PROSPECTION OF NEW GENES RELATED TO PHENOL DEGRADATION IN METAGENOMIC LIBRARIES DERIVED FROM PETROLEUM REFINERY SLUDGE

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Abstract:

Phenol and phenolic compounds are the main organic pollutants found in several industrial wastewaters, such as oil refineries. These compounds cause serious damage to the environment and their presence in wastewater may lead to the decrease of microbial taxa richness, thus affecting the biological degradation of such pollutants. Bioremediation has shown to be an excellent alternative approach that utilizes microorganisms for phenol removal from wastewater. In this sense, there has been a great interest in investigating the potential of new microbial metabolism, mainly of yet uncultured microorganisms, aiming to improve the biodegradation activity of pollutant compounds. Thus, our goal was to evaluate the phenol biodegradation potential of metagenomic libraries derived from the microbiota present in wastewater treatment plant (WWTP) by using the pyrosequencing approach, focusing mainly on novel genes involved in the central pathway of phenol degradation. The sludge sample was collected from a pilot membrane bioreactor (MBR) of a petroleum refinery WWTP in Brazil. High molecular weight DNA of sludge sample was isolated, ligated into the pCC2Fos fosmid vector and cloned into *E. coli* EPI300 cells. One hundred clones were selected based on phenol degradation activity, pooled and submitted to fosmid DNA extraction and 454-FLX sequencing. The pyrosequencing run yielded 129,635 sequences and 609 contigs, from which 341 were considered to be large contigs (> 1000 bp). These large contigs were analyzed by using the SEED/MG-RAST platform and the PRODIGAL program, followed by BLASTp and phylogenetic analyses. The MG-RAST analyses showed a broad metabolic profile, with 108 sequences related to the metabolism of aromatic compounds. Nineteen proteins were affiliated to proteins from the phenol degradation pathway, such as phenol hydroxylase subunits, cathecol 2,3-dioxygenase and cathecol 1,2-dioxygenase, some of which were shown to be potential new genes, distantly related to known sequences deposited in the GenBank database. This study revealed that the sludge community under study has a great potential for the degradation of aromatic compounds present in petroleum refinery wastewater treatment system. **Financial support: PETROBRAS, FAPESP.**

Key words: bacterial diversity, metagenome, petroleum refnery, phenol degradation, pyrosequencing analysis