TITLE: COBALT PRECIPITATION IN R2A MEDIA PROMOTED BY *MUCILAGINIBACTER* SAP-B3 ISOLATED FROM SERPENTINIC SOIL.

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ABSTRACT:

Serpentine soils contain high concentrations of metals, especially nickel and cobalt. *Mucilaginibacter* SAP-B3 was isolated from serpentine soils and showed tolerance to 45 mM NiSO4 and 2 mM CoSO4. However, a precipitation of pink granules was observed on solid culture medium containing cobalt. The objective of the present study was to verify if these granules were formed as a result of bacterial activity or if they were formed by changes in the pH of the culture medium. The R2A medium had originally pH 6.54 ± 0.4; since phenol red was used as a pH indicator, the color of the medium was yellow. The inoculated treatments were: R2A solid medium without any metal (control), R2A amended with 2 mM CoSO4 or 2 mM NiSO4. Plates with nickel were used to test whether the precipitation was a general feature of heavy metals. There are two other controls in which R2A plates were prepared, but not inoculated with bacteria: the replica of each treatment and R2A amended with NaOH, increasing the pH. The plates were incubated at 30 ° C for 4 weeks. Plates with alkaline pH with no bacterial inoculum turned red, but no precipitation was observed. All Inoculated control plates turned red on the second day of incubation, whereas R2A plates without inoculum remained yellow. Bacteria growth on R2A with cobalt was slowest among all treatments. However, after three weeks, only the cobalt cultures showed the formation of visible pink granules. The results indicate that the bacterium metabolism changes the pH of the medium, regardless of the presence of any metal. SAP-B3 produces large quantities of Extracellular Polymeric Substances (EPS), and studies suggest that EPS can adsorb metals. It is likely that one of the EPS components complexes with cobalt and forms a poorly soluble product. After growth on liquid media, the EPS was separated from the biomass by centrifugation. EPS from cultures containing cobalt was precipitated with ethanol revealing precipitate with purple-blue color. The observed co-precipitation of the polymer with cobalt was poorly soluble in water. Finally, the study showed that *Mucilaginibacter* SAP-B3 promotes cobalt precipitation indicating that this bacterium may have potential for bioremediation of areas contaminated with metals.

Keywords: heavy metals, EPS, cobalt, nickel, phenol red.

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