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Fungal laccases: from structure-activity studies to environmental applications



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INTRODUCTION

- Fungal laccases (EC 1.10.3.2) are a group of multicopper oxidoreductive enzymes involved in lignin transformation, morphogenesis, pathogenesis and fungal virulence, as well as in the oxidation of organic compounds
- Laccases are widely used in several processes of the dye, paper (pulp bleaching) and textile industries
- A wide range of their substrates are of environmental interest, as recognized pollutants of waters and soils
- Structure-activity studies remain necessary today to highlight the high potential of laccases for environmental applications

Our objective is a better knowledge concerning the structural and catalytic properties of fungal laccases, as well as the mechanisms involved in the regulation of their expression, as the first step to their optimized applications

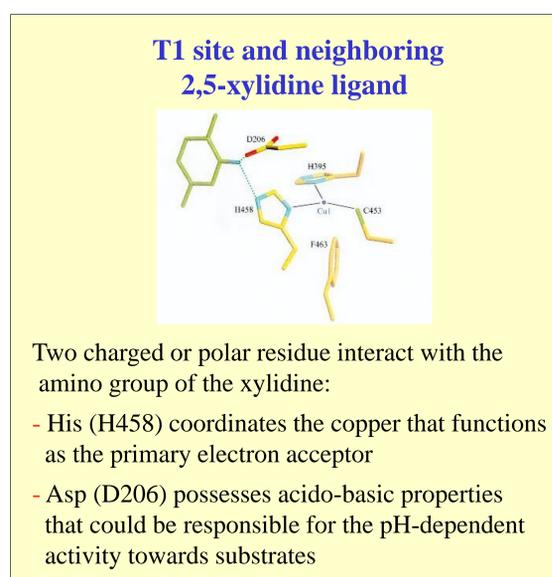
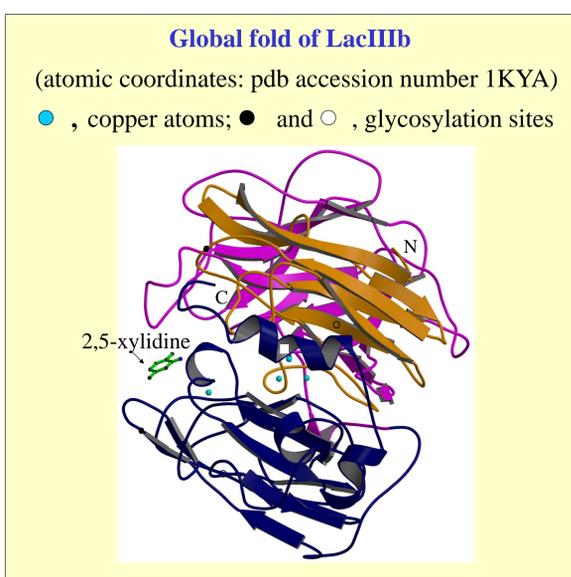
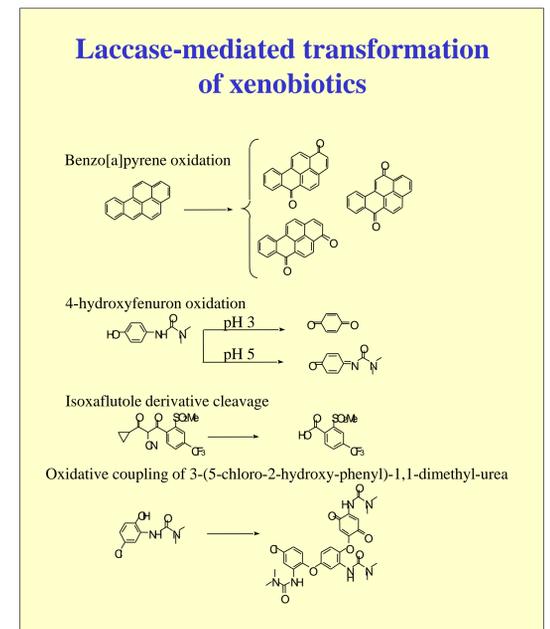
AREAS OF RESEARCH

1 - Involvement of fungal laccases in the biotransformation of xenobiotics^[1-3]

- Fungal laccases are involved in the metabolism of pesticides and polycyclic aromatic compounds
- According to their chemical structures, the enzymes catalyze oxidation reactions leading either to the breakdown of the substrates or to their coupling (polymerization)
- As a consequence, fungal laccases contribute to the fate of xenobiotics in the environment

2 - Structural data of the laccase IIIb secreted by the basidiomycete *Trametes versicolor*^[4]

The crystal structure of the protein has been obtained with 2,5-xylidine bound in its active site



3 - Electrochemical studies to determine the catalytic cycle of the laccase in complex solutions^[3]

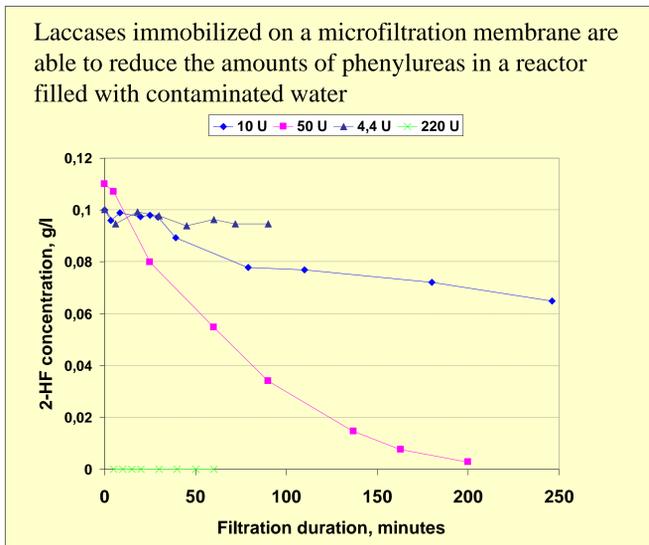
- In some cases, substrate oxidation by laccases needs the presence of a redox mediator (ABTS)
- In complex media, the reaction can be disturbed by compounds such as metals
- This fact strengthens the development of recombinant enzymes with a high redox potential avoiding the use of mediator

4 - Molecular basis of laccase expression^[5]

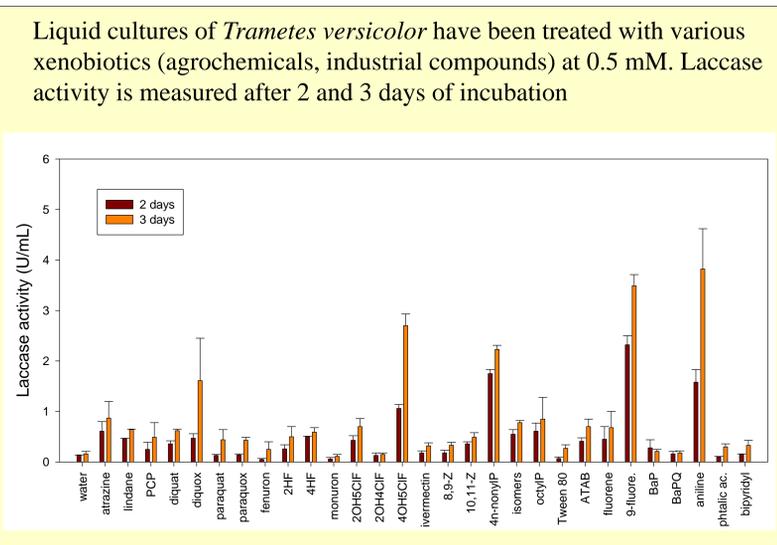
- The gene has been cloned in *Escherichia coli* using the pGEM®-T plasmid
- It has been sequenced and comprises 1563 bases (sequence accession number AF414109), including a peptide signal
- It was then expressed in the yeast *Yarrowia lipolytica* using the pINA1269 vector (Patent INRA/INA-PG), thus allowing the production of an active form of the recombinant enzyme

ENVIRONMENTAL APPLICATIONS

1 - Laccases as tools for bioremediation^[1]

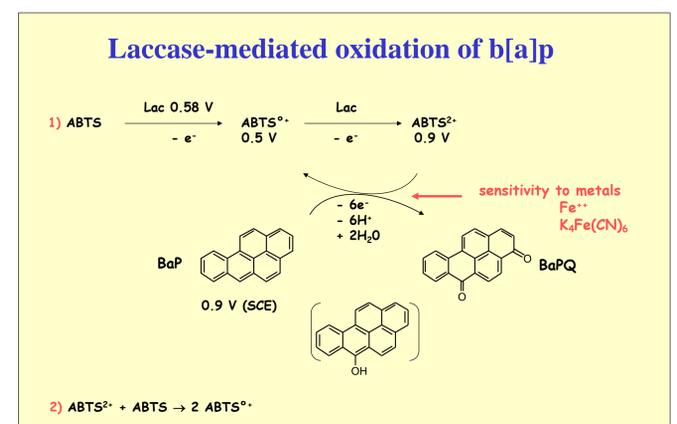


2 - Laccases as biomarkers to assess the impact of chemicals^[6]



- These results allow to develop a strategy of directed mutagenesis to design laccases with:
- a high efficiency in catalysis (high redox potential)
 - a wider variety of substrates
 - a pH of activity optimized for natural media

Additional studies need to be performed with other xenobiotics bound in the active site



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