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C-di-GMP regulation of a novel collagen-binding surface protein in *Clostridium difficile*

I-Hsiu Huang[1], **Tung Sheng Lin**[1], **Zih-Cian Su**[1] and **Guan-Yu Chen**[2]
[1]National Cheng Kung University
[2]Department of biological Sciences and technology, Taiwan

Clostridium difficile is a gram positive, spore forming obligate anaerobic bacteria and is the leading cause of the nosocomial antibiotic associated diarrhea. *C. difficile* infection (CDI) results in diarrhea and severe pseudo membrane colitiss (PMC) and toxic mega-colon. Before pathogens can cause diseaseitneeds to attach to the host cell first. Therefore, our studies focus on non-toxin virulence factors responsible for *C. difficil*e colonization. By bioinformatics analysis, orfCD2831 was predicted to encode for a large collagen-binding protein on the surface of *C. difficile* and was renamed as Cell surface protein 1 (Csp1). ELISA studies demonstrated that recombinant Csp1 binds specifically to collagen Type I. Previous *in vitro* studieshas revealed that the metalloprotease CD2830 cleaves Csp1. Cyclic diguanylate(3', 5'cyclic diguanylic acid) (c-di-GMP), a secondary messenger regulates the virulence and biofilm formation in many bacterial pathogens.Transcriptional analysis has indicated that c-di-GMP can increase the transcriptional level of Csp1 while down regulate CD2830 through riboswitches. The aim of our study then was to elucidate the relationship between c-di-GMP, CD2830 and Ccsp1 in *C. difficile*. In our preliminary data, we used cell fractionation and Western blot to analyze the localization of Csp1. Csp1 was found mostly secreted into the culture supernatant rather than on the cell wall under laboratory condition.Inactivation of CD2830 shifted the localization profile of Csp1 toward cell wall anchoring suggesting that Csp1 is secreted via cleavage by CD2830. Next, artificial induction of the cytoplasmic c-di-GMP level in *C. difficile* resulted in overexpression and prominent cell wall localization of Csp1. In summary, we have identified a novel collagen-binding surface protein in *C. difficile* that is bi-regulated by a metalloprotease and the secondary messenger c-di-GMP. Future works will focus on unraveling the role of Csp1 in *C. difficile* pathogenesis using animal studies.

Biography

I-Hsiu Huang is an Assistant Professor at Department of Microbiology and Immunology Medical College, National Cheng Kung University, Taiwan.

ihsiuhuang@mail.ncku.edu.tw

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