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Cloning and characterization of a tyrosinase gene from the white-rot fungus *Pycnoporus sanguineus*, and overproduction of the recombinant protein in *Aspergillus niger*.

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A new tyrosinase-encoding gene (2,204 bp) and the corresponding cDNA (1,857 nucleotides) from the white-rot fungus *Pycnoporus sanguineus* BRFM49 were cloned. This gene consisted of 7 exons and 6 introns, and encoded a predicted protein of 68 kDa, exceeding the mature tyrosinase by 23 kDa. *P. sanguineus* tyrosinase cDNA was overexpressed in *Aspergillus niger*, a particularly suitable fungus for heterologous expression of proteins of biotechnological interest, under the control of the glyceraldehyde-3-phosphate-dehydrogenase promoter as strong and constitutive promoter. The glucoamylase preprosequence of *A. niger* was used to target the secretion. This construction enabled the production of recombinant tyrosinase in the extracellular medium of *A. niger*. The identity of the purified recombinant protein was confirmed by N-terminal amino-acid sequencing. The maturation process was shown to be effective in *A. niger* and the recombinant enzyme was fully active with a molecular mass of 45 kDa. The best transformant obtained, *A. niger* D15#26-e, produced extracellular tyrosinase activities of 534 and 1,668 U l⁻¹ for monophenolase and diphenolase, respectively, which corresponded to a protein yield of ca. 20 mg l⁻¹.