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CHARACTERIZATION OF MANGANESE OXIDATION COUPLED TO OXYGEN REDUCTION IN *Pseudomonas resinovorans* MOB-513

Parra, Lucía¹ - Rodríguez Simón, Carlos Norberto² - Robledo, Alejandro Javier² - Gottig, Natalia¹ - Busalmen, Juan Pablo²

1) Instituto de Procesos Biotecnológicos y Químicos de Rosario (IPROBYQ-CONICET-UNR) Rosario-Santa Fe-Argentina.

2) Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA-CONICET-UNMDP) Mar del Plata-Buenos Aires- Argentina

Contacto: parra@iprobyq-conicet.gob.ar

Groundwater often contains manganese (II) levels that exceed drinking water standards, posing a significant concern for human health. Additionally, Mn(II) contributes to aesthetic and organoleptic issues and can accelerate corrosion in water distribution networks. Removal of Mn(II) is based on its oxidation to form insoluble oxides that can be filtered out of the water. This may be achieved by physico-chemical methods or by a more eco-friendly strategy that involves biological treatments. The success of biological sand filter technology depends on the presence of Manganese Oxidizing Bacteria (MOB) that can form biofilms and efficiently oxidize the metal, such as the environmental isolate *Pseudomonas resinovorans* MOB-513. Although there is evidence of chemolithoautotrophic growth in some strains, it is still debated whether the electrons from Mn(II) enter the electron transport chain to sustain growth, or if oxidation is merely a detoxification mechanism. The relationship between Mn(II) oxidation and biofilm formation has been studied in MOB-513. Previous studies showed that c-di-GMP, a second messenger crucially involved in *Pseudomonas* biofilm formation, increases biofilm formation and Mn(II)-oxidizing capabilities in MOB-513. To further investigate the role of c-di-GMP in Mn(II) oxidation, a transposon mutagenesis in MOB-513 was performed. To further studies, two mutants that lost their Mn(II) oxidation capability were selected, one defective in Type IV Pili membrane platform PilC protein and the other in the biofilm formation regulator AlgR. Also, one mutant that overexpresses a protein with a GGDEF domain, involved in c-di-GMP synthesis, which showed a higher capability to oxidize Mn(II) than MOB-513 WT, was chosen. In this work, and to gain information about coupling of Mn(II) oxidation and oxygen reduction, vials were inoculated with MOB-513 WT or mutants in the presence or absence of Mn(II) as the sole electron source and were incubated statically. Oxygen levels in vials were measured over time with an optical sensor. After incubation, manganese oxide (MnOx) production was quantified with Leucoberberline Blue (LBB), and residual Mn(II) was measured with linear voltammetry. To characterize manganese oxidase activity, different cell fractions obtained through cell lysis and centrifugation were incubated with Mn(II) and MnOx production was quantified. A correlation between Mn(II) oxidation and oxygen reduction was

found. Oxygen consumption was higher in Mn-oxidizing bacteria than non-oxidizing bacteria. Interestingly, one of the non-oxidizing mutants, MOB-513-*algR*::Tn, recovered manganese oxidase activity after cell lysis, suggesting an underlying mechanism of protein translocation or complex formation dependent on AlgR. On the other hand, MOB-513-*pilC*::Tn did not regain the ability to oxidize manganese after lysis, highlighting the importance of Type IV pili for Mn(II) oxidation.

Palabras clave: Manganese oxidation - Biofilm - Oxygen reduction - Pseudomonas