The C-terminal domain of *Clostridioides difficile* TcdC is exposed on the bacterial cell surface

**Introduction**

*Clostridioides difficile* is an anaerobic gram-positive bacterium that can produce the large clostridial toxins, TcdA and TcdB, encoded within the pathogenicity locus (PaLoc). The PaLoc also encodes the sigma factor TcdR, that positively regulates toxin gene expression, and TcdC, a putative negative regulator of toxin expression. TcdC is proposed to be an anti-sigma factor; however, several studies failed to show an association between tcdC genotype and toxin production. Consequently, TcdC function is not yet fully understood. Previous studies have characterized TcdC as a membrane-associated protein with the ability to bind 6-qual dashes structures. The binding to the DNA secondary structures is mediated through the OB-fold domain present at the C-terminus of the protein. This domain was previously also proposed to be exposed on the bacterial cell surface.

**TcdC C-terminal domain is predicted to be extracellular**

Figure 3 – Detection of C-terminal His6™ tag. A) Representation of the Western blot analysis. The protein of interest (less band) found at the C-terminus to the His6™ tag through the ECL detection (grey lines) are indicated. The positions of molecular mass markers (molecular mass standards) are indicated. The band present at the C-terminus to the His6™ tag is indicated (grey). B) Line scan detection (grey lines) for the C-terminal His6™ tag detected for the 100 ng protein and induced with 10 ng/mL for 45 minutes. Optical density normalized luciferase activity (OPL0508) is shown relative to the value of the 0 ng/mL sample. C) The western blot of TcdC-His6™-terminal represented at the same time (grey lines). The average of biological quadruplicate measurements are shown, with error bars indicating the standard deviation from the mean. Significance was defined as higher than probability (p-value) of 0.05.

**Cytosine of TcdC are exposed on the bacterial surface**

Figure 4 – TcdC C51 is important for membrane association. A) TcdC C51 affects membrane anchoring. The C51 residue is conserved in TcdC orthologues from *C. difficile* (Shi et al., 2014), *C. innocuum* (Hoskins et al., 2015), and *C. perfringens* (Han et al., 2016). The mutation of C51 to alanine results in a 5% luciferase activity, compared to the wild-type (W.T.). B) The C51A mutant is less exposed on the bacterial cell surface, as indicated by the green arrow, compared to the wild-type (orange arrow). C) The C51A mutant is less exposed on the bacterial cell surface, as indicated by the red arrow, compared to the wild-type (green arrow).

**Conclusions**

- **TcdC C-terminal is exposed on the bacterial surface**
- **Membrane localization of TcdC is dependent on C51**
- **Prevaling model of TcdC as a cytoplasmic anti-sigma factor is challenged**

**Possible topologies of TcdC**

Figure 5 – TcdC topology models. TcdC is located in the outer membrane with an extracellular C-terminal region. The location of the C-terminal of TcdC cell surface location is unknown. In the topology model 1, TcdC is inside the outer membrane and not accessible extracellularly or intracellularly. In the topology model 2, the C-terminal is present on the intracellular environment of the bacteria, and the cytochrome c terminal, locations (orange line) are indicated.