

**TITLE:** DELETION OF TYPE II SECRETION SYSTEM GENES COMPROMISES OUTER MEMBRANE INTEGRITY AND ABOLISHES SECRETION OF MANY DEGRADING ENZYMES IN *Chromobacterium violaceum*

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**ABSTRACT:**

Bacterial pathogens use sophisticated protein secretion nanomachines to deliver toxins, effectors, and hydrolytic enzymes into the environment. Among the six secretion systems (named type I to VI) already identified in Gram-negative bacteria, the type II secretion system (T2SS) translocates via periplasmic-step many extracellular toxins and degradative enzymes, many of which contribute to pathogenesis in both plants and animals. *Chromobacterium violaceum*, a Gram-negative bacterium that occurs in soil and water and can act as a human opportunistic pathogen, has an operon of 12 genes encoding a potential T2SS. In this work, we obtained four mutant strains with in-frame null deletion of *gspD* (major outer membrane protein), *gspE* (ATPase), *gspC-N* (deletion of the entire T2SS operon) and *pilD* (prepilin peptidase). Growth curves performed in LB medium showed that all the mutant strains have impaired growth relative to the wild-type strain. The deletion of the T2SS genes also resulted in defects in the outer membrane, as demonstrated by growth curves added with the antibiotic polymyxin B. Furthermore, all mutant strains, except  $\Delta pilD$ , had a decrease in biofilm production. Degrading substrate plate assays performed in the defined medium M9 supplemented with specific substrates (chitin, gelatin, blood agar, and skim milk) revealed that all the mutant strains had decreased activity of chitinase, gelatinase, hemolysin, and protease, indicating that the *C. violaceum* T2SS is required for secretion of these enzymes. We analyzed the protein content of the culture supernatant from late-stationary-phase cultures of wild-type and mutant strains grown in LB broth at 37°C by SDS-PAGE. Wild-type and mutant strains displayed different extracellular protein profiles. Together, our results indicate that *C. violaceum* has a functional T2SS required to maintain outer membrane integrity and involved in secretion of several extracellular enzymes.

**Keywords:** *Chromobacterium violaceum*, type II secretion system, secreted toxins, outer membrane, extracellular enzymes

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