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Enzyme activities of *Chlorophyllum molybditis* and *Cortinarius melliolens* at different sporophore stages

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Abstract

The intracellular enzyme activities of *Chlorophyllum molybditis* (Mayer *ex.* Fr.) Massee and *Cortinarius melliolens* Fries were determined at different stages of sporophore maturity. In both mushroom species, total amylase, α-amylase, proteinase, lipase, peroxidase, catalase and polyphenol oxidase activities were increased with sporophore maturity. In contrast, glucose-6-phosphatase activity was higher in the young sporophore than in very young and mature ones. All the enzymes assayed except cellulase and β-amylase showed greater activity in the pilei than in the stipes. *C. molybditis* showed greater total amylase, α-amylase, cellulase, proteinase, catalase and glucose-6-phosphatase activities than *C. melliolens.*

Introduction

During development from primordia to mature sporocarps, biochemical changes occur in both the sporocarps and the substrate on which the sporocarp is growing. These biochemical changes are brought about by oxidising and hydrolytic enzymes produced by the developing sporocarp (Norkrans 1957, Yamaguchi *et al.* 1970, Paranjpe and Chen 1979). Enzymes convert insoluble substrate into soluble components that could be utilized nutritionally by the growing sporocarp (Jablonsky 1981). In *Volvariella volvacea,* β-glucosidase and cellulase activities have been shown to increase from pinhead stage to the egg stage during the development of sporocarp (Wang 1982). Kadiri and Fasidi (1990a) have also showed that the activities of α-amylase, proteinase, cellulase, lipase, peroxidase and polyphenol oxidase increased from very young to mature sporophores in *Pleurotus tuber-regium,* and *Tricholoma lobayensis.*

In our previous investigation, we have shown that the crude fibre, glycogen, lipid and protein contents of *Chlorophyllum molybditis* and *Cortinarius melliolens* increased with the sporophore development (Fasidi and Kadiri 1990, Kadiri and Fasidi 1990b). In the present investigation the intracellular enzyme activities of

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C. molybditis and *C. melliolens* were examined at different stages of sporophores to show which stages and which parts are metabolically most active.

Materials and methods

Fresh sporophores of *C. molybditis* and *C. melliolens* were harvested at three developmental stages according to the method used by Gruen and Wong (1982) Hammond (1981) and Hammond and Nichols (1975). These developmental stages were designated as follows: very young stage (pileus, less than 3 cm for *C. molybditis* and less than 2.6 cm for *C. melliolens*); young stage (pileus, 3 - 4.9 cm for *C. molybditis* and 2.6 - 3.9 cm for *C. melliolens*); mature stage (pileus 5 - 10 cm for *C. molybditis* and 4 - 7 cm for *C. melliolens*).

Enzyme extraction: 1 g of frozen pileus or stipe tissue was ground with an extracting buffer in a cooled mortar. The ensuing suspension was centrifuged at 18 000 *g* for 30 min (at 2 °C). 0.1 M sodium acetate buffer (pH 5.0) was used for extracting amylase and lipase and 0.05 M sodium phosphate buffer (pH 6.0) for proteinase. Peroxidase and catalase were extracted in 0.3 M phosphate buffer (pH 6.8) and polyphenol oxidase in 0.1 M sodium phosphate buffer (pH 5.0). Water was used for extracting glucose-6-phosphate, and 0.07 M K_2HPO_4 (pH 5.0) for cellulase.

Cellulase activity: 1 cm^3 of the enzyme extract and 9 cm^3 of 1 % carboxymethyl-cellulose in 0.05 M phosphate buffer (pH 5.0) were incubated for 1 h at 30 °C (Singh and Kunene 1980). The enzyme action was stopped with 3,5-dinitrosalicylic acid (DNSA) and the amount of reducing sugar formed was determined (Denison and Koehn 1977).

Amylase activity: For determination of total amylase activity 1 cm^3 of the enzyme extract was incubated with 1 cm^3 of 1 % solution of starch in 0.1 M acetate buffer (pH 5.0) at 70 °C for 15 min. The enzyme activity was terminated by adding DNSA and the quantity of reducing sugar formed was determined (Swain and Dekker 1966).

For determination of α-amylase activity 5 cm^3 of the amylase extract were heated at 70 °C for 15 min to denature β-amylase (Wilson 1971). 1 cm^3 of the heated extract was incubated with 1 cm^3 of 1 % solution of starch in 0.1 M acetate buffer (pH 5.0) at 27 °C for 1 h. The enzyme activity was stopped with DNSA and the quantity of reducing sugar formed was determined as for total amylase activity.

Polyphenol oxidase activity: To 1 cm^3 of the extract, 3 cm^3 of 0.01 M catechol and 0.01 M proline (both in phosphate buffer, pH 6.5) was added. The enzyme action was terminated 1 h later by adding 2 cm^3 of 10 % trichloroacetic acid (Yamaguchi *et al.* 1970). The activity of the active form of the enzyme in 1 cm^3 of enzyme extract was taken as the amount of quinone formed by measuring the absorbance at 525 nm (Yamaguchi *et al.* 1970).

Other enzyme activities: Proteinase activity was determined according to McDonald and Chen (1965), lipase activity after the method of Yong and Wood (1977),

peroxidase after Keilin and Hartree (1951), catalase after Feinstein (1949) and glucose-6-phosphatase activity after Swanson (1955) with determination of Pi by the method of Russell (1940).

Protein content was estimated by the method of Lowry *et al.* (1951) with casein as the standard protein.

Results and discussion

Total amylase, α-amylase, cellulase, lipase, peroxidase, proteinase and polyphenol oxidase activities were found to increase from very young to mature sporophores in both *C. melliolens* and *C. molybditis* (Tables 1 and 2). The increase in enzyme activities with sporophore maturity is similar to the observed pattern of distribution of nutrients in the sporophores of these mushrooms (Fasidi and Kadiri 1990, Kadiri and Fasidi 1990b). A similar trend was detected by Yamaguchi *et al.* (1970) and Paranjpe and Chen (1979) for polyphenol oxidase and cytochrome oxidase activities of *Agaricus bisporus* sporophores.

With regard to β-amylase and glucose-6-phosphatase, the young sporophore was found to possess a higher activity than the mature and very young sporophore (Tables 1 and 2). The same activity distribution was obtained by Moore *et al.* (1979) for NADP-glutamate dehydrogenase in the young *Coprinus lagopus*. Glucose-6-phosphatase is a respiratory enzyme and thus the observed higher activity indicates a faster respiratory rate in the young sporophore. This could be expected because the spore formation in young sporophore is associated with high respiratory rate and great utilization of respiratory metabolites.

Total amylase, α-amylase, proteinase, lipase, peroxidase, catalase, polyphenol oxidase, and glucose-6-phosphatase activities were higher in the pilei than in the stipes (Tables 1 and 2). This is consistent with the finding of Yamaguchi *et al.* (1970) for polyphenol oxidase activity in *A. bisporus*. This implies that the pilei of *C. melliolens* and *C. molybditis* are more active metabolically than their respective stipes. This agrees with our initial finding that the pilei store more nutrients than the stipes (Fasidi and Kadiri 1990, Kadiri and Fasidi 1990b). On the other hand, for cellulase and β-amylase this trend was reversed. This may be due to the fact that the stipe, being the support for pileus needs more reinforcement of carbon skeletons than the pileus. In our earlier work, we found that the stipes stored more crude fibres and iron than their respective pilei (Fasidi and Kadiri 1990, Kadiri and Fasidi 1990b).

Chlorophyllum molybditis showed greater cellulase, total amylase, α-amylase, proteinase, catalase, and glucose-6-phosphatase activities than *C. melliolens* (Tables 1 and 2). This may be due to the fact that *C. molybditis* which normally attains a much larger sporophore size than *C. melliolens* requires more sugars and amino acids for its growth and implies that *C. molybditis* is more active metabolically than *C. melliolens.*

In summary, the enzyme activities increased with sporophore maturity and the pileus was more likely to undergo a faster depreciation in flavour, food quality and nutrients.

Table 1. Intracellular cellulase, total amylase, α-amylace, β-amylase and proteinase activities in various sporophore stages of *Chlorophyllum molybditis* and *Cortinarius melliolens*.

Mushroom species and stage	Cellulase activity [mg(glucose) h^{-1} mg^{-1}(protein)]	Total amylase activity [mg(maltose) h^{-1} mg^{-1}(protein)]	α-amylase activity [mg(maltose) h^{-1} mg^{-1}(protein)]	β-amylase activity [mg(maltose) h^{-1} mg^{-1}(protein)]	Proteinase activity [mg(maltose) h^{-1} mg^{-1}(protein)]
C. molybditis					
very young pileus	0.31 e	1.6 d	1.6 d	0.04 c	2.1 e
very young stipe	0.43 d	1.3 d	1.1 d	0.22 b	1.6 e
young pileus	0.65 c	3.6 b	3.3 b	0.25 b	5.8 b
young stipe	0.68 bc	2.9 c	2.3 c	0.58 a	3.1 d
mature pileus	0.75 b	5.6 a	5.3 a	0.27 b	8.1 a
mature stipe	0.95 a	3.9 b	3.7 b	0.22 b	4.0 c
C. melliolens					
very young pileus	0.19 e	1.4 c	1.3 c	0.11 e	1.1 c
very young stipe	0.31 bc	1.0 d	0.8 d	0.22 d	0.9 c
young pileus	0.25 d	1.9 b	1.6 b	0.28 c	1.9 b
young stipe	0.34 ab	1.4 c	0.9 d	0.45 a	1.2 c
mature pileus	0.28 cd	2.7 a	2.3 a	0.38 b	3.2 a
mature stipe	0.37 a	1.8 b	1.5 bc	0.26 cd	2.0 b

Table 2. Intracellular lipase, peroxidase, catalase, polyphenol oxidase, and glucose-6-phosphatase activities in various sporophore stages of *Chlorophyllum molybditis* and *Cortinarius melliolens*.

Mushroom species and stage	Lipase activity [cm^3(0.02 M NaOH) h^{-1} mg^{-1}(protein)]	Peroxidase activity [mg(purpuro-gallin) h^{-1} mg^{-1} (protein)]	Catalase activity [mg($NaBO_3$. 4 H_2O) h^{-1} mg^{-1}(protein)]	Polyphenol oxidase activity [mg(quinone) h^{-1} mg^{-1}(protein)]	Glucose-6-phosphate activity [mg (phosphate) h^{-1} mg^{-1} (protein)]
C. molybditis					
very young pileus	2.8 d	1.1 d	1.2 d	1.6 d	1.8 c
very young stipe	1.0 e	1.0 d	0.9 e	1.4 d	1.7 c
young pileus	5.0 b	2.5 b	2.2 b	3.8 b	3.6 a
young stipe	3.9 c	1.8 c	1.8 c	2.6 c	2.6 b
mature pileus	5.9 a	2.9 a	2.7 a	4.8 a	2.7 b
mature stipe	4.1 c	1.9 c	1.8 c	2.7 c	1.8 c
C. melliolens					
very young pileus	2.6 c	1.2 d	0.44 bc	1.8 bc	1.6 bc
very young stipe	2.0 d	0.9 c	0.40 e	1.4 d	1.5 c
young pileus	3.1 b	1.6 ab	0.75 a	2.0 b	2.0 a
young stipe	2.7 c	1.4 c	0.49 b	1.6 cd	1.6 bc
mature pileus	3.6 a	1.7 a	0.79 a	2.6 a	1.8 ab
mature stipe	3.3 ab	1.5 bc	0.72 a	1.9 b	1.4 c

Data followed by the same letter(s) within any mushroom group are not significantly different at $P = 0.01$ by Duncan's multiple range test.

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