

Brown mycelial mat as an essential morphological structure of the shiitake medicinal mushroom *Lentinus edodes* (Agaricomycetes)

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Abstract

© 2017 Begell House, Inc. We show here, to our knowledge for the first time, that the brown mycelial mat of the xylophilic shiitake medicinal mushroom, *Lentinus edodes*, not only performs a protective function owing to significant changes in the ultrastructure (thickening of the cell wall, increased density, and pigmentation of the fungal hyphae) but also is a metabolically active stage in the development of the mushroom. The cells of this morphological structure exhibit repeated activation of expression of the genes *lcc4*, *tir*, *exp1*, *chi*, and *exg1*, coding for laccase, tyrosinase, a specific transcription factor, chitinase, and glucanase, which are required for fungal growth and morphogenesis. This study revealed the maximum activity of functionally important proteins with phenol oxidase and lectin activities, and the emergence of additional laccases, tyrosinases, and lectins, which are typical of only this stage of morphogenesis and have a regulatory function in the development and formation of fruiting bodies.

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Keywords

Basidiomycetes morphogenesis, Brown mycelial mat, Lectins, *Lentinus edodes*, Medicinal mushrooms, Phenol oxidases, Ultrastructure

References

- [1] Maltseva OV, Niku-Paavola ML, Leontievsky AA, Myasoedova NM, Golovleva LA. Ligninolytic enzymes of the white rot fungus *Panus tigrinus*. *Biot Appl Biochem*. 1991;13(3):291-302.
- [2] Revin VV, Kadimaliev DA, Shutova VV, Samuilov VD. Wood lignin modification by the fungus *Panus tigrinus*. *Appl Biochem Microbiol*. 2002;38(5):450-3.
- [3] Przybylowicz P, Donoghue J. *Shiitake growers handbook: the art and science of mushroom cultivation*. Dubuque (IA): Kendall Hunt Publishing; 1988.
- [4] Stamets P. *Growing gourmet and medicinal mushrooms*. Berkeley (CA): Ten Speed Press; 1993.
- [5] Okeke BC, Paterson A, Smith JE, Watson-Craik IA. The relationship between phenol oxidase activity, soluble protein and ergosterol with growth of *Lentinus* species in oak sawdust logs. *Appl Microbiol Biotechnol*. 1994;41(1):28-31.
- [6] Wasser SP, Weis AL. *Shiitake mushrooms [Lentinus edodes (Berk.) Sing.]*. In: Nevo E, editor. *Medicinal mushrooms*. Haifa (Israel): Peledfus Press; 1997.
- [7] Bender S, Lonergan GT, Backhaus J, Cross RF, Dumitrach-Anghel CN, Baker WL. The antibiotic activity of the edible and medicinal mushroom *Lentinus edodes* (Berk.) Sing. *Int J Med Mushrooms*. 2001;3(2-3): 118-25.

- [8] Reshetnikov SV, Wasser SP, Tan KK. Higher Basidiomycota as a source of antitumor and immunostimulating polysaccharides. *Int J Med Mushrooms*. 2001;3(4):361-94.
- [9] Feofilova EP. Mycelial fungi as a source for obtaining new medical products with immunomodulating, antitumoral, and wound healing activities. *Immunopathol Allergol Infectol (Moscow)*. 2004;1:27-33.
- [10] Vetchinkina EP, Nikitina VE. Morphological patterns of mycelial growth and fruition of some strains of an edible xylotrophic basidiomycete *Lentinus edodes*. *Izv Samar Nauch Tsentr Ross Akad Sci*. 2007;9(4):1085-90.
- [11] Vetchinkina EP, Gorshkov VY, Ageeva MV, Gogolev YV, Nikitina VE. Activity and expression of the laccase, tyrosinase, glucanase and chitinase genes in the morphogenesis of *Lentinus edodes*. *Microbiology*. 2015;84(1):78-89.
- [12] Garibova LV, Zav'yalova LA, Aleksandrova EA, Nikitina VE. Biology of *Lentinus edodes*. I. Morphologo-cultural and physiologo-biochemical characteristics. *Mikol Fitopatol*. 1999;33(2):107-10.
- [13] Reynolds ES. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol*. 1963;17(1):208-12.
- [14] Osterman LA. Methods for investigation of proteins and nucleic acids: electrophoresis and ultracentrifugation. Moscow (Russia): Izdatel'stvo "Nauka"; 1981.
- [15] Sakamoto Y, Nakade K, Sato T. Characterization of the post-harvest changes in gene transcription in the gill of the *Lentinula edodes* fruiting body. *Curr Genet*. 2009;55(4):409-23.
- [16] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248-54.
- [17] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227(5259):680-5.
- [18] Sammons DW, Adams LD, Nishizawa EE. Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels. *Electrophoresis*. 1981;2:135-40.
- [19] Weber K, Osborn M. The reliability of molecular weight determination by dodecyl-sulfate-polyacrylamide-gel electrophoresis. *J Biol Chem*. 1969;244(16):4406-12.
- [20] Gaal' E, Med'eshi G, Veretski L. Electrophoresis in the separation of biological macromolecules. Moscow (Russia): Izdatel'stvo "Mir"; 1992.
- [21] Pomerantz SH, Murthy VV. Purification and properties of tyrosinases from *Vibrio tyrosinaticus*. *Arch Biochem Biophys*. 1974;160(1):73-82.
- [22] Slomczynski D, Nakas JP, Tanenbaum SW. Production and characterization of laccase from *Botrytis cinerea* 61-34. *Appl Environ Microbiol*. 1995;61(3):907-12.
- [23] Lutsik MD, Panasyuk EN, Lutsik AD. Lectins. Lvov (Ukraine): Izdatel'stvo "Vishcha shkola"; 1981. 24. Herrera-Estrella A, Chet I. Chitinases in biological control. In: Chitin and chitinases. Jolles P, Muzarell R, editors. Basel (Switzerland): Birkhausen Verlag; 1999.
- [24] Cabib E, Bowers B, Sburlati A, Silverman SJ. Fungal cell wall synthesis: the construction of a biological structure. *Microbiol Sci*. 1988;5(12):370-5.
- [25] Fontaine T, Hartland RP, Beauvais A, Diaquin M, Latge JP. Purification and characterization of an endo-1,3- β -glucanase from *Aspergillus fumigatus*. *Eur J Biochem*. 1997;243(1-2):315-21.
- [26] Eulgem T, Rushton PJ, Robatzek S, Somssich I. The WRKY superfamily of plant transcription factors. *Trend Plant Sci*. 2000;5(5):199-206.
- [27] Ulker B, Somssich IE. WRKY transcription factors: from DNA binding towards biological function. *Curr Opin Plant Biol*. 2004;7(5):491-8.
- [28] Chen YH, Yang XY, He K, Liu MH, Li JG, Gao ZF, Lin ZQ, Zhang YF, Wang XX, Qiu XM, Shen YP, Zhang L, Deng XH, Luo JC, Deng XW, Chen ZL, Gu HY, Qu LJ. The MYB transcription factor superfamily of *Arabidopsis*: expression analysis and phylogenetic comparison with the rice MYB family. *Plant Mol Biol*. 2006;60(1):107-24.
- [29] Evans C. Laccase activity in lignin degradation by *Coriolus versicolor*. In vivo and in vitro studies. *FEMS Microbiol Lett*. 1985;27(3):339-43.
- [30] Baldrian P. Fungal laccases - occurrence and properties. *FEMS Microbiol Rev*. 2006;30(2):215-42.
- [31] Baldrian P, Valásková V. Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiol Rev*. 2008;32(3):501-21.
- [32] Dashtban M, Schraft H, Syed TA, Qin W. Fungal biodegradation and enzymatic modification of lignin. *Int J Biochem Mol Biol*. 2010;1(1):36-50.
- [33] Leatham GF, Stahmann MA. Studies on the laccase of *Lentinus edodes*: specificity, localization and association with the development of fruiting bodies. *J Gen Microbiol*. 1981;125:147-57.
- [34] Feofilova EP. Fungal cell wall. Moscow (Russia): Izdatel'stvo "Nauka"; 1983.
- [35] Whitaker JR. Polyphenol oxidase. In: Food enzymes: structure and mechanism. Wong DWS, editor. New York (NY): Chapman and Hall; 1995.
- [36] Thurston CF. The structure and function fungal laccase. *J Microbiol*. 1994;140(1):19-26.

- [37] Das N, Sengupta S, Mukherjee M. Importance of laccase in vegetative growth of *Pleurotus ostreatus* (Florida). *Appl Environ Microbiol.* 1997;63(10):4120-2.
- [38] Wood DA. Production, purification and properties of extracellular laccase of *Agaricus bisporus*. *J Gen Microbiol.* 1980;117:327-38.
- [39] Vetchinkina EP, Pozdnyakova NN, Nikitina VE. Laccase and lectin activities of intracellular proteins produced in a submerged culture of the xylotrophic basidiomycete *Lentinus edodes*. *Curr Microbiol.* 2008;57(4): 381-5.
- [40] Vetchinkina EP, Pozdnyakova NN, Nikitina VE. Laccase and lectin activities of intracellular proteins produced in a submerged culture of the xylotrophic basidiomycete *Lentinus edodes*. *Curr Microbiol.* 2008;57(4): 381-5.