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Pycnoporus species as new biotechnological agents for the production of laccase and tyrosinase of biotechnological interest.

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Among microorganisms that degrade the lignocellulosic compounds of wood, only white-rot filamentous fungi, such as *Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes versicolor* and *Pycnoporus cinnabarinus* mineralize lignin to CO_2 and H_2O. The genus *Pycnoporus* was already studied for its ability to produce natural aroma such as vanillin or benzaldehyde (bitter almond aroma). In this study, we showed that it is particularly suitable for the production of phenoloxidases of biotechnological interest: laccase and tyrosinase. Laccases (p-diphenol oxygen oxido-reductases, EC 1.10.3.2) are blue multi-copper enzymes that catalyze the reduction of molecular oxygen into water, coupled to one-electron oxidation of a wide range of phenolic substrates. Laccases have numerous biotechnological applications including pulp biobleaching and waste water treatment in the paper industry, synthesis of polymers by reticulation of phenolic compounds and soil bioremediation. Tyrosinases (monophenol, o-diphenol:oxygen oxidoreductases, EC 1.14.18.1) are copper-containing enzymes that catalyse two different reactions: the ortho-hydroxylation of monophenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity), both using molecular oxygen. Tyrosinases can have numerous biotechnological applications including waste water treatment with phenol removal, synthesis of high-value o-diphenolic drugs, healthy antioxidant molecules, and formation of biopolymers by cross-linking of polysaccharide-protein or protein-protein. The practical application of laccase or tyrosinase in biotechnology requires large quantities of enzyme. Therefore, we screened various Pycnoporus strains, originated from various geographical areas (particularly from tropical Chinese regions), as potential overproducers of laccase and tyrosinase. Chinese white-rot fungal isolates, from tropical environments, were identified as a source of high laccase-producers. The strain P. sanguineus BRFM 49 showed the highest tyrosinase productivity (45.4 and 163.6 U g proteins-1 day-1 for monophenolase and diphenolase, respectively) and was subsequently chosen for tyrosinase purification. The **P. sanguineus** BRFM 49 tyrosinase was biochemically characterized. The N-terminal amino acid sequence was determined as IVTGPVGGQTEGAPAPNRLEIN. This tyrosinase was shown to be effective for the bioconversion of p-tyrosol and p-coumaric acid into the antioxidants p-hydroxytyrosol and caffeic acid, respectively, and it could also catalyse the cross-linking formation of a model protein.