empty pBAD33 plasmid was also used to transform MC1000 for a control. The growth of the 2 different strains in different conditions was tracked by measuring absorbance over 16 hours with a plate reader (figure 3). This was repeated, using knockouts for OmpF and OmpC membrane proteins (figure 4 and 5).

Absorbance of MC1000 *E.coli* in repressing (glucose) and inducing (arabinose) conditions

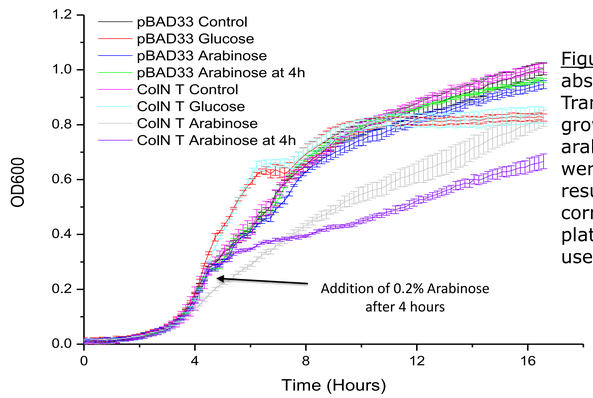


Figure 3: Plate reader absorbance results. Transformed *E.coli* were grown overnight in 0.2% arabinose. The bacteria were spun down and resuspended in the correct solution for the plate reader. LB alone was used as a control.

The MC1000 plate reader results (figure 3) show growth was significantly reduced in the MC1000 ColN-T when induced with 0.2% arabinose from the beginning or after 4 hours.

Absorbance of *E.coli* strains in 0.2% Glucose

The graph in figure 4 shows reduced growth of MC1000 compared with OmpF-/OmpC-. The pBAD33 and ColN-T lines are almost identical for each strain suggesting the colicin gene was successfully repressed.

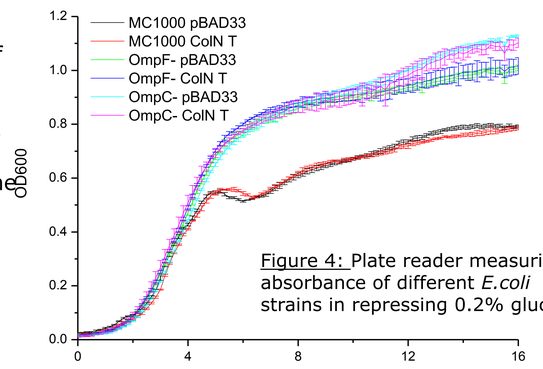


Figure 4: Plate reader measuring absorbance of different *E.coli* strains in repressing 0.2% glucose.

Absorbance of *E.coli* strains in 0.2% Arabinose

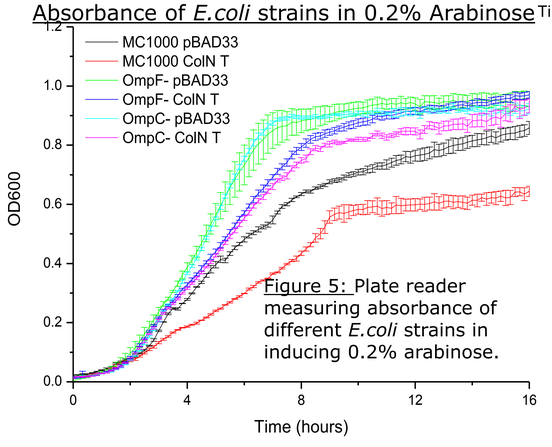


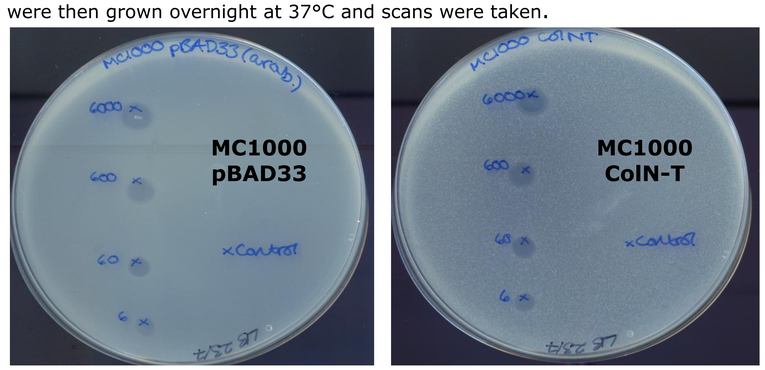
Figure 5: Plate reader measuring absorbance of different *E.coli* strains in inducing 0.2% arabinose.

The graph in figure 5 shows a significant change in the MC1000 strain, with the growth of MC1000 ColN-T reduced. However the OmpF-/OmpC- knockouts only showed a slight reduction, suggesting they may be immune to ColN-T, perhaps being involved in it's function.

Over expression of the T domain

Pommier et al. found that inducing over expression of some colicin domains protected the cells from exogenous colicins[3]. When the ColN-T is targeted to the periplasm it binds TolA translocation proteins in the inner plasma membrane which then become saturated. This prevents TolA from binding full length ColN when added externally. To test this, expression of the ColN-T was induced in E.coli overnight and then used in a spot test with full length colicin N. MC1000 with empty pBAD33 plasmid was used as a control.

Figure 6: MC1000 ColNT/ T domain were grown overnight in inducing conditions (0.2% arabinose). The cultures were then mixed with melted sloppy agar, poured onto regular LB agar plates and left to set. Colicin N was diluted and 2µl of each concentration was spotted on the plate (6000ng-600ng-60ng-and 6ng) The plates were then grown overnight at 37°C and scans were taken.



Both the ColN-T MC1000 and the control pBAD33 MC1000 showed the same result (figure 6). The lawn of bacteria shows clear areas of inhibition where the ColN was spotted, these zones increase in diameter as the concentration of the colicin increases. The buffer control shows no inhibition of the E.coli growth on either plate. These results imply that over expression of the T domain does not protect from the full length colicin N.

Conclusions

•The purification of ColN-T was successful and shows 2 bands on the western blot suggesting the protein undergoes modification.

•ColN-T seems to be able to kill E.coli when targeted to the periplasm (see figure 3), despite being the domain responsible for colicin transport across the bacterial membrane.

•These early results (figure 4 and 5) suggest ColN-T works via the OmpF or OmpC membrane protein when targeted to the periplasm because the growth of the knockouts with ColN-T only slightly reduced under inducing conditions.

•The over expression of ColN-T does not appear to give any protection from the full length colicin N but further investigation is needed of lower ColN concentrations.

References

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3. Pommier S., Gavioli M., Cascales E., Lloubès R.(2005) Tol-dependent macromolecule import through the *Escherichia coli* cell envelope requires the presence of an exposed TolA binding motif. J. Bacteriol. 187:7526–7534