



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-xylydine, and on the replacement of neighboring interacting residues

RESULTS

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

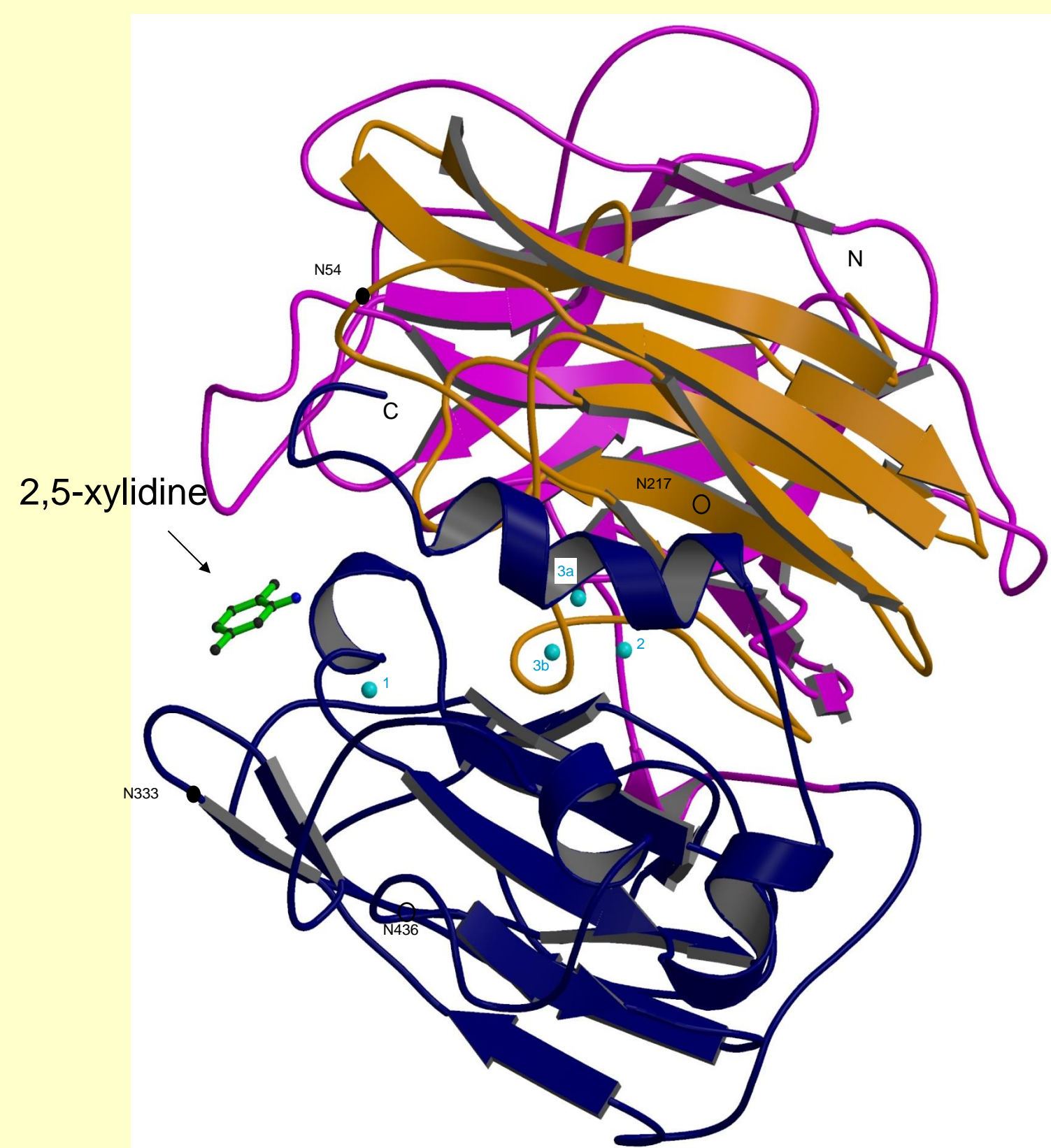
- The gene has been cloned in *Escherichia coli* using the pGEM®-T plasmid
- It has been sequenced and comprises 1563 bases (sequence accession number AF414109), including a peptide signal
- It was then expressed in the yeast *Yarrowia lipolytica* using the pINA1269 vector (Patent INRA/INA-PG), thus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

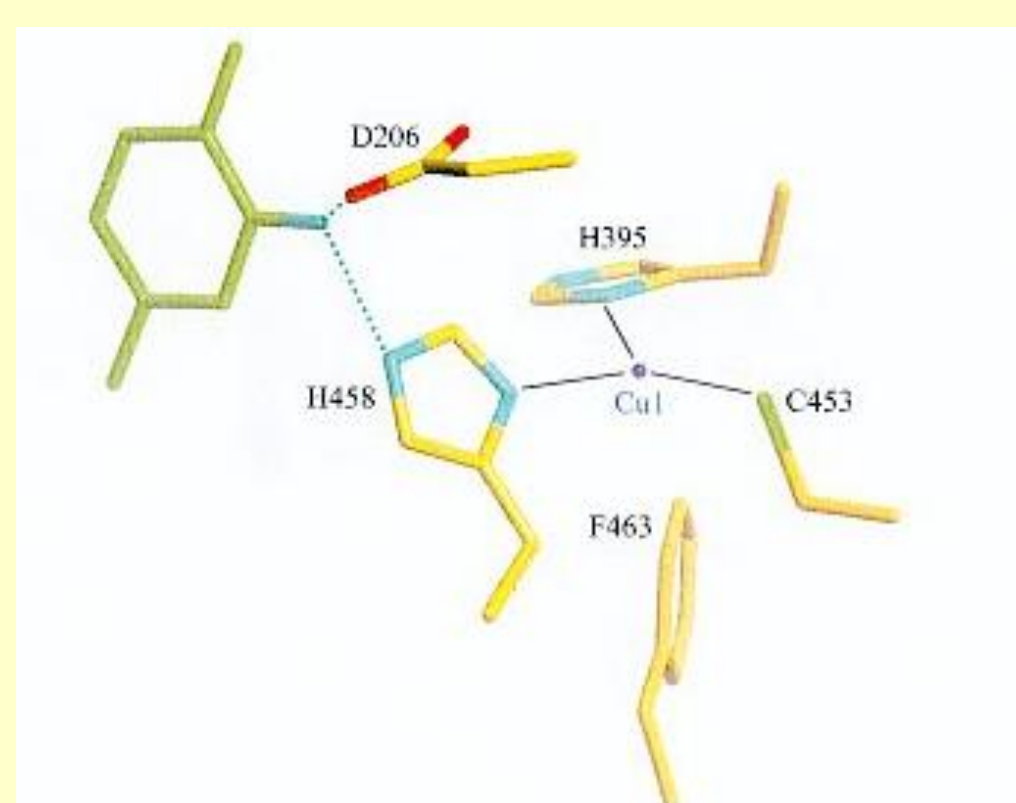
(atomic coordinates: pdb accession number 1KYA)

●, copper atoms; ● and O, glycosylation sites



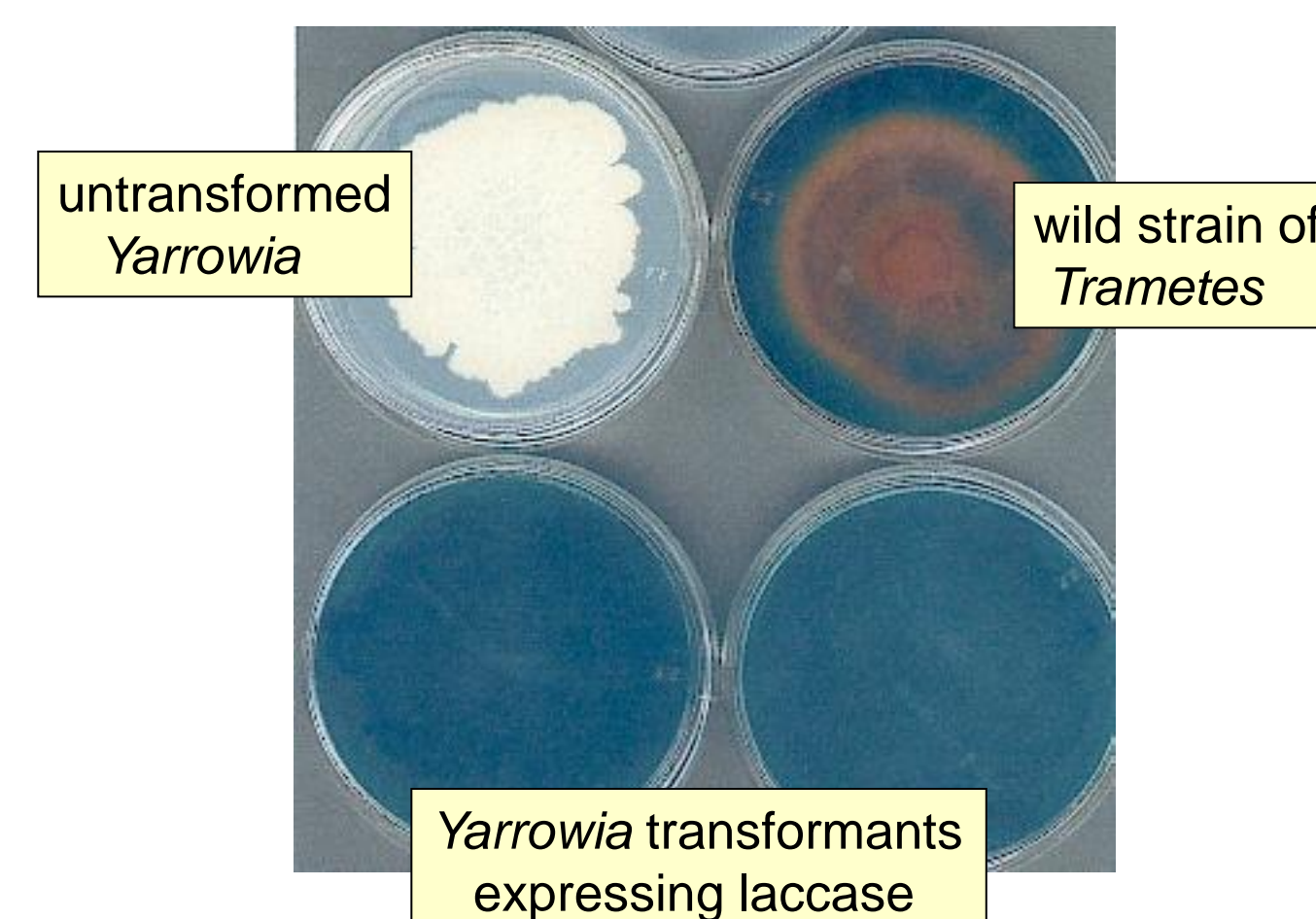
The crystal structure of the protein has been obtained with 2,5-xylydine bound in its active site

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



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

- 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



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						
Tversicolor	INGK	GRSPSTTTADLS	VISVT	PGKRYR	FLVSLSCDPNYT	FSIDGNNMTIIE	TD	SIN	APLVVDS	IQ	F	
Ccinereus	INGK	GRVGGPAAELSL	VNVVE	QGGKRYR	FLVSLSCDPNWT	FSIDGNNMTIIE	TD	SIN	APLVVDS	IQ	F	
Pcinnabarinus	INGL	GRITGTAPDLAVIK	VTQ	GKRYR	FLVSLSCDPNHT	FSIDGNNMTIIE	TD	SIN	APLVVDS	IQ	F	
Malbomyces	INGT	AVNPNTGEGQYAN	VVLT	TPGKRRLR	LLNTSTENHFOV	SLVNTMTIVIAAD	MVFPVNMATV	DS	SL	FLA		
Athaliana	INGP	GDLYNCSTKETTV	VVPL	INSGET	SLLRVINAAALNQ	PLFTVANAKLTIVV	GADASYLR	KPFTT	KVLM	IG		
Rvernificera	INGE	LDLYNCSTKETTV	RLSV	QPNKTYL	RLRVNAVLNEEK	FGIAKHTLTVVAO	DASYLR	KPINT	SYIM	IT		
Apeudoplatanus	INGP	GALFDCSTGTTFL	LPV	KKG	ETYLRL	RLINAILNEEMF	FGIAOENLRVVG	T	DGFY	TKPINT	EYIM	IT

- 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*; Ccinereus = *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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