

NRC Institute for Biological Sciences

Clostridium difficile* Toxin A-Specific Single Domain Antibodies*The Business Opportunity**

Clostridium difficile is a gastrointestinal pathogen that is a leading cause of hospital-related infections in developed nations. It is responsible for *C. difficile*-associated disease (CDAD) in humans, with symptoms ranging in severity from mild cases of antibiotic associated diarrhea to fatal colitis. The recent emergence of hypervirulent, antibiotic-resistant, *C. difficile* strains with increased morbidity, mortality and recurrence rates have warranted the development of novel, non-antibiotic, treatment regimes.

The development of antibodies targeting virulence factors - such as bacterial toxins - would help reduce the selection pressure that antibiotics place upon pathogens and also be useful to control the infection's recurrence.

Clostridium difficile is a Gram-positive pathogen that produces two primary virulence factors, enterotoxins A and B, which are responsible for CDAD. Monoclonal antibodies specific for toxin A and toxin B have been shown to effectively treat CDAD and prevent disease relapse in animal models and in humans.

A superior alternative to monoclonal antibodies for anti-toxin immunotherapy is to use variable heavy-chain single-domain antibodies (VHHs) isolated from *Camelidae* species. These VHHs maintain many characteristics of conventional mAbs, including high target affinity and specificity, with the added advantages of small size (~15 kDa), easy genetic manipulation, amenable to library screening and selection, inherent thermal and proteolytic stability, and high-yield, low-cost recombinant production in bacteria, yeasts, plants, and mammalian cells. In particular, toxin-specific VHHs engineered to resist the acidic pH and proteases of the gastrointestinal tract would make these VHHs efficacious oral therapeutics.

The Technology

NRC-IBS has developed a panel of high affinity *C. difficile* toxin A-specific llama VHH antibodies which are potent neutralizers of toxin A. In addition, they are resistant to the harsh conditions of the gastrointestinal tract and thus

promise to be effective orally as a therapeutic agent for CDAD.

The *C. diff.* toxin A-specific VHHs characteristics include:

- very high affinities against *C. difficile* toxin A;
- Potent neutralizers of toxin A (from the pathogenic strains) and super-potent when used in combinations;
- Highly resistant to stomach's acidic pH (pH 2.0) and the major gastrointestinal proteases pepsin, trypsin and chymotrypsin;
- Highly thermostable, with T_m's in the 80's (several VHHs) and as high as 96°C;
- production in very high amounts in *E. coli*.

These toxin-specific VHHs are attractive as systemic therapeutic agents but also have a strong potential as orally-delivered therapeutics.

Patent Position

Provisional patent application – PCT NRC-IBS case 12212

The Markets

C. difficile is the most common cause of infectious diarrhea in hospital patients in the industrialized world. CDAD affects hundreds of thousands of patients and costs over \$3 billion every year in North America.

Technology Transfer Possibilities

- Technology License
- Joint development of the technology and its applications.

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