

TITLE: *Chromobacterium violaceum* REQUIRES PRODUCTION AND UPTAKE OF DISTINCT ENDOGENOUS CATECHOLATE-TYPE SIDEROPHORES FOR IRON ACQUISITION AND VIRULENCE

AUTHORS: BATISTA, B.B.; SANTOS, R.E.R.S.; RICCI-AZEVEDO, R.; 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:

Bacteria rely on siderophores as high-affinity iron acquisition systems to supply their iron demand. Siderophores stand out in the arsenal required for bacterial pathogens steal iron from their hosts. It is unknown how the human opportunistic pathogen *Chromobacterium violaceum* uptake iron. In this work, we show that *C. violaceum* produces at least two catecholate-type siderophores, here named chromobactin and viobactin, each of which required to sustain *C. violaceum* growth in iron-restricted conditions *in vitro* and maintain *C. violaceum* virulence *in vivo*. An *in silico* analysis in the genome of *C. violaceum* revealed that genes related to synthesis and uptake of chromobactin (*cba*) and viobactin (*vba*) are located within two secondary metabolite biosynthetic gene clusters. Using a combination of gene deletions, siderophore activity, and growth stimulation assays, we revealed that chromobactin and viobactin are catecholate siderophores synthesized from the common precursor 2,3-DHB on two non-ribosomal peptide synthetase (NRPS) enzymes (CbaF and VbaF). Moreover, we identified the TonB-dependent receptors (CbuA and VbuA) involved in the uptake of each siderophore. Infection assays in mice revealed that both the synthesis and the uptake of chromobactin and viobactin are required for the virulence of *C. violaceum* since only the mutant strains that do not produce any siderophores or are unable to uptake both of them were attenuated for virulence. The absence of both receptors caused extracellular siderophore accumulation, reduction of neutrophil extracellular trap (NET) production by neutrophils, and marked virulence attenuation in mice. These data indicate that in addition to acquiring iron, siderophores modulate the host immune response. As disrupting the balance of production and uptake of siderophores was detrimental for *C. violaceum* virulence, our data provide an initial basis for anti-siderophore-based treatment strategies against this human pathogen.

Keywords: *Chromobacterium violaceum*, iron uptake, siderophore biosynthesis, siderophore acquisition, bacterial virulence

Development Agencies: FAPESP; CAPES; FAEPA