Toxin Secretion in *Clostridium difficile.*

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Clostridium difficile is a major nosocomial pathogen and the principal causative agent of antibiotic-associated diarrhea. The toxigenic *C. difficile* strains that cause disease secrete virulence factors, toxin A and toxin B that cause colonic injury and inflammation. *C. difficile* toxins have no export signature and are secreted by an unusual mechanism that involves TcdE, a holin-like protein. We isolated a TcdE mutant of the epidemic R20291 strain with impaired toxin secretion that was restored by complementation with functional TcdE. In the TcdE ORF, we identified three possible translation start sites; each translated isoform may play a specific role in TcdE controlled toxin release. We created plasmid constructs that exclusively express only one of the three TcdE isoforms and complemented the TcdE mutant with these isoforms. Western blot analysis of the complemented strains demonstrated that TcdE is translated efficiently from the start codon at the 25^{th} and 27^{th} positions in the predicted ORF, producing proteins with 142 ($TcdE_{142}$) and 140 ($TcdE_{140}$) amino acids, respectively. The effects of all three TcdE isoforms in *C. difficile* cell viability and toxin release were determined and were found to be different from each other. Our preliminary data also suggest that the peptide coded by the small ORF (*tcdF*) downstream of *tcdE* may play a role in toxin release in *C. difficile*. More experiments are in progress to unravel this unique protein secretion pathway in this important nosocomial pathogen.