TITLE: THE *Chromobacterium violaceum* TYPE VI SECRETION SYSTEM IS REQUIRED FOR INTERBACTERIAL COMPETITION AND AFFECTS INTERLEUKIN 1 BETA RELEASE IN PRIMARY MACROPHAGES

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ABSTRACT:
Secretion systems are specialized nanomachines responsible for protein translocation from the bacterial cytoplasm to the extracellular milieu. In Gram-negative bacteria, these secretion systems are classified as type I to VI. The type VI secretion system (T6SS), a widespread contractile apparatus, deliveries effector proteins directly into bacterial and eukaryotic target cells in a contact-dependent manner. In this work, we aim to characterize the T6SS of *Chromobacterium violaceum*, a free-living beta-proteobacterium able to act as an opportunistic pathogen in humans. *In silico* analysis revealed that the genes encoding the core components of the T6SS machinery and potential T6SS effectors are found in the *Chromobacterium violaceum* genome in a large cluster organized in two operons. Null mutant strains were generated by allele exchange with an in-frame deletion of the T6SS core components *hcp*, that form the inner tube released into the target cell; *vipA* and *vipAB*, that form a contractile sheath that propels this inner tube; and *clpV*, an ATPase responsible for disassemble the contracted sheath. These mutant strains and the wild-type strain were challenged in interbacterial competition assays against 15 different species, including Gram-positive and different classes of Gram-negative bacteria. *C. violaceum* wild-type killed most target bacteria and the killing activity against *Pseudomonas aeruginosa, Escherichia coli,* and *Stenotrophomonas maltophilia* was markedly decreased using the four T6SS mutant strains, indicating a pivotal role of the *C. violaceum* T6SS in bacterial competition. Supernatant analysis by western blot revealed that the loss of core components in the mutant strains affect the inner tube release to the extracellular milieu, thus impairing the whole system activity. The T6SS mutant strains did not show obvious virulence attenuation using an *in vivo* infection murine model. However, infection assays with primary macrophages revealed that the T6SS mutant strains induce increased Interleukin 1 beta release, possibly by caspase-1 activation, and caused a barely increase in cytotoxicity, as seem by LDH release assay. Together, these results provide a first clue to understanding the role of T6SS in *C. violaceum* interactions with other bacteria and host cells.

Keywords: *Chromobacterium violaceum*, bacterial secretion systems, type VI secretion system, interbacterial competition, bacterial virulence.

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