

Comparison of Different Methods of Microorganism's Identification from a Copper Mining in Pará, Brazil

Authors : Louise H. Gracioso, Marcela P.G. Baltazar, Ingrid R. Avanzi, Bruno Karolski, Luciana J. Gimenes, Claudio O. Nascimento, Elen A. Perpetuo

Abstract : Introduction: Higher copper concentrations promote a selection pressure on organisms such as plants, fungi and bacteria, which allows surviving only the resistant organisms to the contaminated site. This selective pressure keeps only the organisms most resistant to a specific condition and subsequently increases their bioremediation potential. Despite the bacteria importance for biosphere maintenance, it is estimated that only a small fraction living microbial species has been described and characterized. Due to the molecular biology development, tools based on analysis 16S ribosomal RNA or another specific gene are making a new scenario for the characterization studies and identification of microorganisms in the environment. News identification of microorganisms methods have also emerged like Biotyper (MALDI / TOF), this method mass spectrometry is subject to the recognition of spectroscopic patterns of conserved and features proteins for different microbial species. In view of this, this study aimed to isolate bacteria resistant to copper present in a Copper Processing Area (Sossego Mine, Canaan, PA) and identifies them in two different methods: Recent (spectrometry mass) and conventional. This work aimed to use them for a future bioremediation of this Mining. Material and Methods: Samples were collected at fifteen different sites of five periods of times. Microorganisms were isolated from mining wastes by culture enrichment technique; this procedure was repeated 4 times. The isolates were inoculated into MJS medium containing different concentrations of chloride copper (1mM, 2.5mM, 5mM, 7.5mM and 10 mM) and incubated in plates for 72 h at 28 °C. These isolates were subjected to mass spectrometry identification methods (Biotyper - MALDI/TOF) and 16S gene sequencing. Results: A total of 105 strains were isolated in this area, bacterial identification by mass spectrometry method (MALDI/TOF) achieved 74% agreement with the conventional identification method (16S), 31% have been unsuccessful in MALDI-TOF and 2% did not obtain identification sequence the 16S. These results show that Biotyper can be a very useful tool in the identification of bacteria isolated from environmental samples, since it has a better value for money (cheap and simple sample preparation and MALDI plates are reusable). Furthermore, this technique is more rentable because it saves time and has a high performance (the mass spectra are compared to the database and it takes less than 2 minutes per sample).

Keywords : copper mining area, bioremediation, microorganisms, identification, MALDI/TOF, RNA 16S

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