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Towards the engineering of fungal laccases for an environmental application. 1st European congress on Engineering Enzymes for biocatalysis by using directed evolution

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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 numerous xenobiotics
- 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, and are recognized pollutants of waters and soils
- Laccases are potential useful tools to improve the chemical quality of polluted media through bioremediation^[1]

Our objective is the design of laccases with:

- a high efficiency in catalysis (high redox potential)
- a wider variety of substrates
- a pH of activity optimized for natural media



The present poster reports the preliminary studies intended to allow a better knowledge of the laccase IIIb (LacIIIb) from the white-rot basidiomycete *Trametes versicolor*, as the first step of its engineering

Our approach is based on the knowledge of the cavity enclosing a weak reducing substrate of the enzyme, the arylamine 2,5-xylylidine, and on the replacement of neighboring interacting residues

RESULTS

I - Expression of LacIIIb by *Yarrowia lipolytica* ^[4]

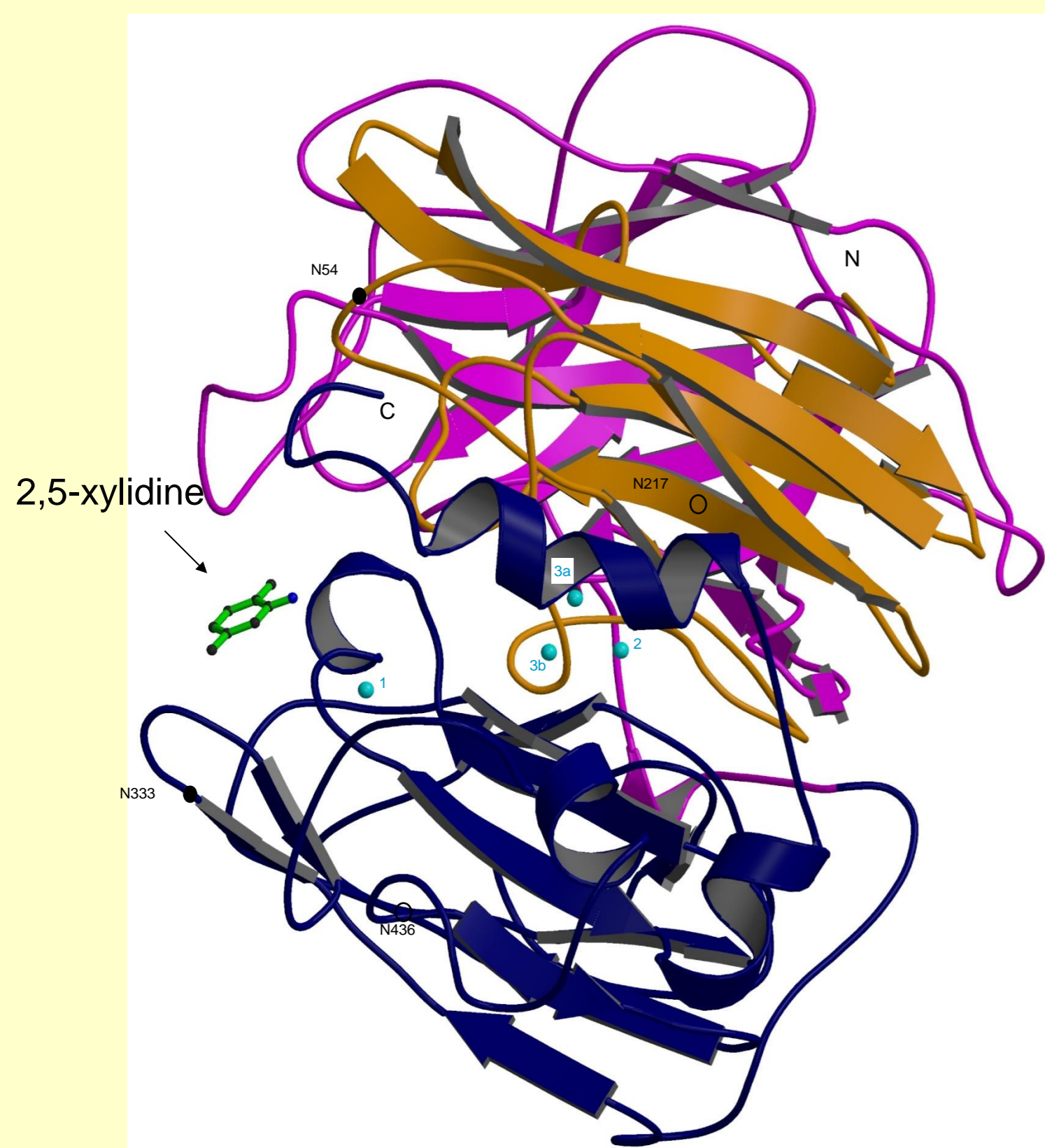
- The gene has been cloned in *Escherichia coli* using the pGEM®-T plasmid
- It has been sequenced and comprises 1563 bases (sequence accession number AF414109), including a peptide signal
- It was then expressed in the yeast *Yarrowia lipolytica* using the pINA1269 vector (Patent INRA/INA-PG), thus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

2 - Structural data of the wild protein ^[5]

Global fold of LacIIIb

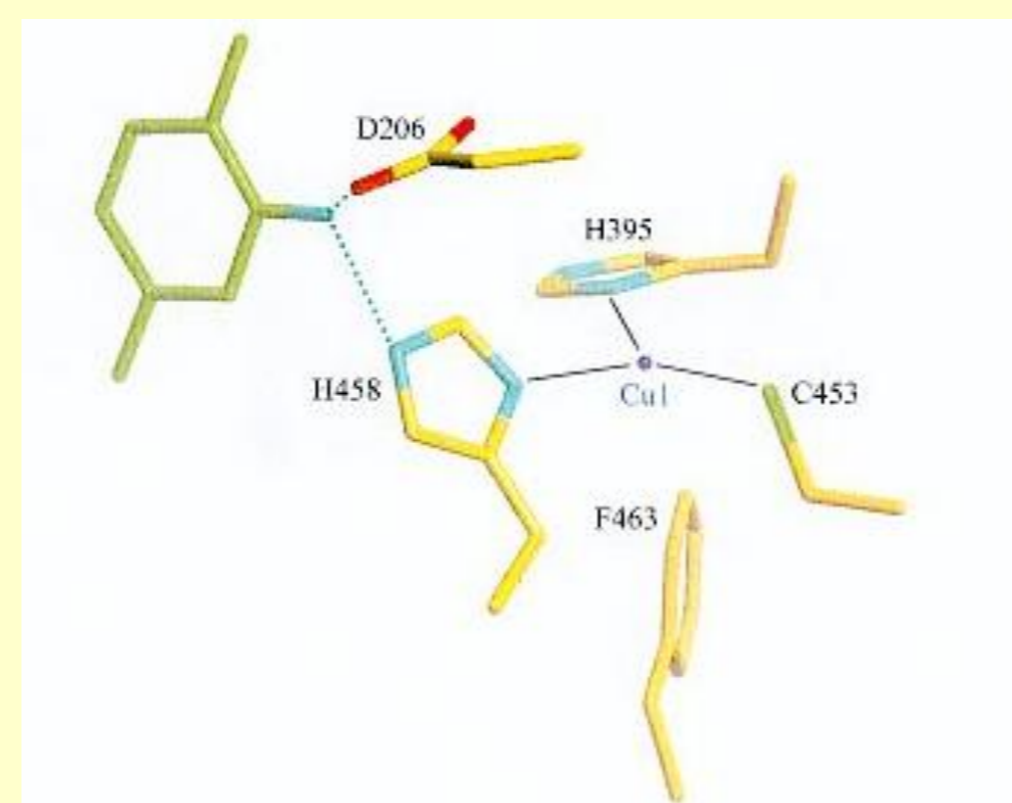
(atomic coordinates: pdb accession number 1KYA)

●, copper atoms; ● and O, glycosylation sites



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

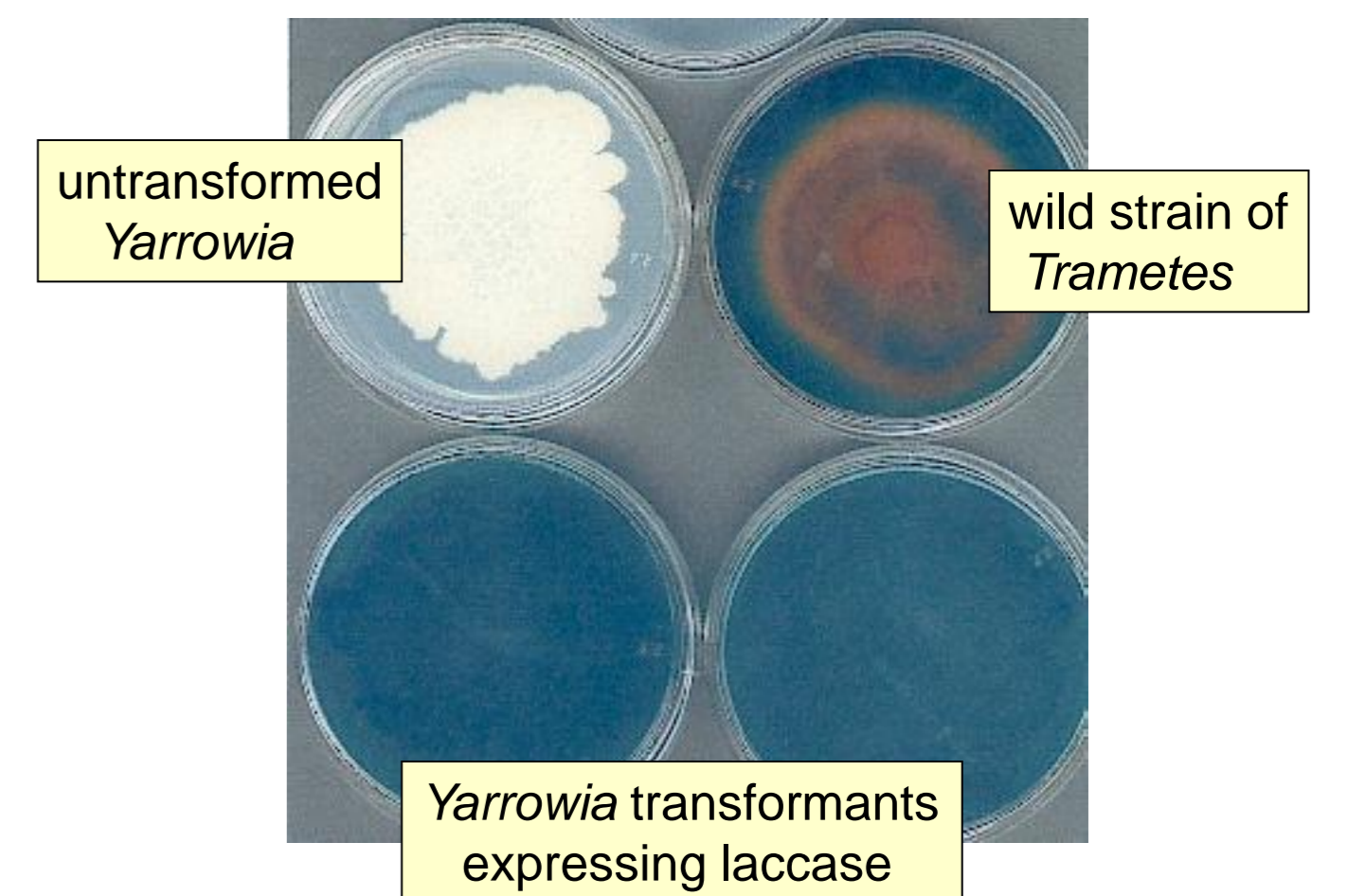
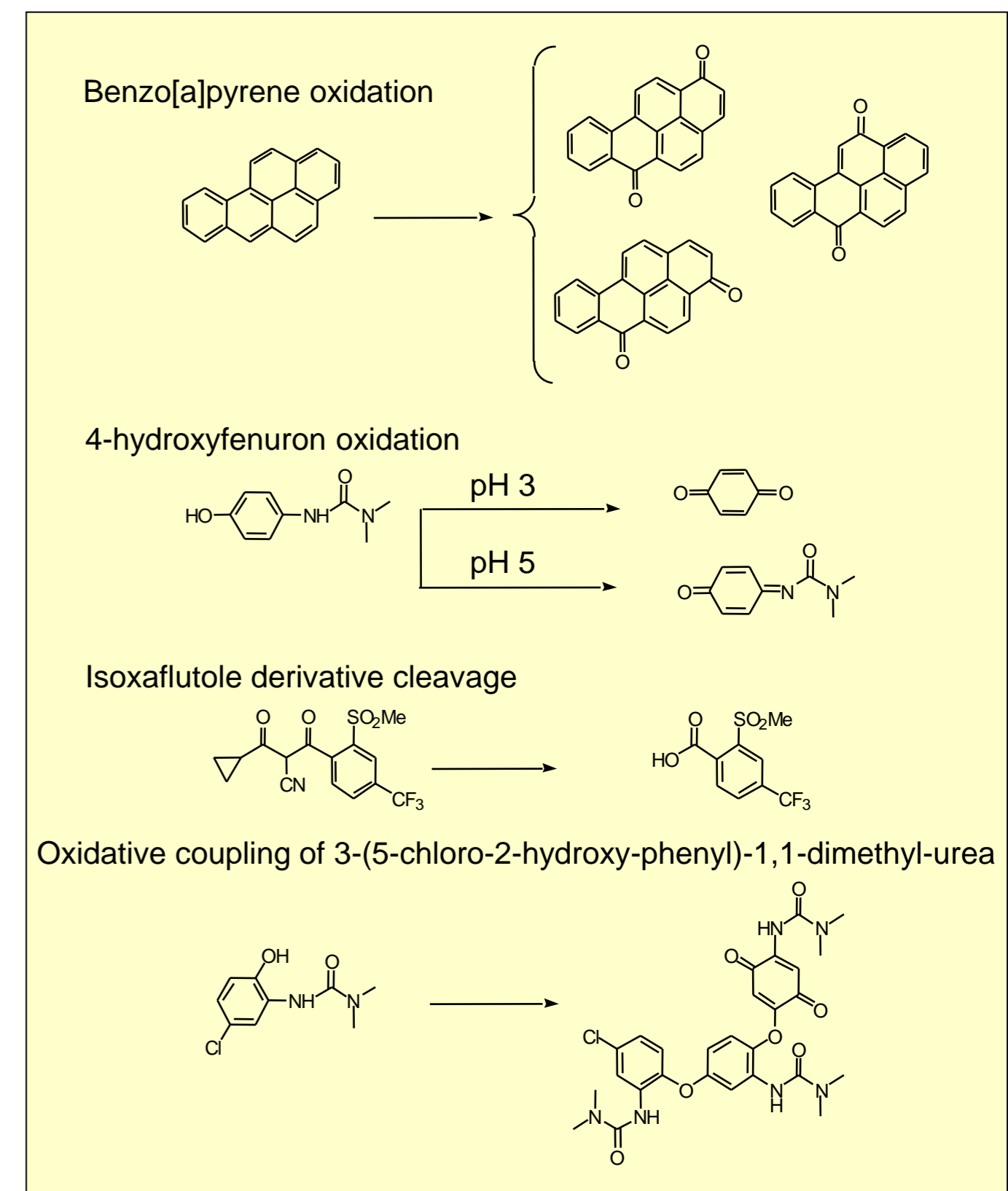
3 - T1 site and neighboring 2,5-xylylidine ligand



Two charged or polar residue interact with the amino group of the xylylidine:

- His (H458) coordinates the copper that functions as the primary electron acceptor
- Asp (D206) possesses acido-basic properties that could be responsible for the pH-dependent activity towards substrates

Laccase-mediated transformation of xenobiotics ^[1-3]



5 - Strategy of directed mutagenesis

- To a residue with another negative chain: D206E
- To a residue with a polar uncharged chain: D206N
- To a residue with an uncharged chain: D206A

4 - Sequence alignment of laccases from fungi and higher plants

	180	190	200	210	220	230
<i>Tversicolor</i>	INGK	GRSPSTTTADLS	VISVT	FGKRV	RLVLSL	CDPNYTF
<i>Ccinerereus</i>	INGK	GRYVGGPAEELS	VNVVE	QGKKV	RLVLSL	CDPNWQF
<i>Pcinnabarinus</i>	INGL	GRTGTAPSDLA	VIKVTE	QGKRV	RLVLSL	CDPNHFS
<i>Malbomyces</i>	INGT	AVNPNTGEGQYAN	VLTLP	GKRHL	RLVLSL	CDPNHFS
<i>Athaliana</i>	INGQ	FDLYNCSKRETV	VVPLNS	GETSL	LRVINAAL	NQPLFF
<i>Rvernificera</i>	INGEL	GDLYNCSKRETV	RLSVQ	PNKTYL	LRVNAVL	NEEKF
<i>Apeudoplatanus</i>	INGQ	GALFDCSTGTT	FLPVK	KGETYL	LRVINAAL	NNEEM

- Biological roles of laccases from fungi and higher plants appear to be opposite: fungal laccases are involved in ligninolysis whereas enzymes from plants catalyze the initial polymerization of monolignols during lignification
 - Fungal laccases have a negatively charged residue at the 206 position (Asp, D or Glu, E), whilst those of higher plants have an asparagine (Asn, N)
- Fungi: *Tversicolor* = *Trametes versicolor*; *Ccinerereus* = *Coprinus cinereus*; *Pcinnabarinus* = *Pycnoporus cinnabarinus*; *Malbomyces* = *Melanocarpus albomyces*
- Higher plants: *Athaliana* = *Arabidopsis thaliana*; *Rvernificera* = *Rhus vernicifera*; *Apeudoplatanus* = *Acer pseudoplatanus*

CONCLUSIONS

- We describe here the first crystal structure of a laccase complexed with a reducing substrate. Relevant residues of the reducing center can thus be localized
- Previously based only on sequence alignment analysis, the directed mutagenesis of laccases has shown only very slight results in improving enzyme efficiency ^[6]. The present knowledge of structural data will supply new and promising leads
- However, the high redox potential of some fungal laccases remains unexplained

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