TITLE: THE MULTIPLE VgrG PROTEINS OF THE TYPE VI SECRETION SYSTEM OF *Chromobacterium violaceum* HAVE REDUNDANT AND SPECIFIC FUNCTIONS

AUTHORS: LEAL, F.C.; ALVES, J.A.; DA SIVA NETO, J.F.

INSTITUTION: DEPARTAMENTO DE BIOLOGIA CELULAR E MOLECULAR E BIOAGENTES PATOGÊNICOS, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, UNIVERSIDADE DE SÃO PAULO (AVENIDA BANDEIRANTES, 3900 CEP:14049-900, RIBEIRÃO PRETO, SP, BRAZIL)

ABSTRACT:
Bacteria export proteins out of their cells employing multiprotein devices called secretion systems. The type VI secretion system (T6SS) deliveries toxic effectors inside target cells by expelling a puncturing apparatus composed of Hcp, VgrG, and PAAR proteins. It is not yet known precisely how the effector proteins bind to VgrG or the reason for the existence of multiple copies of VgrG in some bacteria. Recently, our group has shown that the Gram-negative bacterium *Chromobacterium violaceum* has a functional T6SS with antibacterial activity. In this work, we aim to investigate the *vgrG* genes of the *C. violaceum* T6SS. *In silico* analysis revealed that *C. violaceum* has six *vgrG* genes (*vgrG1* to *vgrG6*), two of them located inside the main T6SS cluster and the other four genes found in different genomic regions. Amino acid sequence alignment revealed a high sequence identity among the six VgrG proteins (from 70% to 93%). Each of the six VgrGs had the same domain organization, being composed of two canonical VgrG domains, and an additional C-terminal domain (DUF2345) putatively involved in the interaction with effectors. We constructed null mutant strains with individual deletion of *vgrG1*, *vgrG2*, *vgrG3*, *vgrG4*, *vgrG5*, and *vgrG6*. In addition, we generated mutant strains deleted of *vgrG1/2*, *vgrG1/2/3*, and *vgrG1/2/3/4*, while the last two strains deleted of five or all *vgrGs* are being constructed. Competition assays of the single null mutants of *vgrG*s against bacteria of different classes showed that these strains remained capable of killing several target bacteria, similar to that observed in the wild-type strain. One exception was the *vgrG3* mutant, which showed loss of antibacterial activity against *Pseudomonas aeruginosa* similar to the levels of the mutant *hcp*, a control strain that lack a functional T6SS. Thus, VgrG3 seem to be loaded with effectors for the specific killing of *P. aeruginosa*. Western blot assays revealed the presence of Hcp in the culture supernatant of all single *vgrG* mutants, indicating that the T6SS machinery remained functional in the absence of individual VgrG proteins. These data suggest that the *C. violaceum* VgrG proteins seem to have a redundant role in assembling a functional T6SS with antibacterial activity and that some VgrGs may be dedicated for delivery of specific effectors.

Keywords: bacterial secretion systems, type six secretion system, VgrG paralogs, bacterial competition, *Chromobacterium violaceum*, effector proteins.

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