TITLE: EVALUATION OF NICKEL TOLERANCE BY BACTERIA OF THE GENUS *MUCILAGINIBACTER* ORIGINATED FROM SERPENTINE SOIL

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ABSTRACT:

Serpentine soils are rich in heavy metals, especially nickel. Microorganisms adapted to these soils have mechanisms to tolerate high concentrations of metals. Our previous work showed that a Brazilian serpentine soil bacterium was tolerant to 16 mM $NiSO_4$. This bacterial isolate belongs to the genus *Mucilaginibacter* and our genomic analysis revealed an Average Nucleotide Identity (ANI) of 92% with *M. gossypii*. This bacterium and its close relative *M. gossypiicola* have already been tested for nickel and cadmium toxicity, but they tolerated only 1 mM of these heavy metals. The objective of the present study was to evaluate the tolerance to nickel in *Mucilaginibacter* SAP B3 obtained from serpentine soil and to determine its Maximum Tolerated Concentration (MTC). *Mucilaginibacter* SAP B3 was initially cultured in R2A medium in the presence or absence of 5 mM $NiSO_4$. These bacteria were then transferred to solid R2A medium containing concentrations of $NiSO_4$ ranging from 2.5 to 45 mM. Growth was assessed using the drop-dilution method; the highest dilution presenting colony growth was recorded as a measure of bacterial growth. In addition, the highest concentration of nickel in which growth was still observed was determined as the MTC. Bacteria preincubated in medium containing nickel showed a MTC of 45 mM NiSO4. In contrast, bacteria originating from a nickel-free pre-inoculum exhibited a delay in colony formation of at least 24 h, compared to samples pre-inoculated with nickel. Moreover, the bacteria pre-inoculated on nickel-free medium presented a lower MTC of only 30 mM. Therefore, the presence of nickel in the pre-inoculum improves the general tolerance to nickel in *Mucilaginibacter* SAP B3. It is not yet known which mechanisms are involved in the observed tolerance, but *Mucilaginibacter* SAP B3 produces large amounts of Extracellular Polymeric Substances (EPS). Studies suggest that bacterial EPS adsorbs metals extracellularly, so this may be one of the resistance mechanisms employed by *Mucilaginibacter* SAP B3. Furthermore our genomic analysis of *Mucilaginibacter* SAP B3 showed the presence of *czc* heavy metal efflux pumps. Finally, the present work showed that *Mucilaginibacter* SAP B3 exhibits the highest nickel MTC observed in all *Mucilaginibacter* species, which may be a result of the environmental heavy metal constraint imposed on the bacteria that inhabit serpentine soils.

Keywords: bacterial growth, *czc,* EPS, heavy metal tolerance, nickel.

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