

TITLE: THE *chuPRSTUV* OPERON ENCODES A HEME AND HEMOGLOBIN UPTAKE SYSTEM IN *Chromobacterium violaceum*

AUTHORS: DE LIMA, V.M.; 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 metal for several cell processes, such as respiration and gene regulation. It is found in high amounts into the heme group of hemoproteins like hemoglobin. Bacteria have evolved sophisticated mechanisms for iron acquisition, including production of iron-chelating compounds known as siderophores, and expression of specialized transporters for the uptake of siderophores and hemoproteins. In this work, we aim to characterize the heme uptake and degradation mechanisms in *Chromobacterium violaceum*, a Gram-negative beta-proteobacterium that causes severe opportunistic infections. *In silico* analysis of the *C. violaceum* ATCC 12472 genome revealed the *chuPRSTUV* operon as a candidate for coding a regulatory protein (ChuP), an outer membrane TonB-dependent receptor (ChuR), a heme degradation enzyme (ChuS), and an inner membrane ABC transporter (ChuTUV) for heme. In-frame null mutant strains $\Delta chuP$, $\Delta chuR$, $\Delta chuS$, $\Delta chuTUV$, and $\Delta chuPRSTUV$ were obtained by allelic exchange. We did not find any difference in the growth curve comparing the wild-type (WT) and all mutant strains in M9 broth or M9 with the iron chelator dipyrindyl. Also, no difference in cell viability was observed when the strains were cultured in M9 broth supplemented with concentrations of hemin up to 2 mM for 24 hours. Nutrition tests showed that hemin and hemoglobin as iron sources were unable to stimulate the growth of the $\Delta chuP$ and $\Delta chuPRSTUV$ mutant strains in iron-chelated M9 media. For $\Delta chuR$, weak growth stimulation was detected for hemin. Moreover, the $\Delta chuR$ mutant showed greater hemolysis in sheep blood agar compared to the WT and other mutant strains. Interestingly, the $\Delta chuP$ and $\Delta chuPRSTUV$ strains displayed increased halos in the CAS assay, which is related to a greater siderophore activity. Preliminary data suggest that the $\Delta chuS$, $\Delta chuTUV$, and $\Delta chuPRSTUV$ strains had decreased virulence *in vivo* when mice were infected intraperitoneally with 10^6 cells. Together these results indicate that the *chuPRSTUV* operon is involved in hemin and hemoglobin uptake. Furthermore, the ChuP regulatory protein seems to have a role in siderophore activity.

Keywords: iron acquisition, heme uptake, hemoglobin uptake, *Chromobacterium violaceum*, siderophore activity.

Development Agencies: CNPq, FAPESP, CAPES, FAEPA