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L'ingénierie des laccases fongiques et ses applications environnementales

Christian Mougin, Claude Jolival, Pierre Briozzo, Catherine Madzak

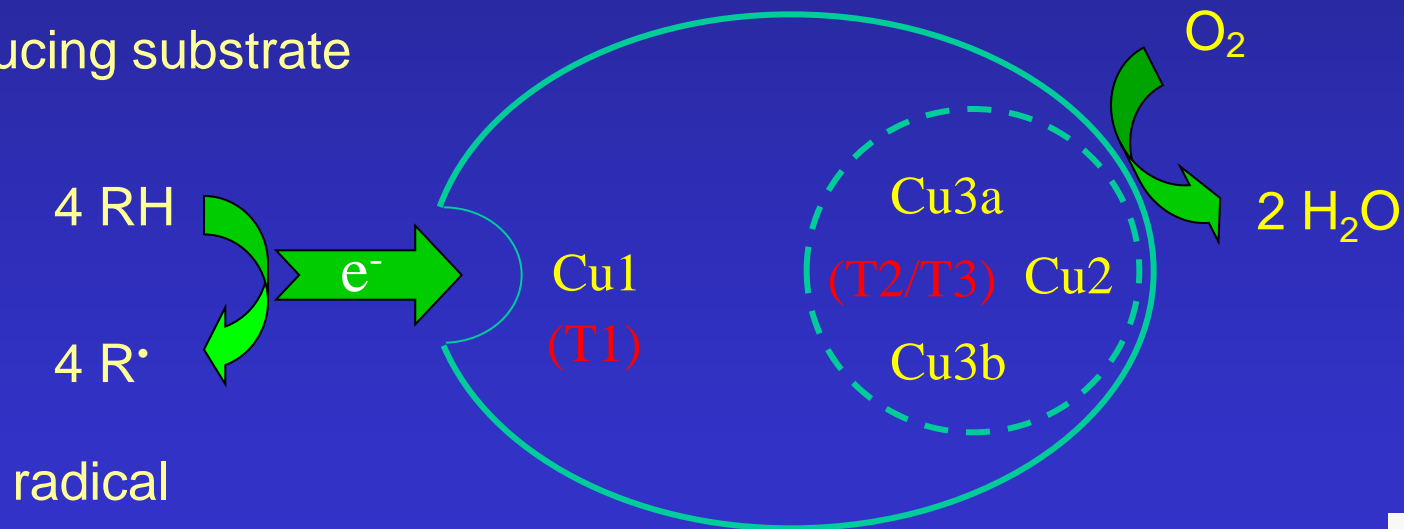
What are laccases ?

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

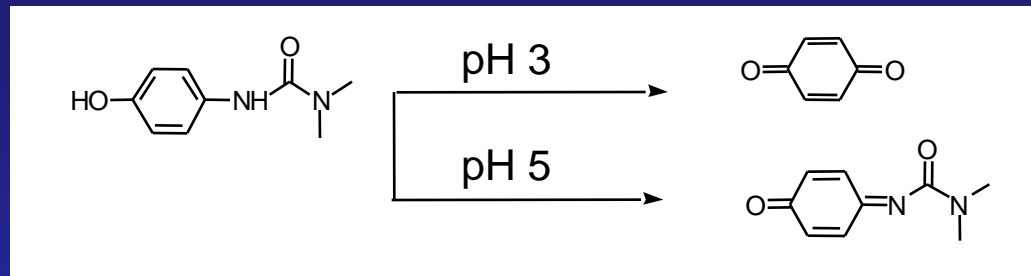
3 - environmental

biotransformation of xenobiotics (pollutants)

→ an increasing interest

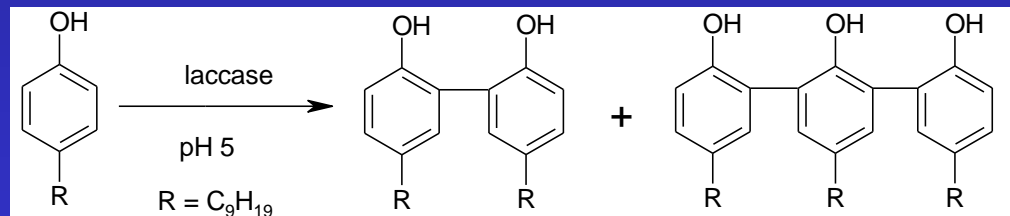
Direct oxidation: phenols, amines

- oxidation of phenylurea herbicides



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

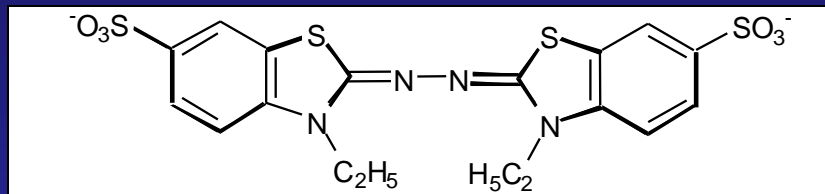
- oxydative coupling of nonylphenol



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

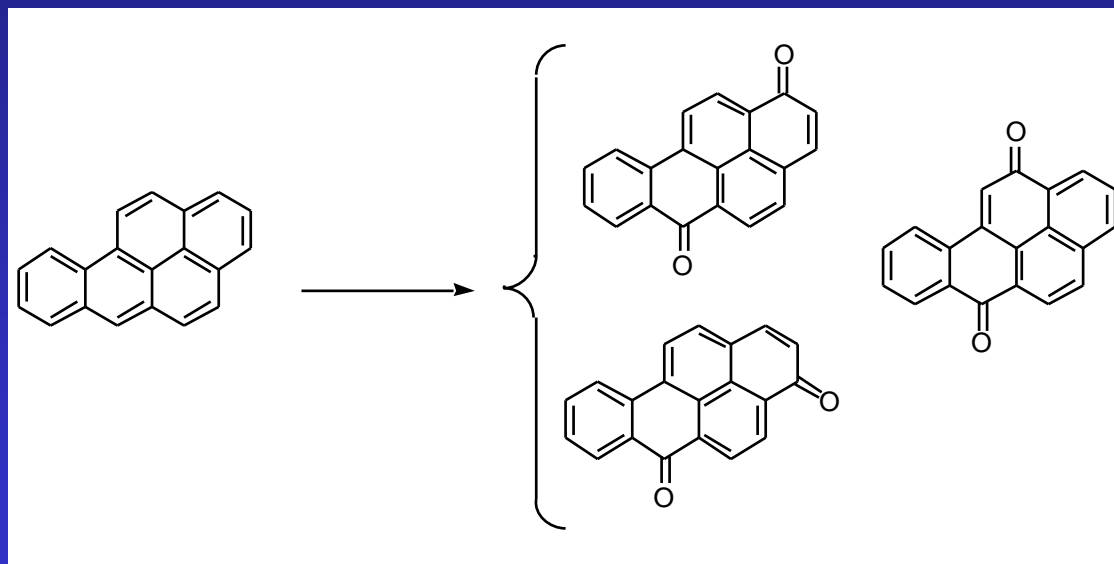
Indirect oxidation

→ 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

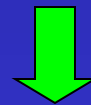
bioremediation of
polluted media

2

assessment of
ecotoxicological impact



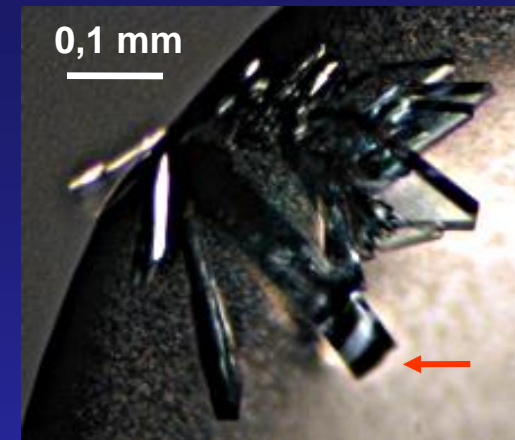
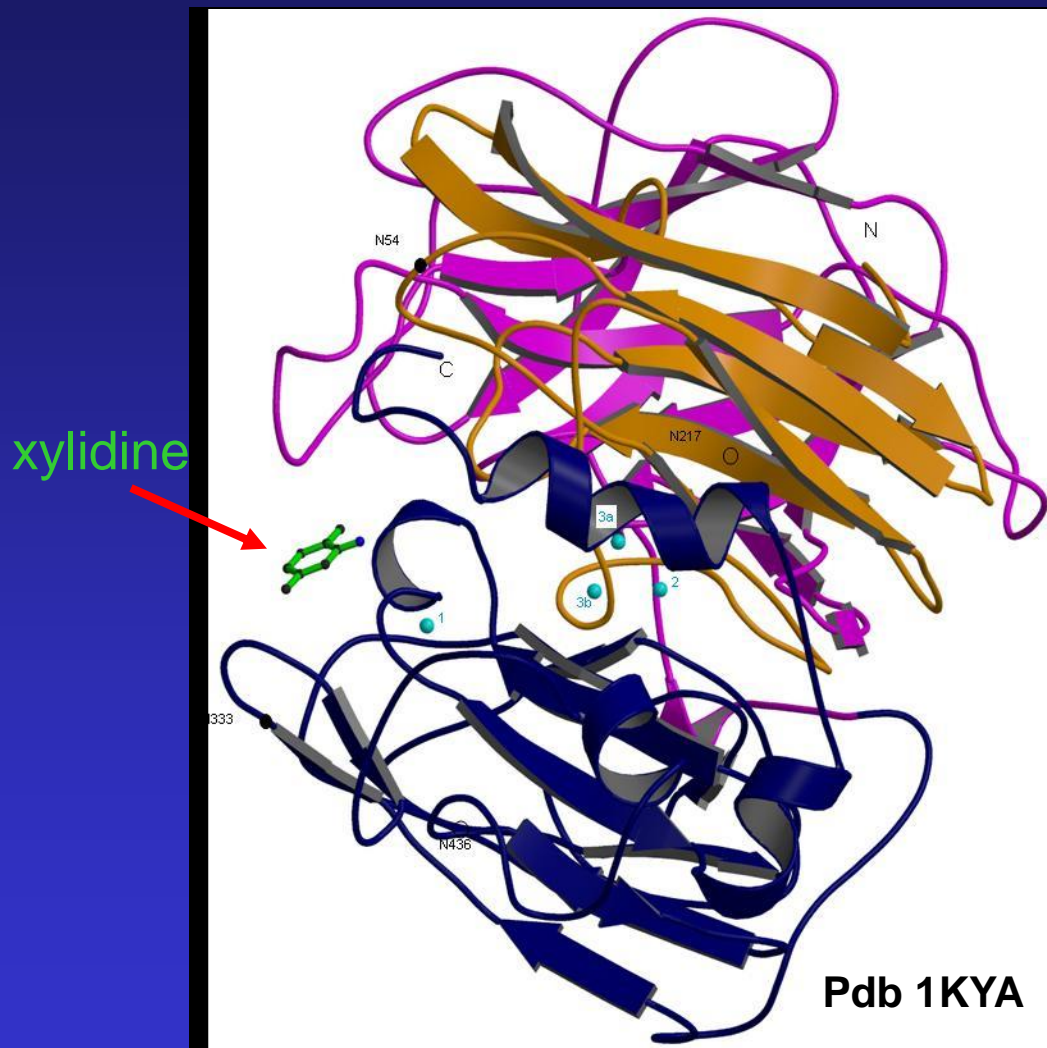
studies concerning fungal laccases



structure (mode of action) gene expression



Crystal structure of the laccase from *T. versicolor*

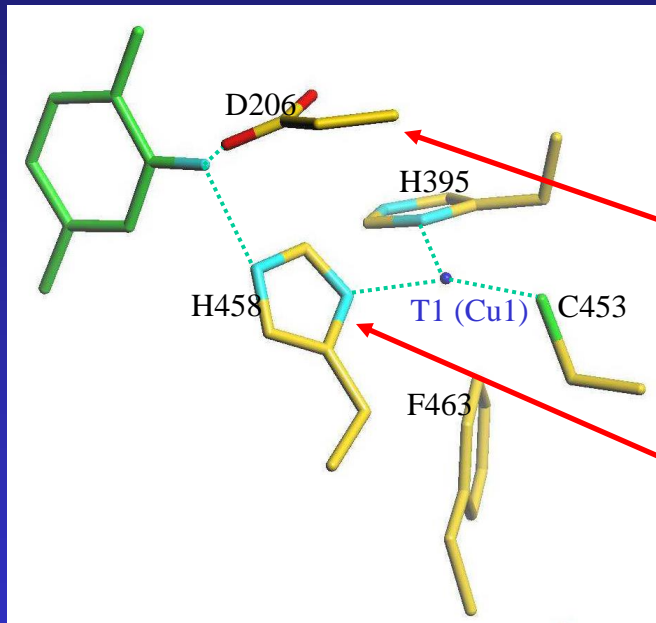


● , copper atoms
● ● , glycosylation sites

- 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

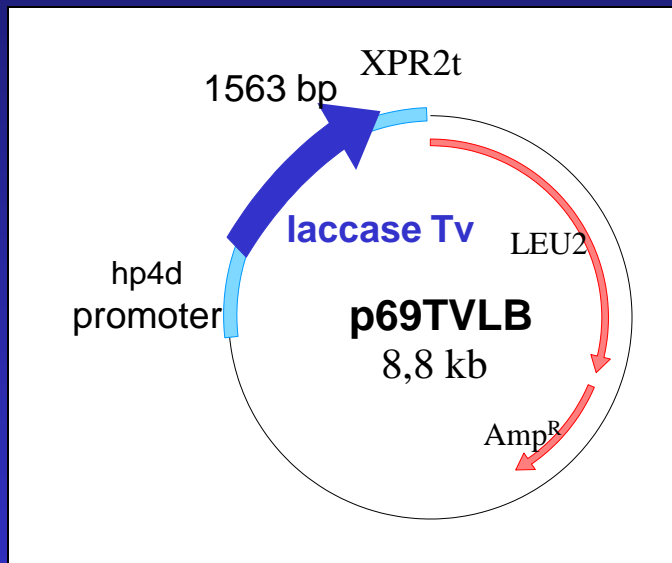


Two aminoacids interact with the amino group of the xylidine:

- 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*



Y. lipolytica T-
transformants

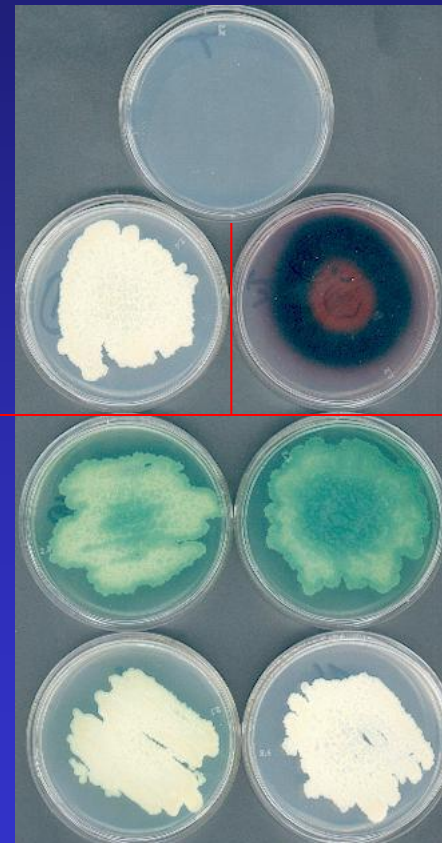
10

8

T. versicolor

4

16



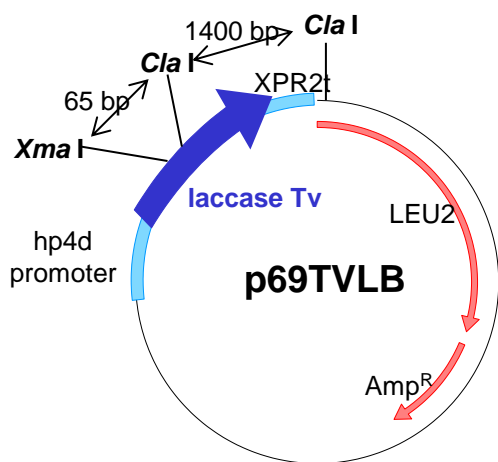
- the recombinant enzyme is characterized by Maldi-TOF

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

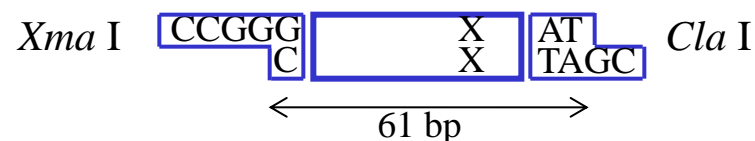
wild enzyme
avantages
 robust, extracellular
 phenols, amines
 activity
 redox mediator
 acidic pH

recombinant enzyme
expected benefits
 conserved properties
 wider substrate specificity
 increased K_m and k_{cat}
 increased E°
 neutral pH

2 - vector preparation



1 - insert design for modified T1 site



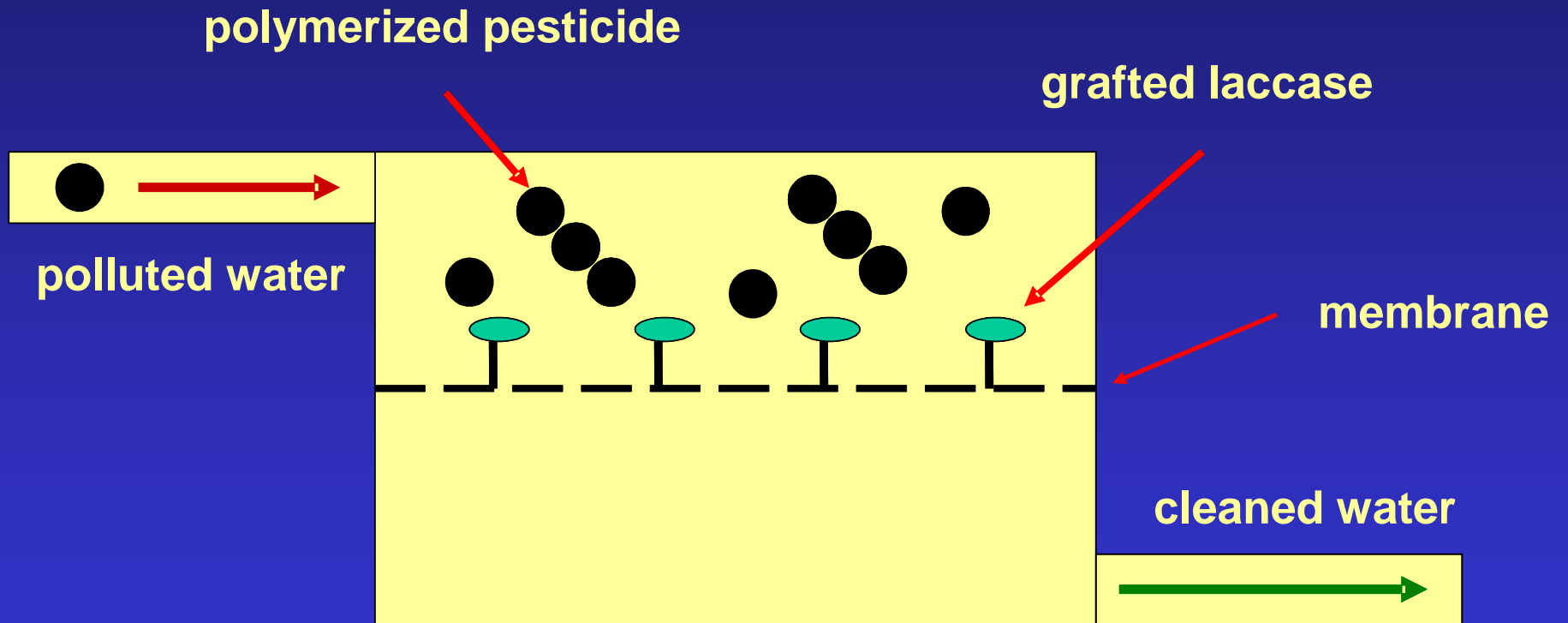
3 - ligation

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

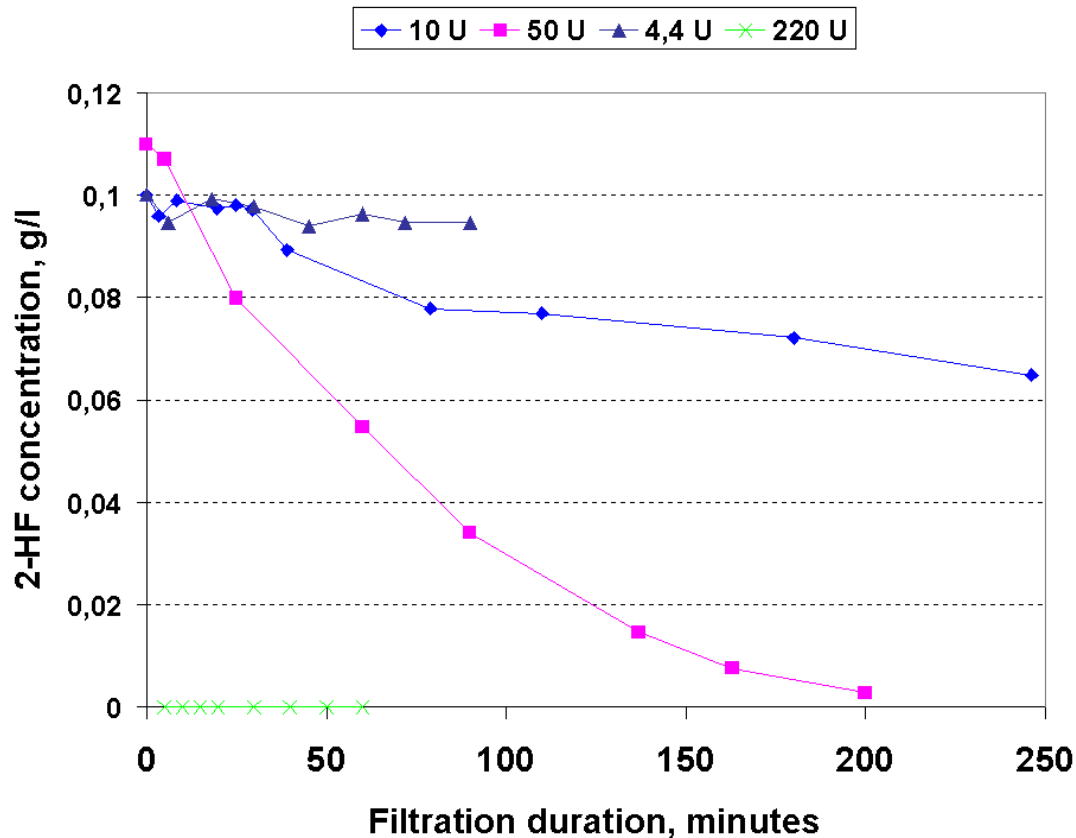
5 - expression in *Y. lipolytica*

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

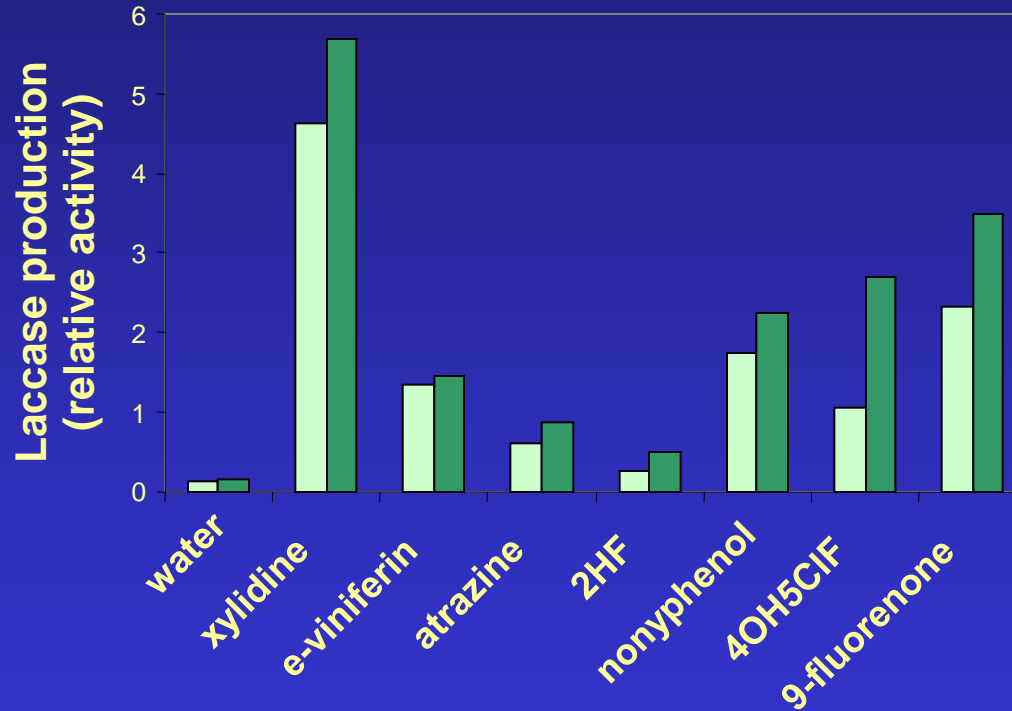
Filtration module: Minitan S
120 cm² planar membranes grafted with 4.4, 10, 50 and 220 laccase activity units.
2-HF initial concentration 0.1 g/L in acetate buffer 0.05 M, pH 5
T=22°C.

Jolivalt 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., Jolival 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

Acknowledgments

V. Chaplain

Metabolic biochemistry and microbiology

C. Jolival, 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

