Analysis of laccase-like enzymes secreted by fungi isolated from a cave in Northern Spain

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Abstract

Laccases belong to a family of multicopper enzymes able to oxidize a broad spectrum of organic compounds. Despite the well-known property of laccases to carry out bleaching and degradation of industrial dyes and polyphenolic compounds, their industrial use is often limited by the high cost, low efficiency, or instability of these enzymes. To look for new microorganisms which produce laccases that are potentially suitable for industrial applications, we have isolated several fungal strains from a cave in northern Spain. Their phenotypic analysis on agar plates supplemented with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) disclosed two laccase-positive strains. Further genotyping revealed that they belonged to the Gliomastix murorum and Conidiobolus thromboides species. The secretion of G. murorum and C. thromboides laccase-like enzymes was then confirmed by zymography. Further identification of these polypeptides by mass-spectroscopy revealed the nature of the laccases and made it possible to predict their functional domains and other features. In addition, plate assays revealed that the laccases secreted by both G. murorum and C. thromboides were capable of degrading industrial dyes (Congo Red, Indigo, and Eriochrome Black T). Homology modeling and substrate docking predicted the putative structure of the currently uncrystallized G. murorum enzyme as well as its amino acid residues potentially involved in interactions with these dyes. In summary, new biochemical and structural insights into decolorization mediated by G. murorum laccase as well as identification of laccase-like oxidase in C. thromboides point to a promising future for these enzymes in biotechnology.

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