



Metagenomic analysis of nickel tolerance in the rhizosphere of the Tinto River

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The Tinto River (Huelva, Spain) is considered a Mars analog, and constitutes an extreme environment with an acidic pH and a high concentration of heavy metals. These extreme conditions are generated by the metabolic activity of chemolithotrophic microorganisms growing in the complex sulfides of the Iberian Pyrite Belt. Previous studies demonstrated the abundance and diversity of eukaryotic microbes in the planktonic phase of the river, in contrast with the low diversity of prokaryotic microbes. Although an impressive work has been realized in the description of the Tinto River ecosystem from different scientific areas, our knowledge is limited about the mechanisms used by the microbial community to live under these extreme conditions, particularly in the presence of heavy metals. Because of this, Tinto River represents an ideal ecosystem to find new useful tools for bioremediation. In this work, we focused in the mechanisms of resistance of this microorganisms to nickel, one of the toxic metals in the rhizosphere of the river. To address this question we first analyzed the composition of the prokaryotic communities in the rhizosphere. The biodiversity found was more complex than the one of the planktonic zone of the river as revealed by the phylogeny of the 16S gene. Most of the sequences matched with uncultured strains and some were similar to species associated to acid mine drainage (AMD) environments (i.e. *Acidiphilium* sp., “*Ferrimicrobium acidiphilum*“ and *Acidobacterium capsulatum*). This complexity was adequate to consider the construction of metagenomic libraries to look for a rich diversity of mechanisms involved in nickel tolerance. We obtained approximately 450.000 clones from five different libraries and fourteen of them grew in the presence of nickel at 2 mM. Among the inserts of these clones we found ORFs coding for membrane proteins including 3 ABC transporters which may play a role in nickel tolerance. The results obtained from the analyses of these sequences are dis-

cussed in this work.