CARBOXYMETHYLCELLULASE AND LACCASE AS ENZYME MARKERS DURING MORPHOGENESIS IN THE *SHIITAKE* MUSHROOM. *LENTINULA EDODES*

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Introduction

The extracellular enzymes, carboxymethylcellulase (CMCase, EC 3.2.1.4) and laccase (1.14.18.1) have been tracked as morphogenetic markers in *Pleurotus sajor-caju* (Tan and Wahab, 1997). We determined whether these enzymes exhibit the same pattern during morphogenesis in *Lentinula edodes*. This has important commercial implication in timing the stimulation for mushroom fruiting. The correlation of CMCase to fruiting subjected to two different environmental regimes was also investigated.

Materials and Methods

L. edodes (strain L 200,) was cultivated on a liquid synthetic medium according to Leatham (1983) in conical flasks, and on a solid sawdust substrate in polypropylene bags. Inoculation was performed with 10-days old mycelial plugs. Spawn-run was at 25°C in the dark with fruiting under an illuminated/fluctuating 25°C/20°C regime for the enzyme studies on solid and liquid media, and fruiting in a dark, constant 22°C regime for the comparative CMCase study in liquid. Enzymes were extracted according to Ganisan et al. (1998), CMCase was assayed with the direct ferricyanide method (Halliwell, 1962) and laccase by oxidation of ABTS (Bourbonnais and Paice, 1988).

Results and Discussion

CMCase specific activity on liquid media (illuminated-fluctuating temperature regime) peaked after four weeks with a value of 10.91 U/mg protein, was maintained for another two weeks (11.16), and then fell to 1.75 by the ninth week. Primordia appeared on the 4th week, implying enzyme activity remained high during fruiting. Activity on sawdust was much lower although following a similar pattern, peaking after nine weeks (coinciding with primordia appearance) with a value of 2.91 and remaining high for the next five weeks. CMCase therefore proved to be a potential fruiting marker as in *Pleurotus sajor-caju*. The low value of CMCase in the lignocellulosic sawdust is expected but the high value in the cellulose-deficient liquid is surprising. CMCase activity in the other darkness-constant temperature regime gave similar results (11.97 units after six weeks) with interestingly, no difference in mushroom yields.

Laccase activity peaked very early on both liquid synthetic medium and sawdust with specific activity of 136.69 U/mg protein and 13.67 U/mg respectively by the second week, when CMCase activity was still low. It then decreased rapidly to 1.54 and 0.26 units respectively by the 7th week. Laccase activity is expectedly higher than CMCase with stimulation by nutrient nitrogen in the liquid and by lignin in sawdust. The lowest point in laccase activity coincided with maximum mycelial growth and primordia formation (7th week). Laccase is therefore another useful fruiting marker, with a sudden drop in activity marking the approach of fruiting.

Conclusions

CMCase and laccase activities are useful morphogenetic landmarks in timing the onset of mushroom fruiting in *L. edodes*. This would be very helpful to mushroom growers in initiating conditions for fruiting as maximum mycelial growth cannot be ascertained visually with high accuracy.

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