Genome-Scale Transcriptional and Metabolic Profiling of *Rhodococcus opacus* R7 During Polyethylene Degradation

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Abstract:

Polyethylene (PE) is the most widely produced synthetic polyolefin, constituting a major fraction of global plastic waste. Its recalcitrance poses critical environmental and biotechnological challenges. Microbial degradation is emerging as a promising strategy to overcome PE persistence.

Among PE-degrading microorganisms, members of the *Rhodococcus* genus stand out for their capacity to degrade hydrocarbons and emerging contaminants, including polyethylene.

Rhodococcus opacus R7 has been demonstrated to grow on untreated PE as its sole carbon and energy source, making it a valuable model for elucidating plastic biodegradation mechanisms. Previous transcriptomic analyses revealed that PE exposure activates key genes involved in the initial oxidation and downstream metabolic processing of polymer-derived intermediates, including three laccase-like multicopper oxidases (LMCO1, LMCO2, LMCO3), an alkane monooxygenase, a cytochrome P450 hydroxylase, and several transporters. Based on these findings, this study investigates the genome-driven molecular response of *R. opacus* R7 under a wide range of PE degradation scenarios, evaluating different cultivation conditions, PE concentrations (1% single-dose vs. 0.4% fed-batch), and the presence of potential inducing substrates.

Physiological and transcriptional analyses showed that pre-cultivation on PE induced a slowdown effect, resulting in altered growth kinetics and increased lipid accumulation in subsequent inocula. Conversely, the 0.4% PE fed-batch condition elicited a broader and more dynamic transcriptional activation, suggesting a finely tuned regulatory system sensitive to substrate availability and concentration. The pre-cultivation on basal mineral medium plus malate and weekly additions of 0.4% PE was identified as the most effective setting, maximizing biomass yield, LMCO enzymatic activity, and lipid accumulation over 28 days. RT-qPCR analyses revealed the up-regulation of additional multicopper oxidases and P450 hydroxylases under specific PE degradation conditions up to 14 days. Screening of potential natural inducers further showed that long-chain *n*-alkanes (e.g., C24) activated diverse LMCOs and oxidative enzymes.

Overall, these integrated approaches advance the understanding of PE biodegradation by *R. opacus* R7, paving the basis for further enzymatic and biochemical investigation.

These insights provide novel insights for developing effective microbial technologies for sustainable plastic waste mitigation and valorization.