Expression of laccase IIIb from the white-rot fungus *Trametes versicolor* in the yeast *Yarrowia lipolytica* for environmental applications

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CLAUDE JOLIVALT¹, CATHERINE MADZAK²,
AGATHE BRAULT³, ELIANE CAMINADE³, CHRISTIAN MALOSSE³, CHRISTIAN MOUGIN³

¹Laboratoire de Synthèse Sélective et Produits Naturels, UMR CNRS 7573, ENSCP, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05.

²Laboratoire de Génétique Moléculaire et Cellulaire, UMR INRA/INA-PG, 78850 Thiverval-Grignon.

³Unité de Phytopharmacie et Médiateurs Chimiques, INRA, route de Saint-Cyr, 78026 Versailles Cedex, France, email: claude-jolivald@enscp.jussieu.fr

INTRODUCTION

- Fungal laccases (EC 1.10.3.2) are involved in lignin transformation, morphogenesis, pathogenesis and fungal virulence, as well as in the oxidation of numerous xenobiotics [1-3]
- 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 [1,3]

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

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

RESULTS: Expression of LacIIIb by *Yarrowia lipolytica*

- The gene encoding LacIIIb 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 and oxidation of guaicol after electrophoretic separation). After tryptic digestion of the transformed *Y. lipolytica* extract, the mass spectrum contains the most significative ions detected and assigned to *T. versicolor* laccase peptides.

Laccase activity detection on agar plates assay. Numbers refer to different transformant clones of *Y. lipolytica* Po1g strain having received p69TVLB plasmid.

Electrophoresis analysis of recombinant laccase IIIb.
 [A] Samples were boiled before loading on the gel. Lane 1 - Laccase IIIb from *T. versicolor*. Lane 2 - Concentrated crude cell free extract from a transformant (clone 4) of *Yarrowia lipolytica* expressing laccase IIIb.
 [B] Samples were not boiled before loading on the gel. Lanes 3 and 6 - Laccase IIIb from *T. versicolor*. Lanes 4 and 7 - Concentrated crude cell free extract from a transformant (clone 4) of *Yarrowia lipolytica* expressing laccase IIIb. Lane 5 - Molecular weight markers.
 Lanes 1 to 5 were stained with Coomassie bleu (R250). Laccase activity was detected in lanes 6 and 7 by colouration with α -naph.

MALDI-TOF spectra
[A] Laccase IIIb from *T. versicolor*.
[B] Analysis of the band corresponding to the activity detected in electrophoresis pattern of crude supernatant from a p69TVLB-derived transformant (clone 4) of *Yarrowia lipolytica* expressing laccase IIIb.

APPLICATION: Towards the engineering of fungal laccases

1- 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

2 – Sequence alignment of laccases from fungi and higher plants

[illegible]

- 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: *Trametes versicolor*; *Cenocetes* = *Coprinus cernuus*; *Corticium cinnabarinus*; *Pycnoporus cinnabarinus*;
Melvinia = *Melvinocarpus albomyces*
 Higher plants: *Thalictrum flavum*; *Arabisopsis thaliana*; *Rumicortia* = *Rhus vernicifera*; *Apeudoplantus* = *Acer pseudoplatanus*

3- Strategy of directed mutagenesis

Three mutants have been obtained comprising:

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

- Their catalytic properties are currently studied.

References

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