







Genome-based approach for the identification of *Rhodococcus* marker genes for biomonitoring of the environmental quality

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Environmental pollution with anthropogenic organic compounds is a global problem and restoring contaminated habitats using biodegradative activities of microorganisms can be an eco-friendly strategy. The genus *Rhodococcus* is a promising group of bacteria suitable for the biodegradation of recalcitrant contaminants. They possess unique adaptation capacities to fluctuating environmental conditions in a wide variety of environments and to thrive under stress conditions. Consistently with these abilities, they usually possess large genomes, high gene multiplicity, redundant and versatile catabolic pathways.

The main goal of this work is the genome analysis of *Rhodococcus* strains with peculiar characteristics in order to identify new genetic determinants involved in the metabolism of more diffused contaminants including hydrocarbons, pesticides, and plastics. These marker sequences can be used for biomonitoring purpose with a culture-independent approach. Different approaches have been used: *in silico* analyses of *Rhodococcus* genomes, the combination of genome-based and transcriptomic analyses, and different functional analysis to determine the role of selected genes.

Among organic contaminants, BTEX is a group of widespread compounds and the *ortho*-isomer of xylenes is one the most common hazardous source of environmental contamination. For these reasons, RNA-seq analysis was performed on *R. opacus* R7 cells grown on *o*-xylene and it showed 542 differentially expressed genes that are associated with the oxidation of aromatic hydrocarbons, stress response, osmotic regulation, and central metabolism. Interestingly, the most up-regulated genes belong to the *akb* gene cluster encoding for the ethylbenzene dioxygenase system. However, R7 genome analysis and the characterization of *o*-xylene metabolites suggested the involvement of other monooxygenases/hydroxylases (*prm* and *phe* genes). Therefore, three oxidative systems encoded by *akb*, *prm* and *phe* genes were selected for functional analyses to define their role. Results revealed a redundancy of converging genes involved in *o*-xylene metabolism that can be used as marker sequences: the *akb* genes are specifically activated in the oxidation of the *o*-xylene ring, while *prm* and *phe* genes are involved in the first step and the phenolic intermediate degradation, respectively.

Among *Rhodococcus* strains, *R. opacus* R7 showed the intriguing ability to grow on different emerging compounds such as pesticides and plastics as only carbon and energy source. Genome-based analyses showed potential marker genes putatively involved in polyethylene degradation. Currently, a transcriptomic approach has been embraced to obtain a wide scenario of genes transcriptionally induced during growth on polyethylene.

These genes will be a useful collection of marker sequences to assess the quality of polluted environments.

