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Transcriptomic analysis of *Rhodococcus opacus* R7 grown on polyethylene by RNA-seq

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Plastic waste management has become a global issue for its excessive use and large disposal that have resulted in the deposition of more than 348 million tons of plastic on earth in 2017. Among other plastics, polyethylene (PE) is the most abundant synthetic plastic worldwide, and low-density polyethylene (LDPE) is mainly used for plastic bags and food packaging making up the largest volume of plastic pollutions. Although polyethylene is one of the most resistant to biodegradation for its hydrophobic properties, few bacteria are able to directly use PE polymers as the sole carbon and energy sources without any physical or chemical pretreatments.

In this context, a combination of genome-level techniques (*omic*-approach) contributed to unravel the complex degradative system behind PE biodegradation to clarify the gene framework involved in PE biodegradation of *Rhodococcus opacus* R7. This strain was selected for its ability to grow on PE as the only carbon and energy source in a short range of time, increasing the total number of cells of almost two orders of magnitude. The RNA-seq allowed uncovering genes putatively involved in the first step of PE oxidation. In-depth investigations through preliminary bioinformatic analyses and enzymatic assays on the supernatant of R7 grown in the presence of PE confirmed the activation of superficial or releasing extracellular enzymes genes encoding for laccase-like enzymes. Moreover, the transcriptomic data allowed identifying candidate genes for the subsequent steps of short aliphatic chain oxidation including *alkB* gene encoding an alkane monooxygenase, *cyp450* gene encoding cytochrome P450 hydroxylase, and genes encoding membrane transporters. PE biodegradative system was also validated by FTIR analysis on R7 cells grown on PE compared to malate condition indicating that PE induces *R. opacus* R7 metabolic changes. In particular, FTIR evidenced the presence of longer lipid hydrocarbon chains in the membranes, and a more intense *beta*-sheet band compared to R7 cells grown on malate.

These findings are noteworthy, and valuable to address the current plastic waste emergency in terms of a novel microorganism able to biodegrade polyethylene and above all new data on genetic determinants involved in this degradation.
The identification of these gene products poses the basis for diverse environmental and biotechnological applications contributing to plastic elimination.