Laccases and their implication in lignification, an in vitro mechanistic study

Betty Cottyn Boitte, Davy Baratiny, Amel Majira, Catherine Lapierre, Lise Jouanin, Nathalie Demont-Caulet, Paul Henri Ducrot, Lise Jouanin, Nathalie Demont-Caulet, Nathalie Demont-Caulet

LACCASES AND THEIR IMPLICATION IN LIGNIFICATION, AN *IN VITRO* MECHANISTIC STUDY

B. Cottyn, D. Baratiny, A. Majira, S. Boutet-Mercey, A. Maia-Grondard, C. Lapierre, L. Jouanin, N. Demont-Caulet, P.-H. Ducrot
[1] *IJPB, UMR1318 Inra/AgroParisTech, Inra, Route de Saint-Cyr 78026 Versailles Cedex, France*
betty.cottyn@versailles.inra.fr

INTRODUCTION

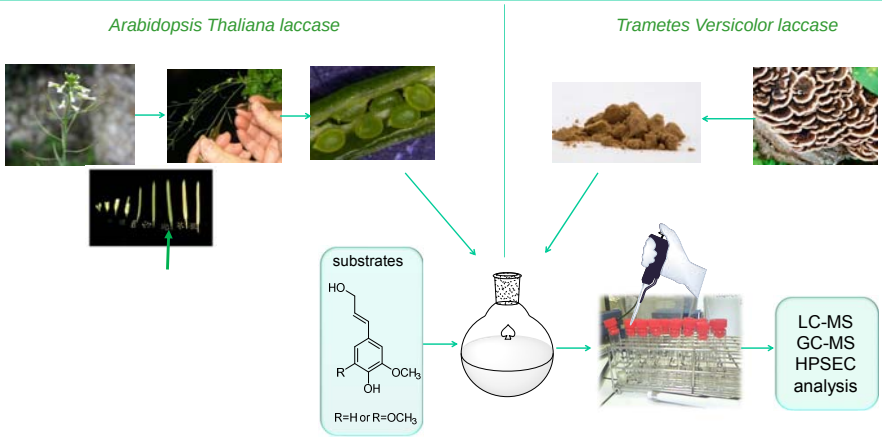
The involvement of plant laccases in lignin formation has been unambiguously established with *Arabidopsis* mutants in which the disruption of the *At*LAC4 and *At*LAC17 genes resulted in altered lignification.[1] By contrast, the laccase-driven polymerization of lignin precursors is still unclear.

EXPERIMENTAL

We monitored the reaction kinetics of coniferyl and sinapyl alcohols in the presence of two distinct laccases. We selected *At*LAC15 (a plant representative reported to be involved in flavonoid oxidation and in lignin polymerization[2]) and the commercially available *Trametes versicolor* laccase (as a representative of high-redox potential fungal laccases studied for their ability to catalyse lignin degradation[3]). The objective of this work was to evaluate laccase substrate specificity, if any, and the impact of laccase type and concentration on the bonding modes of lignin precursors. To this end, we monitored the initial steps of the enzymatically-driven oxidation of lignin precursors.

Microscale *in vitro* oxidation experiments of coniferyl or sinapyl alcohol were carried out with *At*LAC15 rough extracts obtained from transparent testa *tt4* mutant seeds affected in flavonoid synthesis[4] or with the commercially available *Trametes versicolor* laccase.

The reaction was monitored by HPLC combined with electrospray mass spectrometry as well as by high performance size exclusion chromatography of the oligomeric species.

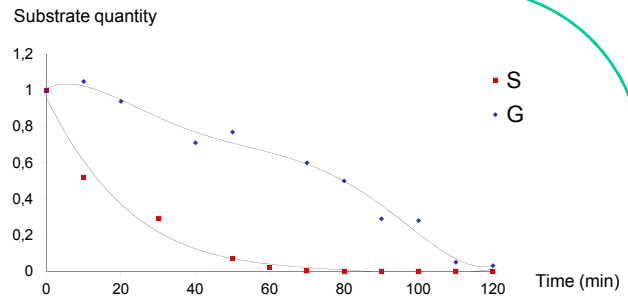


Microscale in vitro oxidation experiments

RESULTS & DISCUSSION

First of all, both laccases induced the rapid disappearance of coniferyl or sinapyl alcohol. A major result was the ability of these enzymes to directly oxidize sinapyl alcohol more rapidly than coniferyl alcohol, contrary to peroxidase-driven oxidation.[5]

Both laccases have shown very similar activities, allowing the formation of oxidative oligomers up to a polymerization degree of 6 at least for a reaction time of 120 minutes. **No specificity is observed.**

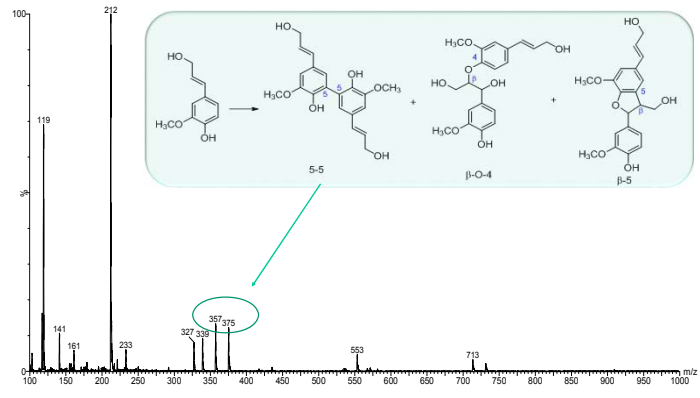


Consumption of substrates by Trametes Versicolor laccase

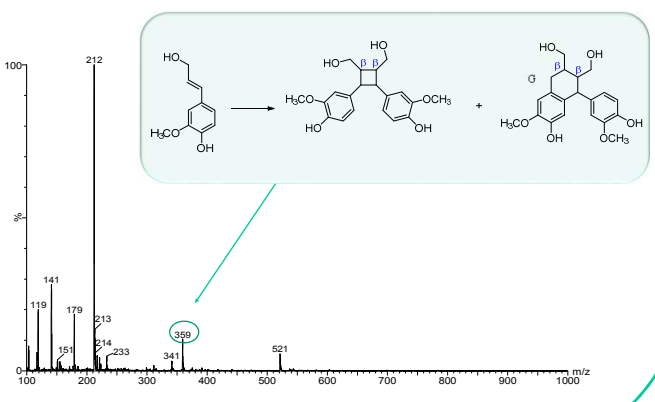
Whatever the enzyme and provided that laccase activity was in sufficient amount (1 laccase unit per 0.55 µmol substrate for *T. versicolor* laccase) the coniferyl alcohol-derived dimers were mainly the β-O-4, phenylcoumaran and pinoresinol dimers while sinapyl alcohol essentially provided syringaresinol. Oligomers up to a 6 polymerization degree could be further obtained within 2-hours.

By contrast, when laccase activity was provided in 100-fold lower level, the dimerization of coniferyl or sinapyl alcohol led to unusual dimers assigned to aryltetraline-type and cyclobutane-type structures. Oligomers up to a 5 polymerization degree could be obtained within 2-hours, giving access this time to organized structures, containing the same repetition units.

High laccase concentration



Low laccase concentration



Global mass spectra obtained with Trametes Versicolor laccase

CONCLUSIONS

Our work demonstrates that laccases may participate in a different way than peroxidases in the first steps of lignin polymerization. The absence of selectivity between a laccase of plant and a laccase from fungus allows us to advocate *Trametes versicolor* laccase as a good model enzyme to study *in vitro* lignification and to obtain a descriptive model of lignin formation.

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