TITLE: Identification and characterization of *Clostridioides difficile* adhesins responsible for laminin-1 recognition.

AUTHORS: Mayara Gil C. Santos,[1] Camilla N. R. Trindade[1], Rossiane C. Vommaro[2], Eliane de O. Ferreira[1] & Regina M. C. P. Domingues[1].

INSTITUTION: [1]Laboratório de Biologia de Anaeróbios, Depto. Microbiologia Médica, IMPPG, UFRJ; [2]Laboratório de Ultraestrutura Celular Hertha Meyer, IBCCF, UFRJ

ABSTRACT:
Clostridioides difficile is an anaerobic bacterium and the leading cause of hospital-acquired diarrhea. While toxins are extensively studied, the contribution of surface proteins to the intestinal colonization is poorly understood, especially those involved in binding to extracellular matrix (ECM) components during the pseudomembranous colitis infection. *C. difficile* can recognize various components of the extracellular matrix (ECM), including collagens and fibronectin. However, its ability to recognize laminin (LMN-1) remains poorly understood. Thus, this study aims to identify and characterize adhesins from the *C. difficile* 630 strain (ribotype 012), reported to recognize LMN-1. Firstly, an adhesion assay was performed by covering immobilized glass coverslips with LMN-1 in 24-well plates and by using the RT012 cultivated in BHI-PRAS. By using this condition, no adhesion was observed, hence a BHI-PRAS supplemented with different concentrations of glucose (0.5%, 1% and 1.8%) was used and the assay repeated. As a result, our studies confirm that *C. difficile* recognized LMN-1 in the presence of different concentrations of glucose and showed higher adhesion to LMN-1 when cells were cultivated in a media supplemented with 0.5% glucose. To evaluate the chemical nature of the adhesin(s), different treatments were made: proteinase k (5- 45 μg/mL), trypsin (20 μg/mL) and sodium periodate (100 mM/mL) and the adhesion LMN-1 re-assayed. Proteinase K treatment has reduced the adhesion in 90%, so different proteins extraction were obtained, whole cell (WP), S-layer (SLP) and flagella. For the protein identification, an immunoprecipitation with magnetic beads (Dynabeads Protein G) bridged to LMN-1 was performed, and each protein extract interacted. After proteins elution and mass spectrometry analysis (Orbitrap), a histidine kinase KdpD (gi|1378719009; *C. difficile* RT 012) protein was identified in the flagellar extract. This protein is responsible for metabolic process, signal transduction by phosphorylation and binding. Further experiments are been carried out to confirm the involvement of KpdD in the LMN-1 recognition. The identification of the binding molecule and cognate receptors will be the target for future strategies and may contribute for understanding *C. difficile* colonization to the colon.

Keywords: *Clostridioides difficile*, Laminin-1, adhesin
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