TITLE: A GLOBAL TRANSPOSON MUTAGENESIS SCREEN IDENTIFIES NOVEL REGULATORY SYSTEMS FOR SIDEROPHORE PRODUCTION IN *Chromobacterium violaceum*

AUTHORS: BATISTA, B.B.; DA SILVA 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:
Iron is an essential micronutrient, necessary as a cofactor in various biological reactions. In bacteria, iron homeostasis involves Fur-mediated derepression of iron uptake systems (such as siderophores) under iron limitation. In this work, we intend to identify and characterize novel iron regulatory systems in *Chromobacterium violaceum*, an environmental bacterium that occasionally causes severe infections in humans. Initially, we validated the use of the plasmid pIT2 to generate transposon mutants in *C. violaceum*, as confirmed by PCR and Southern Blot assays. Unbiased identification of genes related to iron homeostasis was achieved by screening a 10,000-transposon mutant library for siderophore activity in CAS plate assay. From this screening, 161 strains were confirmed by CAS as producing altered levels of siderophores, with 44 mutants having decreased and 117 mutants increased siderophore activity. Sequencing of semi-degenerate PCR products from the mutant strains allowed identification of the transposon insertion sites in the *C. violaceum* genome. Among the strains with increased siderophore activity we identified two possible regulatory systems: one insertion in the gene CV_1057, a probable transcriptional regulator, with an HTH motif of the superfamily Cro and family SinR; and various insertions in the gene CV_2600, a histidine kinase of a two-component system located on the *C. violaceum* Cpi-2 pathogenicity island. We generated a null mutant strain deleted of the gene CV_1057 by homologous recombination. This null mutant strain showed the same phenotype of increased siderophore activity observed for the transposon mutant strain. Further phenotypic characterization indicated that the ΔCV_1057 mutant strain had decreased production of both violacein and biofilm and also had decreased swimming motility. Viability and growth assays indicated that ΔCV_1057 was more sensitive than the wild-type strain to oxidative stress (cumene hydroperoxide and hydrogen peroxide), but was unaffected by SDS treatment. Thus, we found two novel potential regulatory systems (CV_1057 and CV_2600) that mediate repression of siderophore production in *C. violaceum*, with the gene CV_1057 also regulating violacein production, biofilm formation, and swimming motility.

Keywords: transposon mutant library, siderophore, transcriptional regulators, *Chromobacterium violaceum*, iron acquisition.

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