# Isolation and characterization of two novel extracellular multicopper oxidases laccase-like from *Rhodococcus opacus* R7 for their ability to oxidize polyethylene

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**Abstract**:

Synthetic plastic has been widely used in almost every area of our society, often associated with single-use applications. For these reasons, its global production is increased rapidly, reaching 367 million metric tons in 2020. Consequently, managing plastic pollution has become a critical challenge due to ever-growing post-consumer plastic waste and high resistance to (bio)degradation posing severe threats to the environment and organisms.

Among the most produced and employed petroleum-based plastics, polyethylene (PE) accounts for around 60% of the total accumulated plastic waste. Although PE is one of the most recalcitrant for its properties, few bacteria or enzymatic systems can oxidize or/and biodegrade PE polymers. *Rhodococcus opacus* R7 is one of a few strains enable to grow in the presence of PE as the sole carbon and energy source in a short range of time without any physical or chemical pretreatments.

Based on previous RNA-seq analysis performed on *R. opacus* R7 cells grown on PE, two gene products encoding for multicopper oxidases laccase-like (LMCO2 and LMCO3) were selected for their involvement in the first step of PE oxidation. They were cloned into an *E. coli-Rhodococcus* shuttle vector, pTipQC2, to prove their expression in a heterologous strain. Since *E. coli* does not efficiently express *Rhodococcus* genes, they were transferred in *Rhodococcus erythropolis* strain AP. Their expression levels were assessed by a laccase enzymatic assay in the presence of the supernatant or the cell extract of the recombinant strains, and 2,6-dimethoxyphenol as substrate. Their production, purification, and enzymatic assessment demonstrated that LMCO2 and LMCO3 gene products belong to the laccase family showing different biochemical and biophysical features.

On these bases, the biodegradative activity of LMCO2 and LMCO3 on not-pretreated PE was assessed by utilizing GC-MSD and FTIR spectroscopy analyses. PE was already oxidized by LMCO2 and LMCO3 after 6 h of incubation at 60 °C and the oxidation produces oxygenated products including ketones, alcohols, and carboxylic acids at 24 h and even alkyl products at 48 h.

These data together showed for the first time a glimpse of the mechanism of oxidation of PE polymers.