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## **ANALYSIS OF THE MOLECULAR MECHANISMS OF Mn(II) OXIDATION IN *Pseudomonas resinovorans* TO ENHANCE METAL BIOREMEDIATION**

Gaffuri, Marilina<sup>1</sup> - Parra, Lucía<sup>1</sup> - Ottado, Jorgelina<sup>2</sup> - Gottig, Natalia<sup>1</sup>

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

2) Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR) Rosario-Santa Fe-Argentina

Contacto: gaffurimarilina@gmail.com

Groundwater is a crucial drinking water source, but in many areas of Argentina, it contains unacceptable levels of manganese (Mn) in the Mn(II) state. Biological sand filtration is an efficient and eco-friendly method to purify this water and Manganese-Oxidizing Bacteria (MOB) accelerates Mn(II) removal. Previously, we isolated and characterized various MOB, selecting those with high adherence efficiency, biofilm formation, and Mn(II) oxidation capabilities. In one of the isolates, *Pseudomonas resinovorans* MOB-513, the intracellular levels of cyclic dimeric GMP (c-di-GMP) were enhanced by the overexpression of a diguanylate cyclase (DGC), which synthesizes this messenger. Interestingly, we found that c-di-GMP enhances both biofilm formation and Mn(II) oxidation capacity. To investigate the role of c-di-GMP in Mn(II), MOB-513, transposon mutants that lost their ability to oxidize Mn(II) were selected. We found mutants in genes of Type IV Pili (*pil*) involved in bacterial twitching motility, and mutants in the *hk*-2948 (*hk*) gene which encodes a sensor histidine kinase with unknown function. In the upstream region, a gene that encodes a response regulator with a DGC domain, *rr*-2947 (*rr*), is present. To characterize these mutants' twitching mobility, biofilm formation and macrocolony Mn(II) oxidation assays were performed. As expected, *pil* mutants did not display twitching motility, but only *pilC* and *pilH* lost their ability to form biofilm compared to WT strain. Neither *pilC*+DGC nor *pilH*+DGC recovered the ability to form biofilm. Mn(II) oxidation assays on macrocolonies showed that only *pilH*+DGC recovered the ability to oxidize Mn(II). This suggests that both PilC and elevated levels of c-di-GMP are crucial for Mn(II) oxidation. *hk* strain exhibited similar twitching motility than WT strain but showed a significant reduction in biofilm formation. We could not complement the *hk* mutant or overexpress HK-2948 in the WT strain, may be as a consequence of the large size of the protein, or difficulties in its insertion into the membrane. Nevertheless, a *hk*+DGC strain was constructed and it regained the ability to form biofilms and to oxidize Mn(II) compared to *hk*-pEmpty. These results suggest a role of HK-2948 in the increase of c-di-GMP necessary for biofilm and Mn(II) oxidation process. RR-2947 protein levels were increased in WT and *hk* strains by the overexpression of its gene using the pBBR1 vector (WT+*rr* and *hk*+*rr* strains). In WT+*rr*, an increase in biofilm formation and earlier

Mn(II) oxidation were observed compared to WT+pEmpty, and similar to WT+DGC, indicating the involvement of this RR in these process. However, *Δhk+rr* did not shown enhanced biofilm formation and Mn(II) oxidation. These results suggest that HK-2948 is required to activate RR-2947, leading to increased c-di-GMP levels and, consequently, biofilm formation and Mn(II) oxidation. In conclusion, this study suggests that Type IV Pili, HK-2948 and RR-2947 are essential for Mn(II) oxidation.

Palabras clave: *Pseudomonas resinovorans* – Manganese – Biofilm - c-di-GMP – Oxidizing Bacteria