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Characterization of insoluble bioproducts resulting from the transformation of diclofenac by fungal laccase

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A few results have already been published in the form of a scientific paper: 'Characterization of laccases from *Trametes hirsuta* in the context of bioremediation of wastewater treatment plant effluent', accepted August 23, 2023: https://doi.org/10.1016/j.enzmictec.2023.110308. Most of the results presented here will be included in a future publication currently in preparation.

Problem:

Growing urbanization and increased consumption of pharmaceutical products by the public have led to an increase in the load of contaminants of emerging interest (CEI) passing through municipal wastewater treatment plants (WWTPs). These compounds can be released into the environment, where they can have a negative impact.

The success of CEI transformation by WWTPs varies considerably from one compound to another and one process to another. Oxidation processes (OPs) could increase the elimination/transformation of CEI in a given place. Unfortunately, in addition to being costly and environmentally unfriendly, several studies indicate an increase in toxicity after the treatment of several CEI by OP [1]. This is explained by the formation of products that are potentially more toxic than the parent compounds. Finally, in most cases, the compound will end up in aquatic environments in its initial or transformed form and may be transformed again by bio/photo/chemical ways, modifying its toxicity.

Laccase (E.C. 1.10.3.2), an enzyme secreted by white rot fungi, may be an alternative to traditional OPs. It requires no additional reagents to oxidize a given substrate, and no waste is produced. Moreover, several studies indicate high stability in WWTP effluent and a high potential for the transformation of CEI. Several studies indicate a reduction in toxicity after the treatment of pharmaceutical compounds, but also the formation of suspended particles. The formation of these insoluble transformation products could be explained by the oligomerization of the targeted CEI [3]. Unfortunately, these transformation products have not been well characterized and a good understanding of the reduction in toxicity after treatment with laccase is still lacking. In order to determine whether enzymatic treatment could be a future solution for the bioremediation of WWTP effluent, a better understanding of the oligomerization pathway is required.

Objectives:

After purifying and characterizing laccases from one of the most promising strains, *Trametes hirsuta*, we will focus on the oligomerization pathway, and more specifically on oligomer formation, by purifying and characterizing them. These objectives will be developed in the context of bioremediation of a WWTP effluent.

Results:

HPLC-QTOF analysis revealed the formation of numerous diclofenac dimers and trimers. These oligomers differ from each other mainly in the number of hydroxylations, decarboxylations and unsaturations. For example, the main compound identified is a doubly hydroxylated and doubly decarboxylated diclofenac dimer. This study highlights the ability of laccase to reoxidize the substrate, several times in succession, until a highly hydrophobic and insoluble compound is obtained. Thus, the decrease in its bioavailability could explain the reduction in toxicity observed in the literature. Finally, our results indicate that these observations could be valid at low concentrations and in a WWTP effluent.

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