Fungal laccases

*from structure-activity studies
to environmental applications*

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What are laccases ?

oxidases produced by plants (*Rhus vernicifera*)

insects (*Bombyx*)

bacteria (*Azospirillum lipoferum*)

fungi (*Trametes versicolor*)

extracellular glycoproteins containing copper

520 to 550 aa, 60 to 80 kD

high redox potential: 0,7 V

reducing substrate

Functions of fungal laccases

1 - natural

biodegradation of ligno-cellulosic materials
morphogenesis, pathogenesis....

2 - industrial

textile, dye and food industry
pulp paper bleaching

3 - environmental

biotransformation of xenobiotics (pollutants)

→ an increasing interest

Direct oxidation: phenols, amines

- oxidation of phenylurea herbicides

Jolivalt C., Raynal A., Caminade E., Kokel B., le Goffic F., Mougin C., Appl. Microbiol. Biotechnol, 1999, 51:676-681

- oxydative coupling of nonylphenol

Dubroca J., Brault A., Kollmann A., Touton I., Jolivalt C., Kerhoas L., Mougin C., Springer Book, 2003 (in press)

Indirect oxidation

→ a redox mediator is needed:

2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid): ABTS

- benzo[a]pyrene oxidation

Rama R., Mougin C., Boyer F.-D., Kollmann A., Malosse C., Sigoillot J.-C., Biotechnol. Letters, 1998, 20:1101-1104

Our objectives

biotransformation mechanisms, development of tools

1

bioremediation of
polluted media

2

assessment of
ecotoxicological impact

studies concerning fungal laccases

structure (mode of action) gene expression

Crystal structure of the laccase from *T. versicolor*

● , copper atoms
● ● , glycosylation sites

- the laccase is active and contains 4 copper atoms
- a reducing substrate is bound to the active site (presence of a cavity)

T1 site and neighbouring of 2,5-xylidine

Two aminoacids interact with the amino group of the xylidine:

- D206, an acidic residue responsible of pH impact on the transformation rate of xenobiotics?
- H458, an electron acceptor

Expression of the fungal laccase in the yeast *Yarrowia lipolytica*

- the gene encoding the laccase has been cloned and sequenced (Genbank AF414109), then expressed in *E. coli* and *Y. lipolytica*

- the recombinant enzyme is characterized by Maldi-TOF

Y. lipolytica T-
transformants
10

T. versicolor

4

8

16

Site-directed mutation in the *T. versicolor* laccase

wild enzyme

avantages

robust, extracellular
phenols, amines

activity

redox mediator
acidic pH

recombinant enzyme

expected benefits

conserved properties

wider substrate specificity

increased k_{cat}

increased E^0

neutral pH

2 - vector preparation

1 - insert design for modified T1 site

3 - ligation

4 - expression in *E. coli* → 3 mutants

5 - expression in *Y. lipolytica*

6 - protein analysis

A protein-grafted membrane to remove pollutants from waters

Laccase degradation efficiency

■ Removal efficiency of 2-HF in the filtrate increases with the amount of grafted laccases

Filtration module: Minitan S
120 cm² planar membranes grafted with 4.4, 10, 50 and 220 laccase activity units.
2-HF initial concentration 0.1 g/L in acetate buffer 0.05 M, pH 5
T=22°C.

Jolival C., Brenon S., Caminade E., Mougin C., Pontié M., J. Memb. Sci., 2000, 180:103-113.

Assessment of ecotoxicological impact on soil organisms

→ induction of fungal laccases by xenobiotics

treatment of 4-days cultures of *T. versicolor* by xenobiotics at 0.5 mM
measurement of laccase activity after 2 (●) and 3 (●) days

Mougin C., Kollmann A., Jolivalt C., Biotechnol. Letters, 2002, 24:139-142

Assessment of ecotoxicological impact on soil organisms

Towards fungal genomics for applied environmental microbiology?

- 1 - measurement of enzymatic activity: some problems
 - 2 - expression of gene encoding laccase
 - 3 - expression of genes encoding exoenzymes (Lac, MnP, LiP, CdH...)
 - 4 - expression of genes involved in metabolic pathways and cellular cycles
- transcriptomics (gene profiling, DNA microarray) proteomics

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Metabolic biochemistry and microbiology

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C. Malosse, L. Kerhoas, F.-D. Boyer, P.-H. Ducrot, B. Kokel,
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Reactor

M. Pontié