MASTER RESEARCH PROJECT

Project title: An evolutionary process: unraveling the functionality of conserved proteins in different *Shewanella* species

Host laboratory/Institution: Inorganic Biochemistry & NMR / Instituto de Tecnologia Química e Biológica António Xavier

Supervisor: Catarina M. Paquete
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Duration: 1 year

Introduction:

Extracellular electron transfer (EET) pathways allow bacteria to transfer electrons from the cell metabolism to extracellular substrates, such as insoluble compounds and metal oxides. This ability makes these organisms a target of biotechnological research for the development of novel bioremediation processes or generation of energy in microbial fuel cells (MFC). Among the exoelectrogenic organisms, *Shewanella oneidensis* MR-1 is one of the most studied organisms, where its extracellular electron transfer pathway relies on several multiheme *c*-type cytochromes. Among these, the small tetraheme cytochrome *c* (STC) and the flavocytochrome c_3 (FccA) are the two most abundant cytochromes found in the periplasmic space, being both highly conserved among *Shewanella* species. It was demonstrated that these two proteins are responsible for receiving electrons from the cell metabolism, via the inner-membrane tetraheme cytochrome CymA, and delivering them to the decaheme cytochrome MtrA in the outer-membrane for the reduction of solid electron acceptors outside of the cell. Under anaerobic conditions FccA can also function as the terminal reductase of *Shewanella*, being responsible for the reduction of fumarate to succinate.

Detailed thermodynamic and kinetic properties of STC and FccA allowed the elucidation of their electron transfer mechanisms in different *Shewanella* strains. Interestingly, although homologous proteins perform the same physiological function in the different organisms, the electron transfer mechanisms employed by the proteins were different, suggesting different mechanistic pathways and different recognition regions with their physiological partners. In order to understand this difference and to elucidate the functionality of each protein, *Shewanella* knock-out strains co-transformed with different homologous proteins will be constructed and their ability to reduce soluble and insoluble compounds will be evaluated. These results will be pioneer and will allow to understand the functional

conservation of STC and FccA in different *Shewanella* strains and to elucidate their evolutionary relationship.

Work plan:

To elucidate the functional properties of STC and FccA in *Shewanella* strains, a multidisciplinary work plan will be developed. First, molecular biology tools will be used to clone the genes of STC and FccA of different *Shewanella* strains, and electroporation will allow transforming these plasmids into *S. oneidensis* MR-1 ΔSTC or ΔFccA. Once these strains are constructed, their ability to reduce various electron acceptors will be evaluated. This will be achieved by assessing the growth curves profile using different electron acceptors. Both soluble and insoluble electron acceptors will be used, including oxygen, fumarate, iron citrate and iron oxides. The electrochemical properties of *Shewanella* cells will also be elucidated using a bioreactor controlled by a potentiostat. These experiments will be performed under anaerobic conditions using a three-electrode system, and the results obtained will be compared with wild-type organisms in order to assess the functional properties of the proteins.

For more information please visit the WebPages:

http://www.itqb.unl.pt/~louro/

http://www.itqb.unl.pt/research/biological-chemistry/inorganic-biochemistry-and-nmr

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