MSc in Biochemistry
Proposal for Research Project – 2nd Cycle

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TITLE: Um novo sistema bacteriano de resistência ao cobre

Background

*Marinobacter aquaeolei* is a marine halophilic and mesophilic bacterium isolated at the head of oil producing wells on offshore platforms in southern Vietnam. The complete genome was recently sequenced, revealing several uncharacterized proteins and operons, possibly involved in metabolic pathways of hydrocarbons degradation.

Among the proteins identified, a single operon responsible for the regulation of the intracellular levels of copper, was discovered. It was proposed to be involved in the copper resistance of that organism. This operon contains several ORFs, each of which code for a small copper-binding protein, CopC-type, a multicopper oxidase, CopA-type; an outer membrane protein, CopB-type, and a copper-translocating P-type ATPase, and is regulated by a two-component system.

The copper is an essential element that functions as a cofactor in many enzymes, but in excess it can have harmful effects due to metal-catalyzed protein oxidation and generation of reactive oxygen species. Its cellular regulation has to be tightly controlled and thus, it is of extreme importance to understand its mechanism of action and regulation. This operon has a novel and distinct composition, which suggests a different mechanism of copper resistance than the ones recently identified in bacteria. The sequence homology of the proteins that compose this molecular system is very low and unique, which makes it a very interesting target, both to biochemical and structural characterization.

Moreover, copper resistance has recently been connected with increase in virulence. Thus, it is urgent to understand the molecular mechanisms of copper resistance in bacteria.

Objectives and Project Description

Este trabalho terá como objetivo a caracterização bioquímica, e a determinação da estrutura da enzima denominada de CopA, a multicopper oxidase. Assim, irá incluir as seguintes tarefas:

1. Isolamento da CopA através de expressão heteróloga e purificação por técnicas de cromatografia de afinidade.
2. Caracterização bioquímica (espectroscopia de visível, determinação de massa molecular, determinação de cobre/proteína). Caracterização enzimática.

Esta enzima também apresenta interesse biotecnológico na degradação de composto fenólicos.
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As Tarefas 2 e 3 poderão decorrer em paralelo.