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MYCOREMEDIATION OF PCBS DEAD-END METABOLITES: *IN VIVO* AND *IN VITRO* DEGRADATION OF CHLOROBENZOIC ACIDS BY THE WHITE ROT FUNGUS *LENTINUS TIGRINUS*

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Abstract

Chlorobenzoic acids (CBA) are ubiquitous organic contaminants with different degree of chlorination. Besides their use as herbicides, these compounds are often found in the environment in association with polychlorinated biphenyls (PCBs). It is noteworthy, in fact, that the aerobic bacterial co-metabolism of PCBs often leads to the accumulation of CBAs as dead-end products. Several CBA isomers possess endocrine-disrupting activity and exhibit toxicity towards aquatic organisms (*i.e.* ciliates, *Daphnia* sp., algae and fishes) and genotoxicity towards higher plants.

Considering the remarkable bioremediation potential of fungi towards a wide array of aromatic pollutants, the aim of the present work was to assess the CBA-degrading capability of *Lentinus tigrinus* CBS 577.79 and to clarify the CBA-biotransformation mechanisms.

In vivo experiments were performed using axenic cultures, under both stationary and shaken conditions, in either complex or mineral media. Seven day-old cultures were spiked with a mixture of 12 CBA (mono-, di- and tri-CBA) (CBAM) to reach a final concentration of 120 mg/L.

Chlorobenzoates did not exert inhibitory effects on fungal growth and on the production of the ligninolytic enzymes, such as laccase and manganese peroxidase (MnP). In particular, spiking of the CBAM stimulated laccase activity with respect to the relative control (non-spiked cultures). The large majority of CBAs was efficiently removed by *L. tigrinus* cultures with the sole exceptions of 2,6-DCBA, 2,3,6- and 2,4,6-TCBA, the partial depletion of which was only observed in MEG-submerged cultures. The structural identification of CBA degradation products (*i.e.*, chlorinated benzoaldehydes and benzyl alcohols, chlorotoluenes and chlorophenols) suggested that the transformation of these compounds was the result of a combined action of both extracellular and intracellular enzyme systems. *In vitro* experiments with purified laccase and MnP, in the presence and in the absence of redox mediators, showed that these enzymes were not involved in the initial breakdown steps of chlorobenzoates. Conversely, *in vitro* tests carried out with the extracted microsomal fraction confirmed the involvement of cytochrome P450 enzymes in CBA degradation process. Moreover, the *Vibrio fischeri* assay showed that the fungus was able to partially remove the toxicity associated with the CBAM.