

TITLE: CHARACTERIZATION OF IRON TRANSPORT SYSTEMS IN *Caulobacter crescentus*

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Iron is an essential micronutrient, acting mainly as a cofactor for proteins that are involved in a wide range of biological processes. Levels of free iron in the environment can vary considerably, thus bacteria have evolved strategies to maintain iron levels within a physiological and optimum range. Under iron-restricted conditions, iron acquisition systems are used to collect this important nutrient from the environment. In many cases, bacteria solubilize iron (Fe^{3+}) with secreted siderophores. Some bacteria also use iron present in heme, hemoglobin, transferrin and lactoferrin from eukaryotic hosts. In *Caulobacter crescentus*, the active transport across the outer membrane of iron-siderophore complexes involve outer membrane proteins called TonB-dependent receptors and is driven by an inner membrane energy-transducing TonB–ExbB–ExbD protein complex. *C. crescentus* is a proteobacteria that lives in aquatic environments and is a key organism for the study of the cell cycle in prokaryotes, due to its asymmetric cell cycle that results in two distinct daughter cells. Were identified 62 genes in this bacterium encoding outer membrane proteins that are TonB-dependent receptors (TBDR) responsible for the transport of essential nutrients to the cell, which for example sugars, B12 vitamin and iron. We constructed null mutants for genes encoding different TonB-dependent receptors. The flanking regions of these genes were amplified by PCR using specific oligonucleotides, and the respective fragments were cloned in tandem into pNPTS138, a suicide vector in *C. crescentus*. The resulting recombinant plasmids were introduced into *Escherichia coli* S17-1 and subsequently in *C. crescentus* wild type strain by conjugation, resulting in deleted strains for different TonB-dependent receptors after a double recombination event. Southern blotting and PCR were performed to confirm the deletions. Through the construction of these null mutants, we will be able to evaluate the dynamics of iron transport and to characterize the TonB-dependent receptors role in *Caulobacter crescentus*.

Keywords: *Caulobacter crescentus*, iron, siderophores, TonB-dependent receptors

Development Agency: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)