Fungal laccases: from structure-activity studies to environmental applications

Claude Jolivalt, Pierre Briozzo, Catherine Madzak, Christian Mougin

Fungal laccases

from structure-activity studies to environmental applications

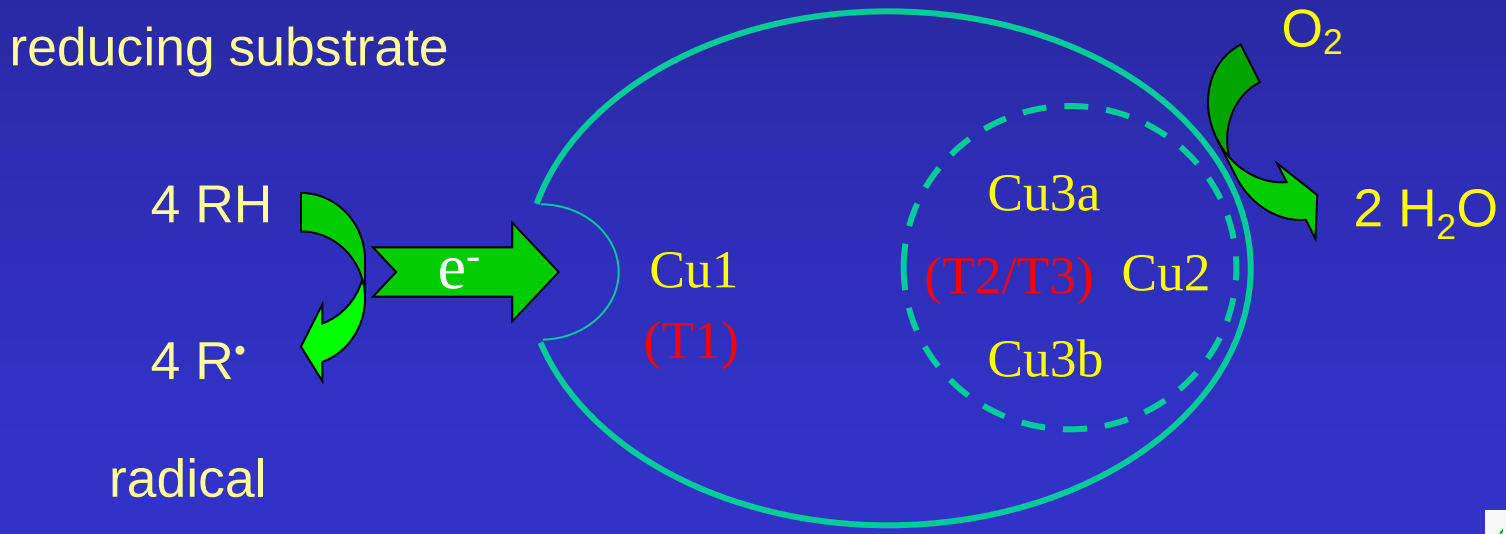
Claude Jolivalt, Pierre Briozzo, Catherine Madzak, Christian Mougin

INRA
Institut National de la Recherche Agronomique

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



Institut National de la Recherche Agronomique

Functions of fungal laccases

1 - natural

biodegradation of ligno-cellulosic materials
morphogenesis, pathogenesis….

2 - industrial

textile, dye and food industry
pulp paper bleaching

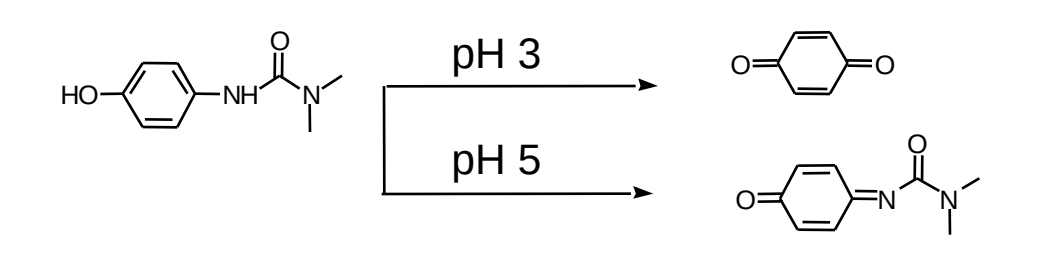
3 - environmental

biotransformation of xenobiotics (pollutants)

→ an increasing interest

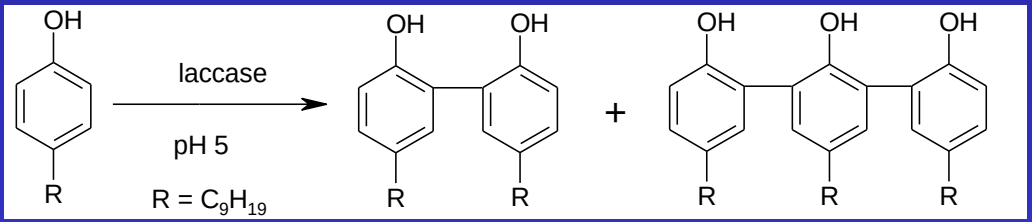
Direct oxidation: phenols, amines

- 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



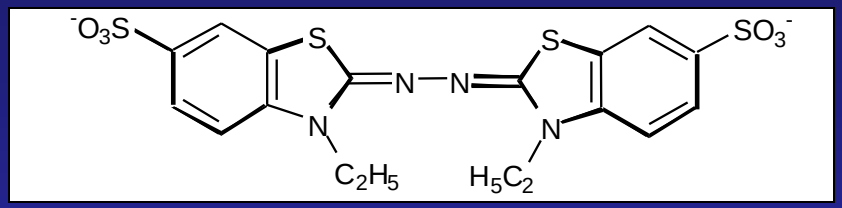
Dubroca J., Brault A., Kollmann A., Touton I., Jolivalt C., Kerhoas L., Mougin C., Springer Book, 2003 (in press)

INRA
Institut National de la Recherche Agronomique

Indirect oxidation

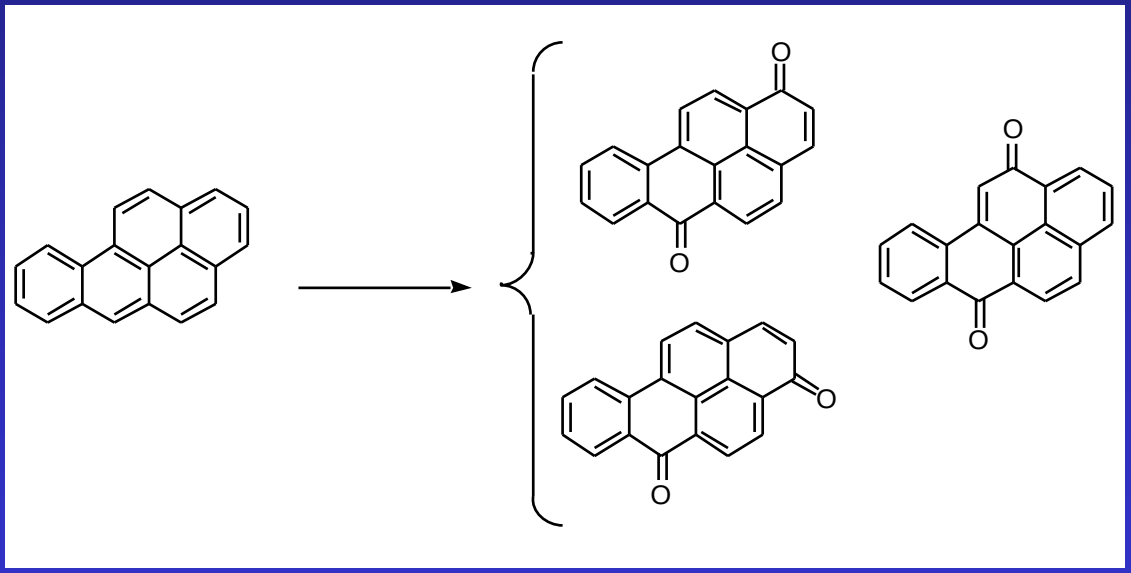
→ a redox mediator is needed: Lac + Med → Med*

Med* + RH → R_{ox} + Med



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

INRA
Institut National de la Recherche Agronomique

Our objectives

biotransformation mechanisms, development of tools

1

bioremediation of polluted media

2

assessment of ecotoxicological impact

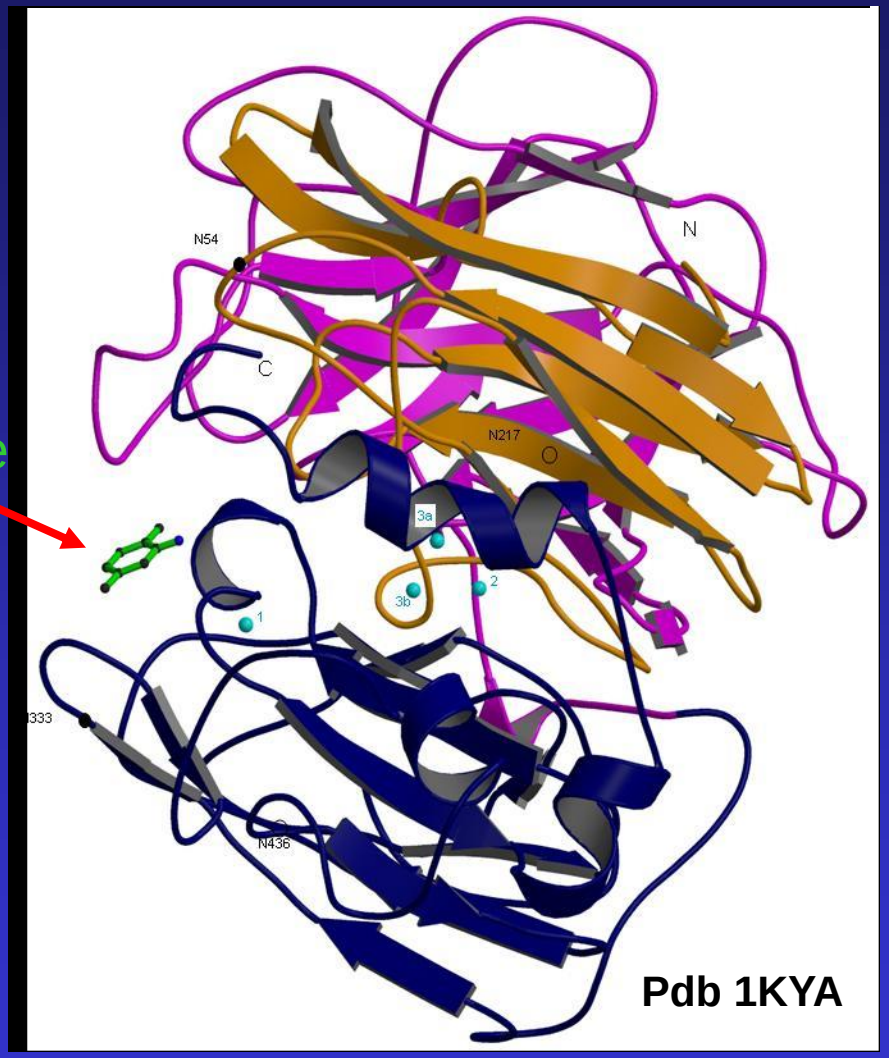
studies concerning fungal laccases

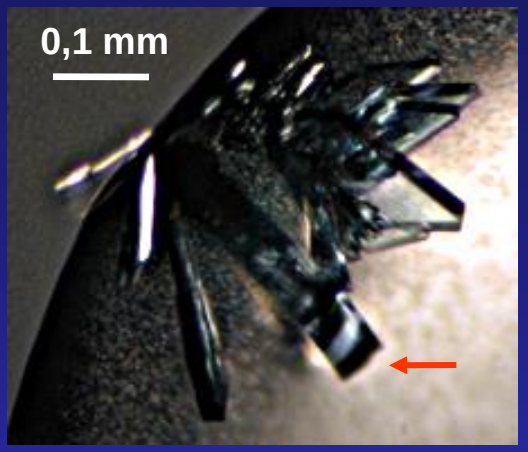
structure (mode of action) gene expression

Institut National de la Recherche Agronomique

Crystal structure of the laccase from *T. versicolor*

xylidine





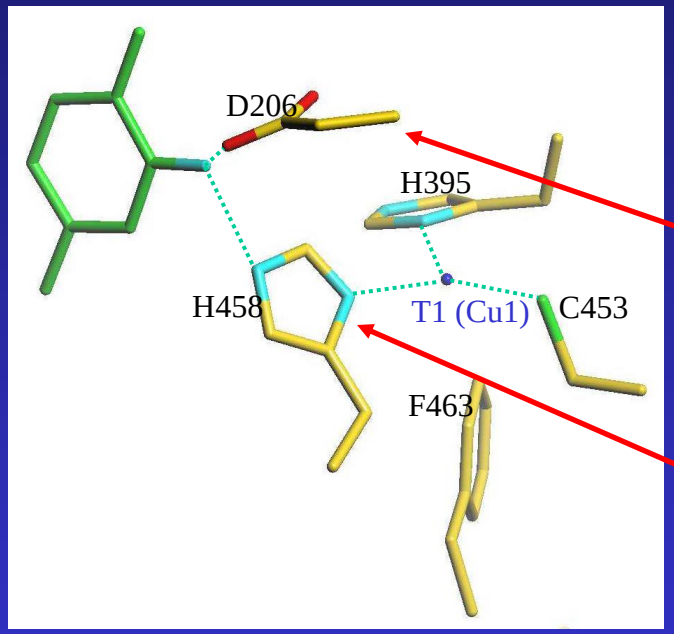
● , copper atoms

● ● , glycosylation sites

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

Bertrand T., Jolivalt C., Briozzo P., Madzak C., Joly N., Caminade E., Mougin C., Biochemistry, 2002, 41:7325-7333.

T1 site and neighbouring of 2,5-xylidine



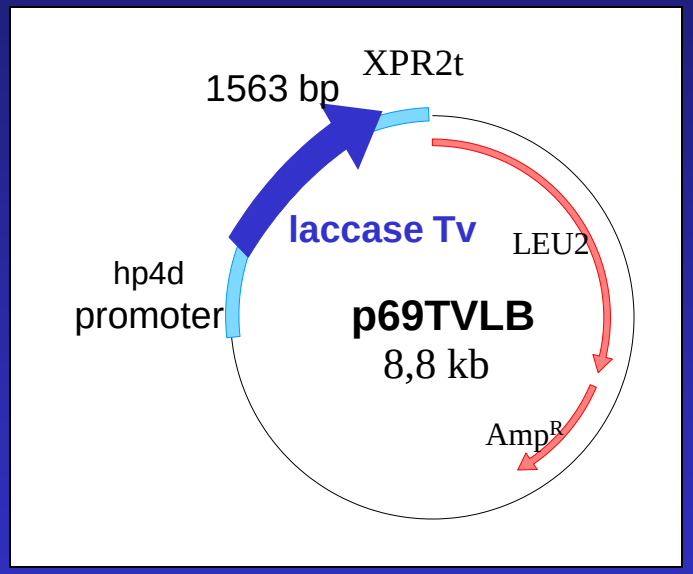
Two aminoacids interact with the amino group of the xylidine:

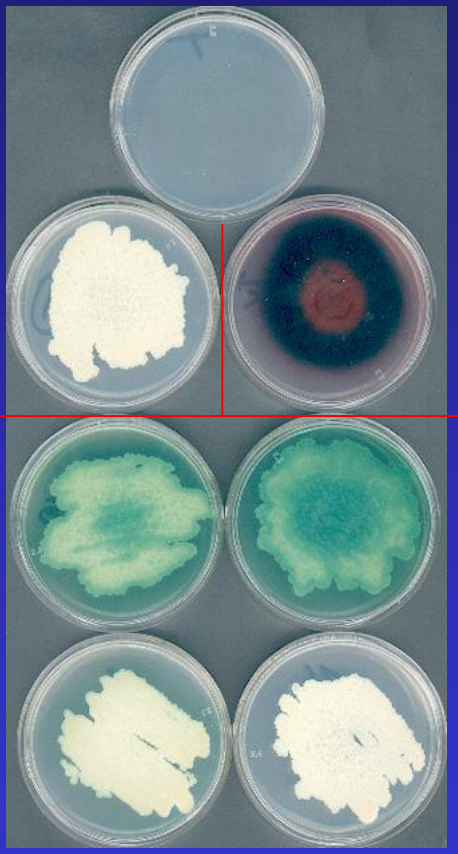
- D206, an acidic residue responsible of pH impact on the transformation rate of xenobiotics?
- H458, an electron acceptor

INRA
Institut National de la Recherche Agronomique

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*





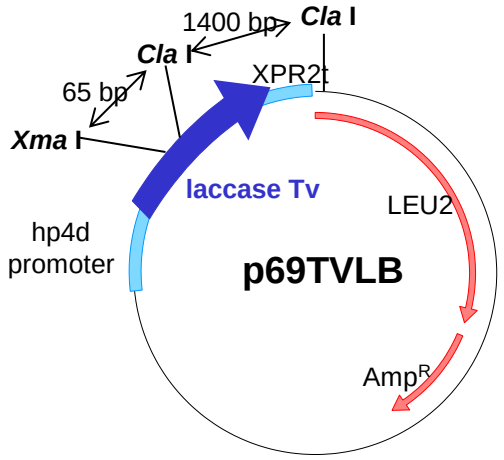
- the recombinant enzyme is characterized by Maldi-TOF

INRA
Institut National de la Recherche Agronomique

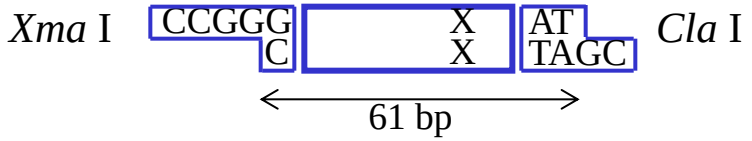
Site-directed mutation in the *T. versicolor* laccase

wild enzyme		recombinant enzyme
avantages	limits	expected benefits
robust, extracellular		conserved properties
phenols, amines		wider substrate specifity
activity		increased k_{cat}
redox mediator		increased E^o
acidic pH		neutral pH

2 - vector preparation



1 - insert design for modified T1 site



3 - ligation

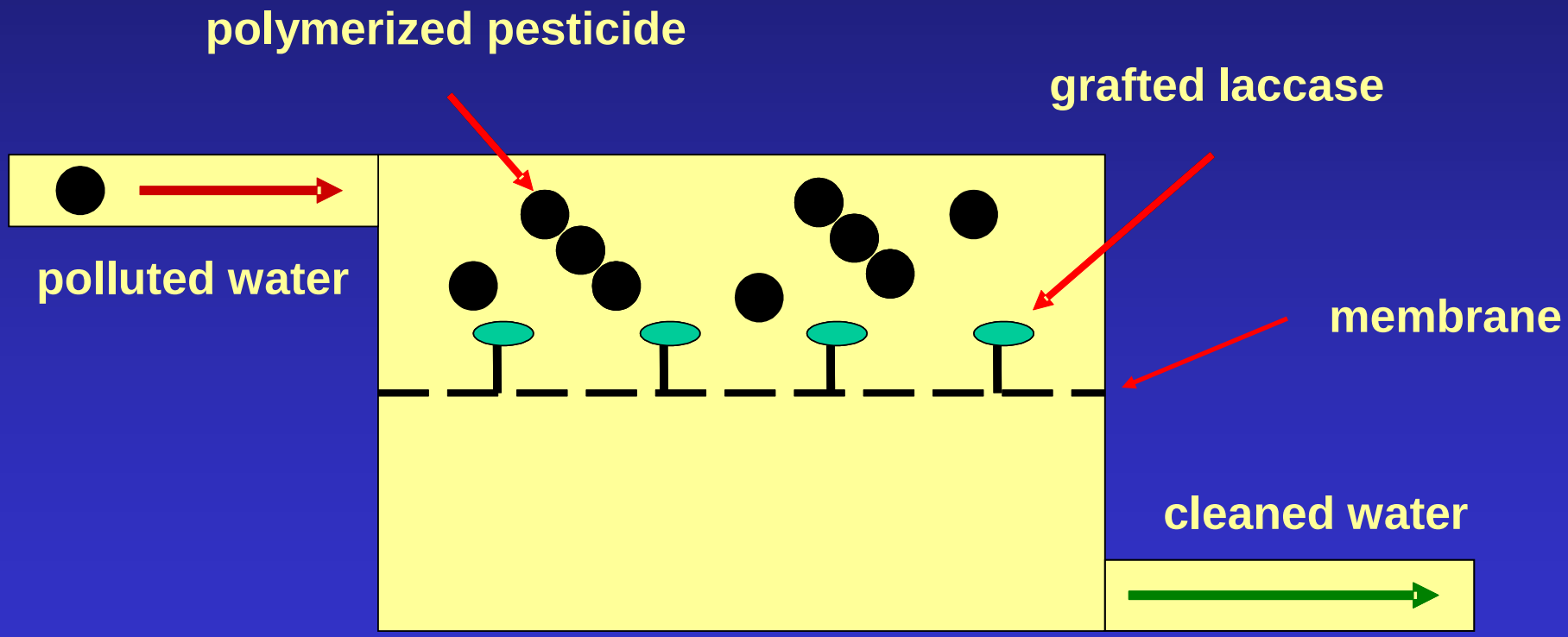
4 - expression in *E. coli* → 3 mutants

5 - expression in *Y. lipolytica*

6 - protein analysis

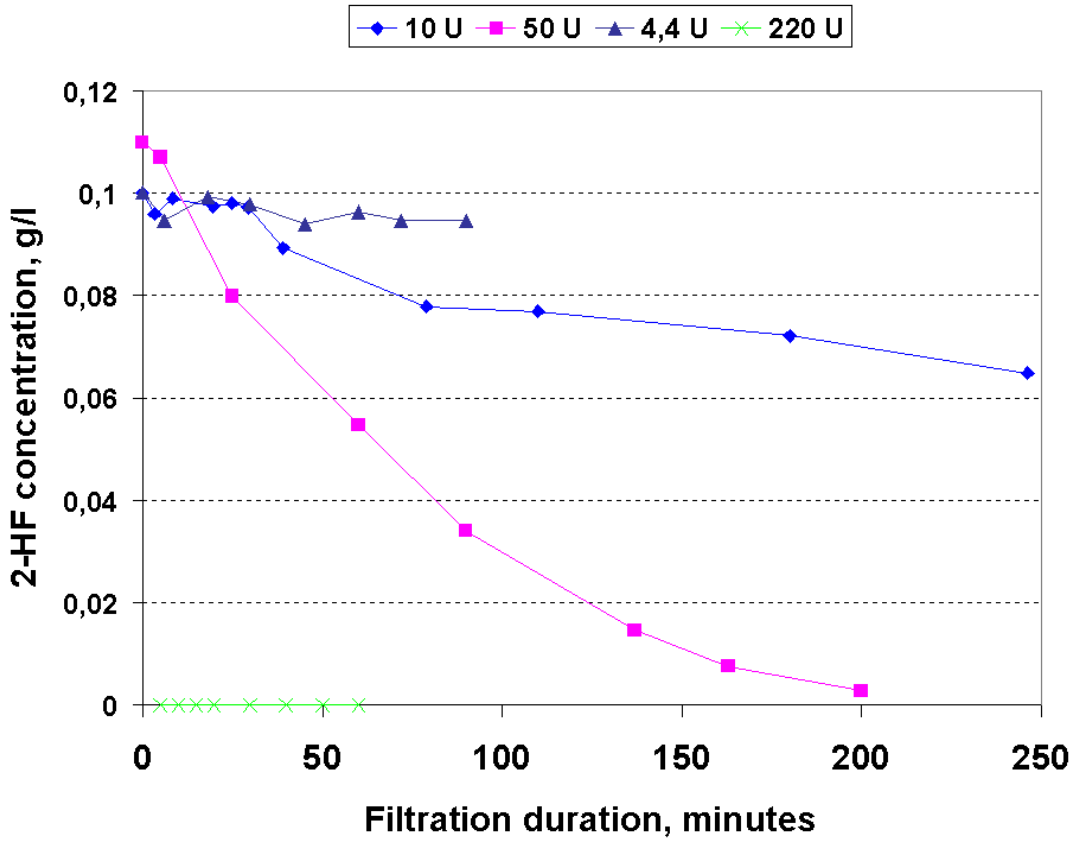
INRA
Institut National de la Recherche Agronomique

A protein-grafted membrane to remove pollutants from waters



INRA
Institut National de la Recherche Agronomique

Laccase degradation efficiency



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

Filtration module: Minitan S 120 cm^2 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.

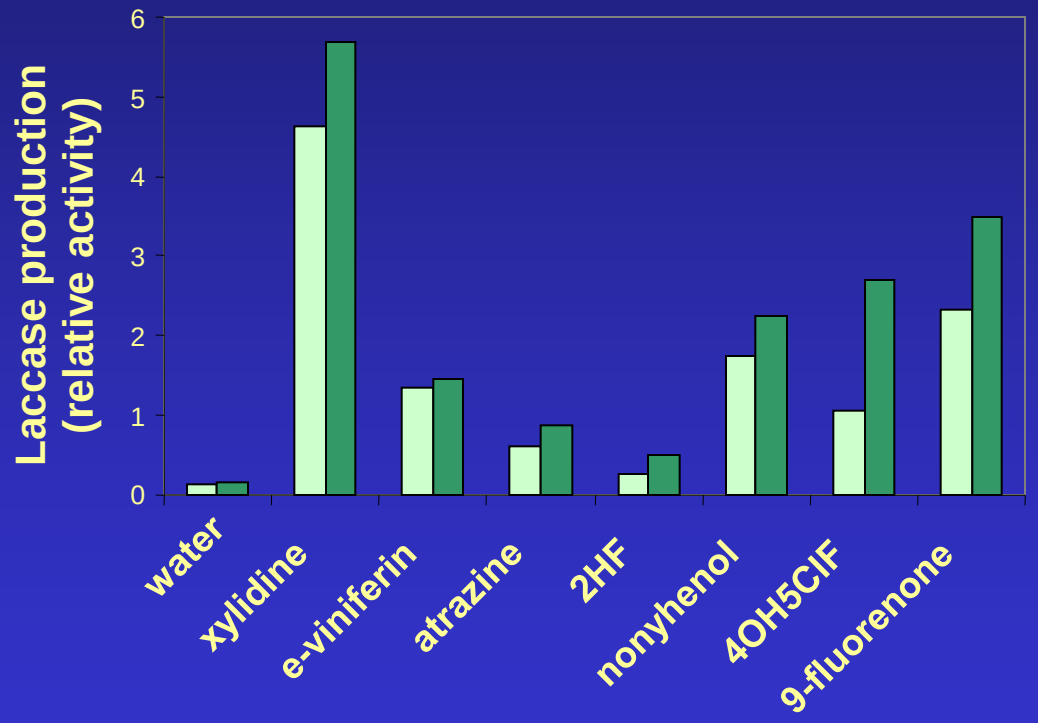
Jolivalt C., Brenon S., Caminade E., Mougin C., Pontié M., J. Memb. Sci., 2000, 180:103-113.

INRA
Institut National de la Recherche Agronomique

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

INRA
Institut National de la Recherche Agronomique

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

Acknowledgments

V. Chaplain

Metabolic biochemistry and microbiology
C. Jolivalt, E. Caminade, A. Kollmann, A. Brault, I. Touton

Structural biochemistry and molecular biology
P. Briozzo, N. Joly, T. Bertrand, C. Madzak

Organic chemistry and mass spectrometry
C. Malosse, L. Kerhoas, F.-D. Boyer, P.-H. Ducrot, B. Kokel, J.-P. le Caer

Reactor
M. Pontié