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TRANSFORMATION OF PHENOLIC POLLUTANTS BY FUNGAL LACCASES PURIFIED OR EXPRESSED IN TOBACCO PLANTS

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Intense pollution of the environment by xenobiotic chemicals continuously released into the biosphere has been one of the biggest concerns for science in the second part of the twentieth century.

Several types of industrial and agricultural wastes contain phenols. Many of these compounds and their derivatives are extremely harmful because they are highly toxic towards living organisms and represent a serious environmental problem.

Conventional methods of remediation of phenol-polluted systems (e.g. solvent extraction, chemical oxidation, adsorption on activated supports, etc.) have some disadvantages due to high cost, time-consuming procedures and formation of toxic residues. Conversely, the use of enzymatic oxidative catalysts is a promising alternative technology to address the clean up of such a waste.

Oxideductases catalyze several biochemical transformations. Among them, laccases have received a great attention because of their possible role in the detoxification of polluted environments, and as catalysts in several applications. They are classified as polyphenoloxidases, i.e. enzymes capable to oxidize a reduced substrate and to start a polymerization process.

Laccases, mainly from fungal origins, are capable to degrade and/or to transform various pollutants into safer products. The potential of these enzymes as decontaminating agents of polluted environments has been verified in studies performed with a large range of xenobiotic substances, representative of pollutants from different origins. Several compounds (substituted phenols, anilines, non phenolic substances) have been partly or totally transformed in polymeric aggregates of different nature and size. Furthermore, it has been demonstrated that the catalytic efficiency of the enzymes towards less-reactive compounds can be greatly improved by additives, which behave as enhancing co-substrates. By contrast, other compounds, eventually present along with the target pollutants, may exhibit not negligible inhibitory effects. Moreover, experiments direct at evaluating the residual activity of the involved enzymes have indicated that some enzyme molecules may be entrapped or adsorbed into the polymeric products, loosing to some extent their activity.

The use of whole plants and/or their root exudates as sources of laccases have been also proposed as an effective tool to deal with the detoxification of phenolic polluted wastes. The ability of these detoxifying agents can be greatly magnified by their expression in a greater extend by metabolic engineering applications. However, plant laccases are much less effective than laccases produced by bacteria and fungi in their oxidative activity on phenols.

The efficiency of various laccases, purified from fungal sources, in the detoxification of either simulated or natural phenolic-polluted waters as well as their possible practical applications in

polluted soil will be addresses. Furthermore, attempts to express a fungal phenoloxidase (POXC) in tobacco plants were successful, after gene transfer mediated by *Agrobacterium tumefaciens*. The *pox*C gene expression was ascertained by Western blot and its activity was checked by appropriate chemical reactions using ABTS as substrate. It has been also shown that the heterologous laccase is released by root exudates into the culture medium, thus giving rise to a new tobacco genotype useful for soil remediation.