

TITLE: DETECTION OF *PPK* GENE AND CORRELATION WITH BIOFILM PRODUCTION IN *Corynebacterium diphtheriae*

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ABSTRACT:

Diphtheria is a bacterial toxemic disease of acute evolution whose etiologic agent is the Gram-positive rod *Corynebacterium diphtheriae*. Among its virulence factors, we may cite the production of diphtheria toxin and the ability to produce pili and to form biofilm. Biofilms are microbial communities of high organization attached to biotic or abiotic surfaces and embedded by complex extracellular matrix consisting of polymeric substances. Polyphosphate (poliP) has a key role in virulence and resistance to environmental stresses in various bacterial species. It has already been demonstrated the involvement of *ppk* gene (responsible for the synthesis of polyphosphatekinase) on biofilm formation in *Pseudomonas aeruginosa*: mutant strains had their motility and biofilm forming capacity reduced. The aim of this study was to detect the *ppk* gene and to correlate it with the production of biofilm in *C. diphtheriae*. So, biofilm formation was investigated in different media and substrates which stimulate or not poliP synthesis. In addition, the presence of the *ppk* gene was analyzed *in silico* and *in vitro*. The three-dimensional structure of the PPK proteins was also predicted. This study also intended to clone the *C. diphtheriae* *ppk* sequence in the TOPO system. As results, it was observed that all samples were able to form biofilms on polystyrene and glass surfaces, but at different intensities. The cultivation in King B broth did not modify the bacterial adherence profile to polystyrene of most of the strains. Strains classified as non-adherent to glass when grown in TSB, began to strongly adhere to this substrate when cultivated in King B broth. Two genes responsible for poliP synthesis were detected in *C. diphtheriae*: *ppk2a* and *ppk2b*. PPK2A and PPK2B proteins share only 40% of identity, displaying distinct three-dimensional structures. The construction of the *ppk2b* mutant was performed. The interruption of the *ppk2b* gene appeared to induce biofilm formation on hydrophilic abiotic surfaces (glass). Cellular adhesion tests, with HEP-2 cells, demonstrated that the mutant and the wild strain showed distinct adhesion patterns. Additional studies are needed in order to better understand the influence of the *ppk2b* gene on virulence and response to stress situations in *C. diphtheriae*.

Keywords: *Corynebacterium diphtheriae*, polyphosphate, polyphosphatekinase, biofilm.

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