

Abstract

The transfer of electrons has been central to biological life processes since the inception of life on earth. Extant bacterial cells sustain themselves by utilizing various sources of energy for carrying out this flow of electrons across membranes to generate ATP for survival, growth, and cell division. One of these processes involving the flow of electrons is the phenomenon of extracellular electron transfer (EET) carried out by electroactive bacteria (EAB). EET is the ability of EAB by which they are able to produce a flow of electrons to electron acceptors external to the bacterial cells leading to the reduction of these extracellular electron acceptors. This thesis aims to investigate and understand the EET ability of the electroactive bacterium *Shewanella oneidensis* MR-1 in the form of its conductive appendages called nanowires as well as study the interaction of the bacterium with three different transition metal ions, namely divalent copper (Cu^{2+}), divalent cobalt (Co^{2+}), and trivalent gold (Au^{3+}) ions, in a three-electrode bioelectrochemical cell.

The analysis of *S. oneidensis* MR-1 cells on a carbon felt working electrode taken from an operating three-electrode bioelectrochemical system, using the relatively uncomplicated technique of field emission-scanning electron microscopy (FE-SEM) revealed the vesicular structure of the conductive nanowires of the bacterium. The electron micrographs showed the detailed vesicular structure of the nanowires on the carbon felt working electrode demonstrating that the vesicular structure reported previously in the absence of EET is preserved during EET as well. The modified protocol employing an acetone : hexamethyldisilazane dehydration step was able to preserve the soft biological structure of the nanowires for FE-SEM imaging and can be used for the examination of nanowires on different types of electrode materials as well as the examination of other similar biological structures. The findings from this study in the three-electrode bioelectrochemical system also informed the fundamental understanding of *S. oneidensis* MR-1 nanowires which are outer membrane and periplasmic extensions of the bacterial cell.

The operating three-electrode bioelectrochemical system with actively EET performing *S. oneidensis* MR-1 was further utilized to develop a workflow to gain an understanding of the interaction of transition metal ions – Cu^{2+} , Co^{2+} , and Au^{3+} – with *Shewanella* cells.

Studying divalent copper ions (Cu^{2+}) using electrochemical techniques such as chronoamperometry and cyclic voltammetry helped in discerning the redox interactions of *Shewanella* and copper. Further, complimenting the electrochemical data with imaging and elemental analysis via scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) and X-ray absorption near edge spectroscopy (XANES) revealed the spatial relationships and speciation fates of the cupric ions. It was found that *S. oneidensis* cells are capable of biologically reducing cupric ions to their lower oxidation states. This phenomenon might have further clinical implications due to horizontal gene transfer causing the spread of EET mechanisms/components across phylogenetic boundaries which must be investigated for any future potential to trigger a rise in resistance against the novel class of metal-based antibiotics called the metalloantibiotics. Moreover, the ability of *Shewanella* to reduce cupric ions can also be translated into the development of biosensing, biomining, and bioremediation technologies.

Divalent cobalt and trivalent gold were the two other important transition metal ions to be subsequently analysed using electrochemical, electron microscopy, and elemental analysis techniques. Examination of *S. oneidensis* MR-1 cells interacting with Co^{2+} and Au^{3+} uncovered the differential response of *Shewanella* when subjected to different metal ions. While the EET ability of *Shewanella* cells was evidently unaffected by the toxicity of the Co^{2+} ions, a significant drop in the flow of current was seen when the system was exposed to Au^{3+} ions. A comparison of the Co^{2+} -exposed and Au^{3+} -exposed carbon felt electrodes subjected to SEM-EDS analysis revealed clear differences in the spatial distribution of the added metal ions and the elemental composition of the electrodes. While the Co^{2+} -exposed electrodes showed a conspicuous absence of cobalt, the Au^{3+} -exposed electrodes were found to be covered with gold nanoparticles in the presence of *S. oneidensis* MR-1 cells and gold microparticles in the absence of the bacterial cells. There was also a stark difference between the survival of planktonic cells from Co^{2+} -exposed and Au^{3+} -exposed systems with the Au^{3+} -exposed systems potentially highlighting a hitherto unexplored characteristic of *Shewanella* towards persister cell formation.

Hence, it was seen that the introduction of transition metal ions in its environment elicits a graded, differential response from *S. oneidensis* MR-1. The bacterium's response to each metal ion highlights the fundamental physiological characteristics of the bacterium while also directing us towards potential translational prospects harnessing the findings presented in this thesis. Overall, this thesis attempts to apply a hermeneutic approach to the discernment of the bacterium *S. oneidensis* MR-1 with the ultimate aim of advancing the current fundamental

understanding of the organism while also highlighting pathways for the application of this understanding.