

**Título: POLYCYCLIC AROMATIC HYDROCARBON OXYGENASES DIVERSITY FROM THE SEQUENCING OF A METAGENOMIC LIBRARY FROM OIL-IMPACTED MANGROVE SEDIMENT**

**Authors** Sousa, S. T. P.<sup>1</sup>, Cabral, L.<sup>1</sup>, Ottoni, J. R.<sup>1</sup>, Domingos, D. F.<sup>1</sup>, Lacerda Júnior. G. V.<sup>1</sup>, Passarini, M.R.Z.<sup>2</sup>, Andreote, F. D.<sup>3</sup>, Oliveira, V. M.<sup>1</sup>

**Institution** <sup>1</sup>CPQBA/UNICAMP - Divisão de Recursos Microbianos (Rua Alexandre Caselatto 999, Vila Betel, CP 6171, 13083-970, Campinas, SP, Brasil), <sup>2</sup>UNILA - Universidade Federal da Integração Latino-Americana (Av. Tarquínio Joslin dos Santos, 1000 - Jd. Universitário, Foz do Iguaçu - PR, 85870-650, Brazil), <sup>3</sup>ESALQ - Escola Superior de Agricultura Luiz de Queiroz (Av. Pádua Dias, 11 - Cx. Postal 9 - Piracicaba – SP)

**Abstract:**

Mangrove is one of the most important biome for maintenance of the biosphere balance and shelters several species of animals, plants and microorganisms. The microbiota present in this ecosystem has a great potential for prospecting of bioactive compounds of social interest. Nevertheless, mangroves have suffered the most diverse human activities with economic and agricultural purposes, including oil spill and industrial wastes. Nowadays, there is a frantic search for enzymes that degrade polycyclic aromatic hydrocarbons, motivated by scientific interest in developing new processes that reduce the environmental impacts caused by oil spills. It is known that a small portion of microbial diversity is accessed from culture-dependent methods (around 5%). To increase this coverage, culture-independent techniques (eg metagenomic libraries and Next-Generation Sequencing) are largely used. Thereby, these techniques can help to elucidate new metabolic pathways, in addition get answers about taxonomic diversity and functional role of the native microbiota. Therefore, this study aimed the investigation of genes involved in the polycyclic aromatic hydrocarbons (PAH) degradation from the sequencing of a metagenomic library constructed from oil-impacted mangrove sediment, located in Bertioga city, São Paulo State. The library was previously constructed using the "Cloning pCC2FOS-Ready Copy Control" kit, following the manufacturer's methodology. A total of 12,800 clones was obtained. Clones were picked in deep well plates containing LB liquid medium with chloramphenicol (12.5 µg/ml) and L-arabinose (0.02%), and incubated at 37°C at 180 rpm during 16 h. The fosmidial DNA was extracted using the QIAGEN Large-construct Kit (Qiagen Sample & Assay Technologies, Germany). The sequencing was performed in HiSeq 2000 platform (Illumina). Sequencing data were uploaded into MG-RAST V3.5 for processing and functional and taxonomic annotation. According to general classification 2% of the sequences of the metagenomic library corresponded to xenobiotics metabolism. The majority divided between benzoate degradation (37,7%), nitrotoluene degradation (29,6%), chlorocyclohexane and chlorobenzene degradation (11%). Also highlighting the sequences classified for PAH degradation, adding up 1% of total. Sequences associated with dioxygenase and monooxygenases were subjected to taxonomic analysis. These results suggest that metagenomic library constructed from oil-impacted mangrove sediment has potential to catch new genes of aromatic compounds oxygenases.

**Keywords:** aromatic hydrocarbons, biodegradation, *in silico* analysis, metagenomic library.

**Acknowledgments:** FAPESP