

**Título: FUNCTIONAL CHARACTERIZATION OF THE DIP1180 AS HEMOLYSIN IN *Corynebacterium diphtheriae* BY GENE DISRUPTION**

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**Resumo**

*Corynebacterium diphtheriae*, the main etiological agent of diphtheria, and its spread occurs by direct contact with skin lesions or respiratory droplets. Diphtheria clinical signs and characteristic symptoms are mainly related to the diphtheria toxin (DT), the most studied *C. diphtheriae* virulence factor. Non-toxicogenic *C. diphtheriae* strains have been frequently isolated from invasive diseases, such as endocarditis, bacteremia, osteomyelitis and catheter-related infections. It suggests the involvement of other determinants in diphtheria bacilli pathogenesis. Bacterial hemolysins play an important role as virulence factor of several infections. Recently, a putative cytotoxin/hemolysin (DIP1180), similar to *Escherichia coli* Hly, was identified in *C. diphtheriae* biovar *gravis* (NCTC 13129 strain). Nevertheless, the cytotoxin effect of DIP1180 is unknown. Therefore, the present study aimed to functionally characterize the gene *DIP1180* and detect it in two toxigenic and five atoxigenic *C. diphtheriae* strains in addition to quantify the hemolytic activity of these isolates. *DIP1180* was detected in all *C. diphtheriae* strains through *in silico* analyses and polymerase chain reaction (PCR). The functional characterization of *DIP1180* was performed through chromosomal disruption of this gene in the NCTC 13129 strain. The mutant obtained (LDCIC-A1) and the other strains were tested for their hemolytic activity in sheep erythrocytes. Presently, *C. diphtheriae* strains showed hemolytic activity at different levels. Moreover, *DIP1180*-interruption caused a significant reduction in the hemolysis of the mutant LDCIC-A1 in comparison to the wild-type strain, indicating the involvement of *DIP1180* in *C. diphtheriae* lysis of sheep erythrocytes. However, the retained hemolytic activity of LDCIC-A1 suggests the participation of other (s) determinant (s) in this process. Additional studies are ongoing in order to identify and characterize other (s) determinant (s) involved in *C. diphtheriae* hemolysis.

Palavras-chave: *Corynebacterium diphtheriae*; cytotoxicity; DIP1180; hemolysin.

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