Título: SEQUENCING OF TOLERANT CLONES FOR HEAVY METAL SELECTED FROM METAGENOMIC LIBRARY CONSTRUCTED FROM OIL-IMPACTED MANGROVE SEDIMENT

Authors Cabral, L.[1], Sousa, S. T. P.[1], Lacerda Júnior. G. V.[1], Noronha, M. F.[1], Ottoni, J. R.[1], Domingos, D. F.[1], Oliveira, V. M.[1]

Institution [1]Divisão de Recursos Microbianos, Research Center for Chemistry, Biology and Agriculture (CPQBA), Campinas University - UNICAMP. Mailbox: 6171. CEP: 13081-970. Campinas. São Paulo. Brazil.

Abstract

Microorganisms found in the environment can adapt to the presence of pollutants, thus developing survival mechanisms. It is know that the soil and sediments has a large microbial diversity. However, traditional cultivation methods are not efficient for cultivation of most microorganisms present in nature. Although improvements on in vitro techniques have been made in order to recover microorganisms from these sites, the knowledge of the mangrove microbiota remains incipient. In this context, the aim of this study was to assess the presence of heavy metal tolerance in one metagenomic library constructed using sediment samples collected from a mangrove area located in Bertioga, State of São Paulo, Brazil. The metagenomic library comprised 13,000 clones and the sampling site was was affected by an oil spill. The functional screening of clones resistant to mercury and nickel was carried out according to methodology described by Freeman et al. (2005) with few modifications. *E. coli* EPI 300 was used as negative control. After 48 h, the growth was measured using Elisa spectrophotometer. The sequencing of fosmidial DNA was done using Ion torrent platform, the assembly of the contigs was performed using IDBA-tran sofware 1.1.0 and the annotation was perfomed in RAST Server (Overbeek et al., 2014). From 1,920 clones tested, were selected of 24 clones with the potential to tolerate metals and were able to grow in the presence of 0.016 mM of mercury and 1 mM of nickel. In the tolerance assay, 6 positive clones presented higher optical density (O.D.) in comparison to the others in the presence of 0.03 to 0,05 mM of mercury and 8 mM of nickel. The results of the annotation in RAST server for assembled contigs presented *Putative oxidoreductase Salicylate Hydroxylase Protein function* and *Chromate transport protein ChrA* function for clone 105 Hg/A5 and clone 13 Hg/C2, respectively. It was expected that enzymes and functions related to the detoxification of mercury and nickel should be found in all clones, however pathways of detoxification of chromate resistance and hydrocarbons degradation maybe are contributing to growing of clones in presence of mercury and nickel. These clones were selected for further studies on metal degradation.

Keywords: *in vitro* techniques; metagenomic library; clones; heavy metal.

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