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Engineering and production of laccase from *Trametes versicolor* in the yeast *Yarrowia lipolytica*

Monnat Theerachat [a,b,c], Stéphane Emond [d], Emmanuelle Cambon [e,f,g], Florence Bordes [e,f,g], Alain Marty [e,f,g], Jean-Marc Nicaud [h], Warawut Chulalaksananukul [b,c], David Guieysse [e,f,g], Magali Remaud-Siméon [e,f,g], Sandrine Morel [e,f,g,*]

[a] *Biological Science Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[b] *Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[c] *Biofuels by Biocatalysts Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[d] *Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, UK*
[e] *Université de Toulouse, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France*
[f] *CNRS, UMR5504, F-31400 Toulouse, France*
[g] *INRA, UMR792, Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France*
[h] *LCNRS, MICALIS, F-78350 Jouy-en-Josas, France*

HIGHLIGHTS

- A laccase gene was cloned in multiple integrations into the genome of *Yarrowia lipolytica*.
- Directed evolution enables to isolate one variant with fourfold activity improvement.
- Laccase variant catalyzes an almost complete decolorization of an amaranth solution.

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ABSTRACT

The *lcc1* gene coding for the laccase from *Trametes versicolor* DSM11269 was cloned into the genome of *Yarrowia lipolytica* using either single or multiple integration sites. The levels of the recombinant laccase activity secreted in the culture media were 0.25 and 1 U ml^{-1} for single and multiple integrations, respectively. The strain with a single integration was successfully used to express variant libraries which were screened on ABTS substrate. The strain encoding the double mutant L185P/Q214K (rM4A) showed a sixfold enhancement in secreted enzyme activity. The catalytic efficiency of the purified rM-4A laccase was respectively increased 2.4- and 2.8-fold towards ABTS and 2,6-dimethoxyphenol, compared to the rWT. Culture supernatants containing either rWT or rM-4A catalyzed the almost complete decolorization of an Amaranth solution (70 nM s^{-1}). Taken together, our results open new perspectives for the use of *Y. lipolytica* as a molecular evolution platform to engineer laccases with improved properties.

1. Introduction

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2), belong to the multi-copper oxidase family, and are widely distributed in fungi, higher plants and bacteria. They catalyze the oxidation of a wide variety of compounds, including mono-, di-, polyphenols, aminophenols, and methoxyphenols with the concomitant reduction of molecular oxygen to water (Morozova et al., 2007; Sirim et al., 2011).

Laccases are also able to oxidize non-phenolic compounds having higher redox potentials, in the presence of small molecules acting as redox mediators such as acetosyringone, a dimethoxy substituted phenol derived from syringyl lignin units (Riva, 2006; Camarero et al., 2005). The laccase mediator system (LMS) is of particular interest in industrial applications such as the biodegradation of xenobiotics, detoxification of industrial waste water and pollutants, textile dye decolorization, pulp delignification and bleaching, modification of the color appearance of food and beverages, organic synthesis, and the construction of biosensors and biofuel cells (Riva, 2006; Morozova et al., 2007; Zeng et al., 2011; Fernández-Fernández et al., 2012). The high redox potential laccase (~780 mV) produced by *Trametes versicolor* is one of the most studied in this enzyme family.

* Corresponding author at: Université de Toulouse, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France. Tel.: +33 (0) 561559491; fax: +33 (0) 561559400.
E-mail address: sandrine.morel@insa-toulouse.fr (S. Morel).

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