Exploring the optimization of polyethylene biodegradation by *Rhodococcus opacus* R7 through its degradative enzymatic systems

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Abstract:
Polyethylene (PE) is the most-produced polyolefin and a major global pollutant. PE biodegradation exerted by microorganisms is suggested to overcome its notorious recalcitrance. Among bacteria with compelling plastic-degrading capacities, bacteria of the *Rhodococcus* genus are well known for the ability to degrade hydrocarbon and emerging contaminants, including polyethylene polymers. Although different bacterial or fungal pure cultures isolated from various environments, and microbial consortia were applied, the biodegradative process and the molecular mechanisms are still unclear. A promising approach is the development of green technologies comprising effective microbial biodegradative strategies exploiting their enzymatic systems.
For this purpose, *Rhodococcus opacus* R7 was selected as a reference strain since in previous studies R7 was demonstrated to be able to grow on untreated PE as the sole carbon and energy source. In addition, its transcriptome was analysed upon growth on PE highlighting the genes involved in the main steps of its metabolic pathway, including three laccases (LMCOs), an alkane monooxygenase, a cytochrome P450 hydroxylase, and a few transporters.
Different growth conditions were tested to evaluate the best performances of the strain including PE concentrations ranging between 0.2% to 0.6%, the addition of a supplementary carbon source to increase the biomass level at the early time of growth, application of sonication during the degradation process, cell wash to detach PE from the cells, and the evaluation of different preculture conditions. The best performances in terms of bacterial cell growth, laccase enzymatic activity chosen as a reference of first PE oxidation, and lipid content were identified: preculture on basal medium (mineral medium added with malate), PE at 0.4% added every week up to 28 days.
Moreover, the main enzymatic systems involved in this process underwent a magnification process. *R*. *opacus* R7 gene expression upon growth on PE was followed over time at 14, 21, and 28 days through RT-qPCR analyses. In addition, each gene involved in PE metabolism was investigated to identify its natural inducer to further potentiate its expression. The expression level was assessed in the presence of different hydrocarbons, lignin-derived compounds and carboxylic acids utilized as the sole carbon and energy source or inducer on the basis of literature data and simultaneous computational investigation on specific enzymatic systems such as R7 LMCOs.
The investigation of PE biodegradative mechanisms paves the basis to mitigate the environmental plastic contamination and further biotechnological applications.