toxins

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Supplementary Materials

A Streamlined Method to Obtain Biologically Active TcdA and TcdB Toxins from *Clostridioides difficile*

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Supplementary Materials and Methods:

DNase assay: To assess whether DNA contamination could be an issue, DNA quantification was done before and after incubation with 70 µg.mL^{-1} DNase I (CAS: 9003-98-9, Sigma Aldrich, MO, USA)for 2 hours at 37°C. In addition, positive control was included by spiking the mixture with 1 µL of a DNA sample at 170.47 ng.µL^{-1}.

Western blot: Purified recombinant toxins rTcdA and rTcdB were separated on 12% Mini-PROTEAN TGX Stain-Free Gels (Bio-Rad, CA, USA) and then transferred to a nitrocellulose membrane using Trans-Blot Turbo Transfer System (Bio-Rad, CA, USA) according to the manufacturer's instructions. Purified recombinant toxins rTcdA and rTcdB were detected using anti-His primary antibody (#2365S, Cell Signaling, MA, USA) diluted at 1:1000 and anti-rabbit horseradish peroxidase (HRP)-linked secondary antibody diluted at 1:20000 (GeneTex GTX213110-01, CA, USA). Antibody binding was revealed with SuperSignal™ West Pico PLUS Chemiluminescent Substrate (reference: 34580, Thermo Scientific, MA, USA) and revelation was performed on Fusion FX7 Imaging System (Vilber Lourmat, Eberhardzell, Germany).

Toxin expression assay: *C. difficile* strains AD3 and AD4 were cultured in 15 mL of BHI medium and allowed to grow overnight in an anaerobic chamber at 37°C and toxin expression was induced by the addition of ATc at 0.1 µg.mL^{-1} or 0.5 µg.mL-1 to 5 mL bacteria culture. Bacteria were centrifuged and culture supernatants were precipitated with 45 g of ammonium sulfate for 100 mL of bacterial culture followed by shaking at room temperature for 5 min. Precipitates were centrifugated (12200 g) for 10 min at 4 °C, and the pellet was resuspended in 100 µL of Laemmli sample buffer. For analysis, supernatant before (S) and after precipitation (CS) were loaded into a sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel using 6% stacking gel and 10% separation gel followed by a standard Coomassie staining.

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