Sociedad Argentina de Microbiología General

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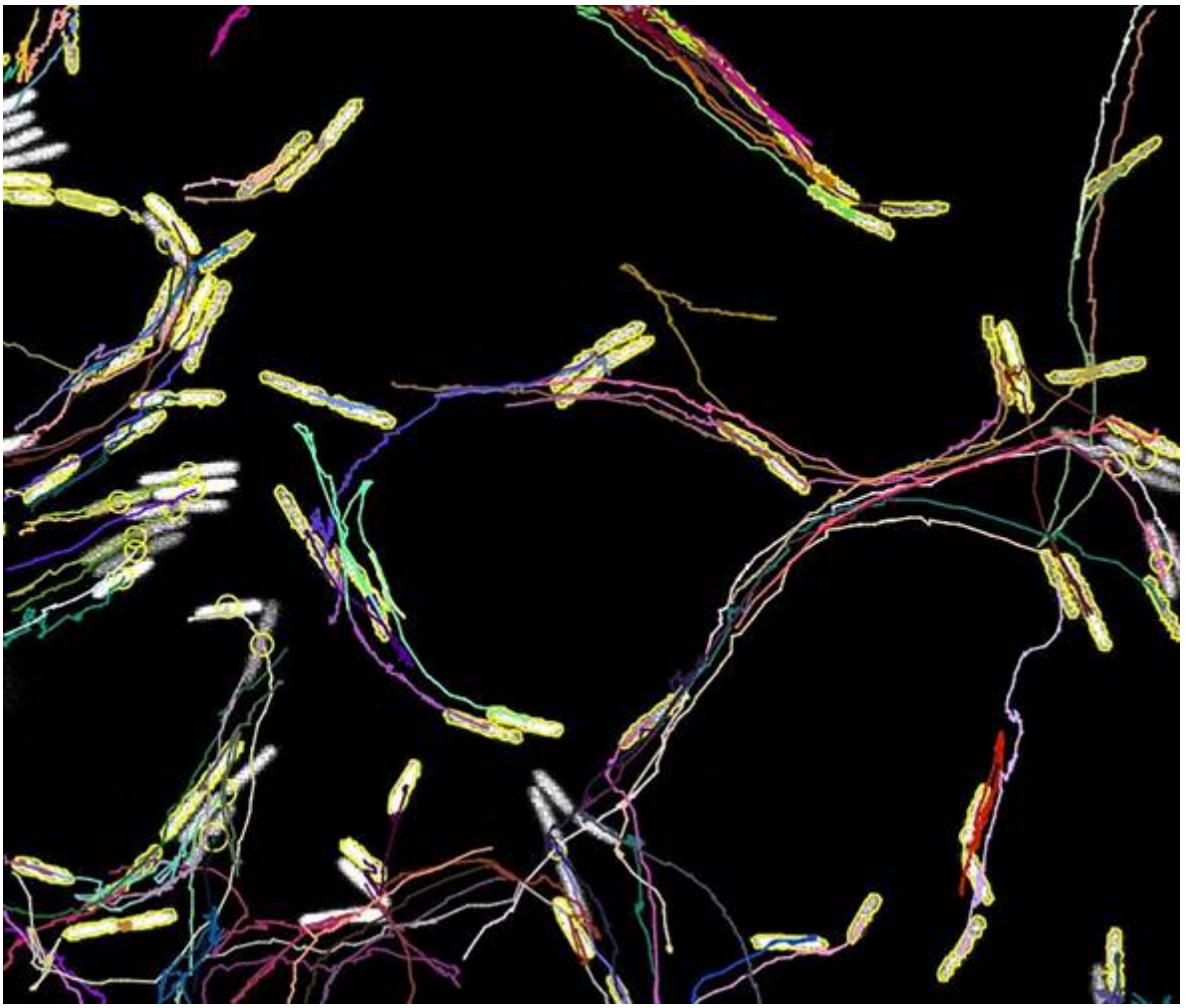

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

23 - Biodegradación, Biorremediación y Biodeterioro

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

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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 synthesize this messenger. Interestingly, we found that c-di-GMP enhance 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) comparted 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, ?*hk*+ *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