MANUSCRIPT OF THE THESIS

Prospecting for the production of a new tyrosinase of biotechnological interest in the genus *Pycnoporus*: from gene expression to applications.

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The purpose of this work was to explore the potentialities of the genus *Pycnoporus* to synthesize a new tyrosinase of biotechnological interest. Firstly, the screening of twenty *P. cinnabarinus* and *P. sanguineus* strains showed, for the first time, tyrosinase activities in the genus *Pycnoporus*. The strain *P. sanguineus* BRFM49 was identified as the best tyrosinase producer and was selected to purify and to characterize the enzyme. This new tyrosinase has some of the general properties of the other fungal tyrosinases: cytosolic localization, neutral optimum pH, existence of several isoforms, absence of N-glycosylation. However, its kinetic and molecular parameters, as well as its amino-acid sequence, are different. The *P. sanguineus* tyrosinase seems also very promising for biotechnological applications such as the synthesis of anti-oxidant molecules and protein cross-linking. Afterwards, two complementary strategies have been exploited in order to improve tyrosinase-production yields. The first strategy, consisting in the selection, by formal genetics, of genetically-stable monokaryons, did not permit a significant increase of tyrosinase activity in P. sanguineus BRFM49. The second strategy consisted in the heterologous expression of tyrosinase. In this context, the tyrosinase gene (2,204 bp) and the corresponding cDNA (1,857 bp) were cloned and characterized. The heterologous production of the *P. sanguineus* tyrosinase was performed in Aspergillus niger, a fungus known for its ability to secrete large amounts of proteins. The tyrosinase cDNA was fused to the preprosequence of A. niger glucoamylase and placed under the control of the strong and constitutive gpdA promoter. For the first time, this construction permitted the production of an active fungal recombinant tyrosinase in the extracellular medium. The transformant A. niger D15#26e, showing the highest yield (50 mg/L), was selected for the purification and the characterization of the recombinant enzyme, which was then compared to the native tyrosinase. As the native enzyme, the recombinant tyrosinase is efficient for protein cross-linking.