Title: CHARACTERIZATION OF EUKARYOTIC-LIKE SERINE/THREONINE KINASES IN *CHROMOBACTERIUM VIOLACEUM*

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Abstract:

Chromobacterium violaceum is a Gram-negative β-proteobacterium widely found in tropical and subtropical areas. In addition to its free-living life style, this bacterium can also act as a human opportunist pathogen. Despite the infection due to *C. violaceum* is rare, the fatality rates are high. Eukaryotic-like serine/threonine kinases (eSTKs) control physiological traits and can confer survival advantages to some pathogenic bacteria during their interaction with the host. In this work, we carried out a search for eSTKs in *C. violaceum* genome using tools of several specialized databases. We are able to find four proteins with similarity to eSTKs. Three of these candidate kinases possess all domains and conserved amino acid residues that define the serine/threonine kinase superfamily, whereas one lacks some subdomains. Two null deletion mutant strains of *C. violaceum* (eSTK1 and eSTK2) were generated by allelic exchange using the suicide vector pNPTS138. Briefly, upstream and downstream gene fragments were PCR-amplified and cloned into pNPTS138 vector. These constructs were introduced in *C. violaceum* ATCC 12472 wild-type strain by conjugation and transconjugants were selected on Luria Bertani (LB) agar plate containing kanamycin/ampicillin. After selection on sucrose LB agar plates, the mutants were screened and confirmed by PCR. To reveal the role of these eSTKs in *C. violaceum* virulence, BALB/c mice (6 weeks old) were intraperitoneally infected with wild-type ATCC 12472, eSTK1 and eSTK2 mutant strains (10^6 CFU). The infected mice showed similar mortality rate and after two days all animals were died, suggesting that eSTK1 and eSTK2 are not essential virulence determinants in *C. violaceum*. Future directions include eSTK3 and eSTK4 mutant strain construction, phenotypic characterization of all eSTKs mutants and gene expression assays. These data will contribute to understand how eSTKs regulate important signaling pathways controlling bacterial physiology and host-pathogen interaction.

Keywords: *Chromobacterium violaceum,* signal transduction, phosphorylation, kinases, virulence

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