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Tyrosinase activities and isoenzymes in the developing stages of three species of Oyster Mushrooms

ANITHA B. KARTHA[2], T. K. ABRAHAM[1] AND N. S. PRADEEP[1]

[1]*Tropical Botanic Garden & Research Institute, Palode, Thiruvananthapuram 695 562, Kerala*
[2]*Department of Biotechnology, University of Kerala, Kariavattom, Thiruvananthapuram*

Tyrosinase activities and isoenzymes were monitored in three species of developing mushrooms from pinhead to mature stage. Catechol oxidase, dopa oxidase and tyrosinase activities decreased during development. The ideal substrate for each species varied. *P. djamour* could utilize tyrosine as a better substrate compared to *P. sapidus* and *P. florida*. *P. florida* could utilize catechol than other two species where as *P. sapidus* used dopa effectively than *P. djamour* and *P. florida*. The electrophoretic profile of dopa oxidase isoenzymes indicated that two forms are present in each developmental stage and the amount of these two forms decreased with maturation of the fruting body and they appeared to contain a single form that could use tyrosine as the substrate. Electrophoresis in the presence and absence of SDS indicated that the isoenzyme patterns of tyrosinase were not affected by SDS

Key Words : Mushroom, *Pleurotus* spp., tyrosinase, catechol, dopa, isoenzyme, assay, electrophoresis

INTRODUCTION

Mushrooms are highly perishable compared to vegetables, meat and fish. Fresh mushrooms have a high rate of metabolic activity, which quickly decline and lead to deterioration. Mushrooms, in particular, contain a very active and relatively abundant tyrosinase (Robb, 1984). This enzyme contributes to the enzyme browning reactions in mushrooms and is of interest to the mushroom and food industry.

Mushroom tyrosinase has been extensively studied in crude extracts, purified forms and in commercial preparations with regard to its physical and enzymatic properties (McCord and Kilara, 1983; Robb and Gutteridge, 1981; Robb, 1984; Sharma and Ali, 1981; Stroth Kamp *et al.*, 1976). The enzyme has also been studied during aging and during post harvest storage (Goodenough, 1978; Murr and Morris, 1975). Investigations have also been carried out to examine ways to increase the shelf life and quality of mushrooms (Burton, 1986; Burton *et al* 1987; Nicholas and Hammond, 1973). Tyrosinase activites and isoenzymes were also studied (Ingebrigsten *et al.*, 1989; Moore and Flurkey, 1989), but much less information is available on the activities, isoenzymes and location of tyrosinase in developing mushrooms. The present study has been conducted with an objective to monitor the tyrosinase activities and isoenzymes in developing mushrooms (*Pleurotus* species) from pin head to mature stage that enables to design future biochemical experiments that inhibit the pathways involved in the enzymic reactions and thus prolong the shelf life of mushrooms.

MATERIALS AND METHODS

Materials

Mushrooms at three developmental stages viz., pin head, immature and mature stages grown on paddy straw were used for different analyses undertaken in the study. The materials used belong to the basidio-

mycetes fungus coming under the order Agaricales and family Lentinaceae. The species selected were *Pleurotus djamour* (RRL, Jammu), *P. sapidus* (MTCC-1807) and *P. florida* (RRL, Jammu).

Enzyme extraction and assay

Enzymes were extracted by homogenising mushroom samples in 25 mM Sodium phosphate buffer pH 6 and centrifuged at 10,000 g for 10 min at 4°C. The supernatants were collected and stored at –70°C for future studies. Active tyrosinase in all samples were assayed using catechol. L-Doppa or L-Tyrosine as the substrates for catechol oxidase, dopa oxidase and tyrosine hydroxylase activity as described by Ingebrigsten *et al.* (1989).

Enzyme Electrophoresis

Enzyme extract (60 μl) loaded on to each of 15 wells of the 1 mm thick non-denaturing gel (7%) was resolved through polyacrylamide gel electrophoresis (PAGE) using Tris Glycine (pH 8.3) buffer system for ~5 hrs. A current of 100 V for stacking gel region and 150 V for separating gel was maintained. Electrophoresis and isoenzyme staining for Tyrosinase (Tyrosinase is the common name for an enzyme that was previously called monophenol mono oxygenase. It is listed as Enzyme 1.14.18.1 in the standard Enzyme Nomenclature) was performed in the presence and absence of SDS (Angleton and Flurkey 1984).

RESULTS AND DISCUSSION

In all the three species of mushrooms, tyrosinase could utilise either catechol, L-dopa or L-tyrosine as the substrate. Catechol oxidase, dopa oxidase and tyrosinase activities decreased during development (Tables 1-3). The pattern of dopa oxidase and tyrosine hydroxylase activities observed were in agreement with the results obtained by Ingebrigsten *et al.* (1989), but catechol oxidase activity showed a different trend. ie, the catechol oxidase activity showed a steady decrease with the development where as Ingebrigsten observed variation in the catechol oxidase activity with development. This may be attributed to the species difference. Crude extracts from each species were subjected to electrophoresis in the presence and absence of SDS.

Table 1 : Catechol oxidase activity

Sample	Catechol
Pleurotus djamour	
bud	0.215
immature	0.133
mature	0.1
Pleurotus sapidus	
bud	0.16
immature	0.13
mature	0.05
Pleurotus florida	
bud	0.72
immature	0.662
mature	0.404

Table 2 : Tyrosinase activity

Sample	Catechol
Pleurotus djamour	
bud	1.893
immature	1.259
mature	1.216
Pleurotus sapidus	
bud	0.682
immature	0.397
mature	0.11
Pleurotus florida	
bud	0.793
immature	0.655
mature	0.25

Table 3 : Dopa oxidase activity

Sample	Catechol
Pleurotus djamour	
bud	0.448
immature	0.316
mature	0.255
Pleurotus sapidus	
bud	0.35
immature	0.318
mature	0.248
Pleurotus florida	
bud	0.185
immature	0.129
mature	0.053

The electrophoretic isoenzyme profile of dopa oxidase and tyrosinase activity at various developmental stages in all the three species were consistent with the data from the enzyme assays (Tables 1-3). The enzyme forms decreased in intensity as the mushrooms matured. Each species showed a single band of enzyme activity, but with slightly different mobility that could utilize tyrosine as the substrate (Figs. 1-3). Dopa oxidase isoenzyme patterns were much different and indicated that all species contained two isoenzyme

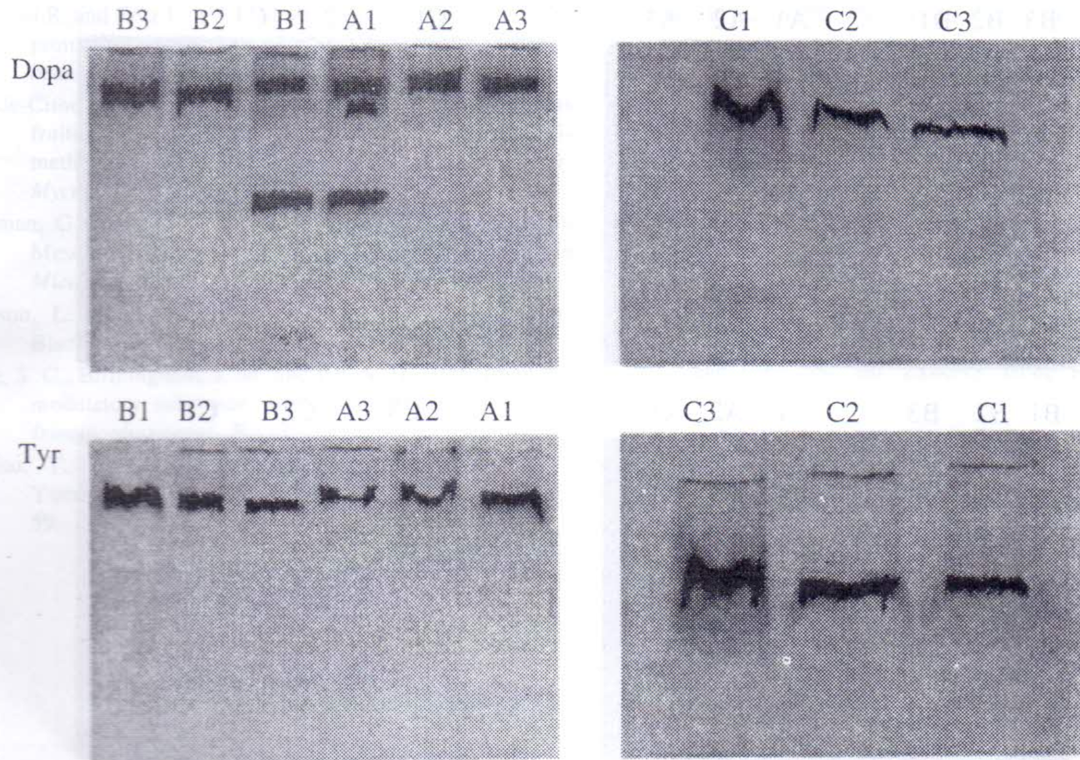

Fig. 1 : Tyrosinase activity in Mushroom (Pleurotus sp) Separated by Page and tested against Dopa and Tyrosine. A-C represents 3 Pleurotus species and 1-3 represents ; 1 : Bud, 2 : Immature and 3 : Mature stages.

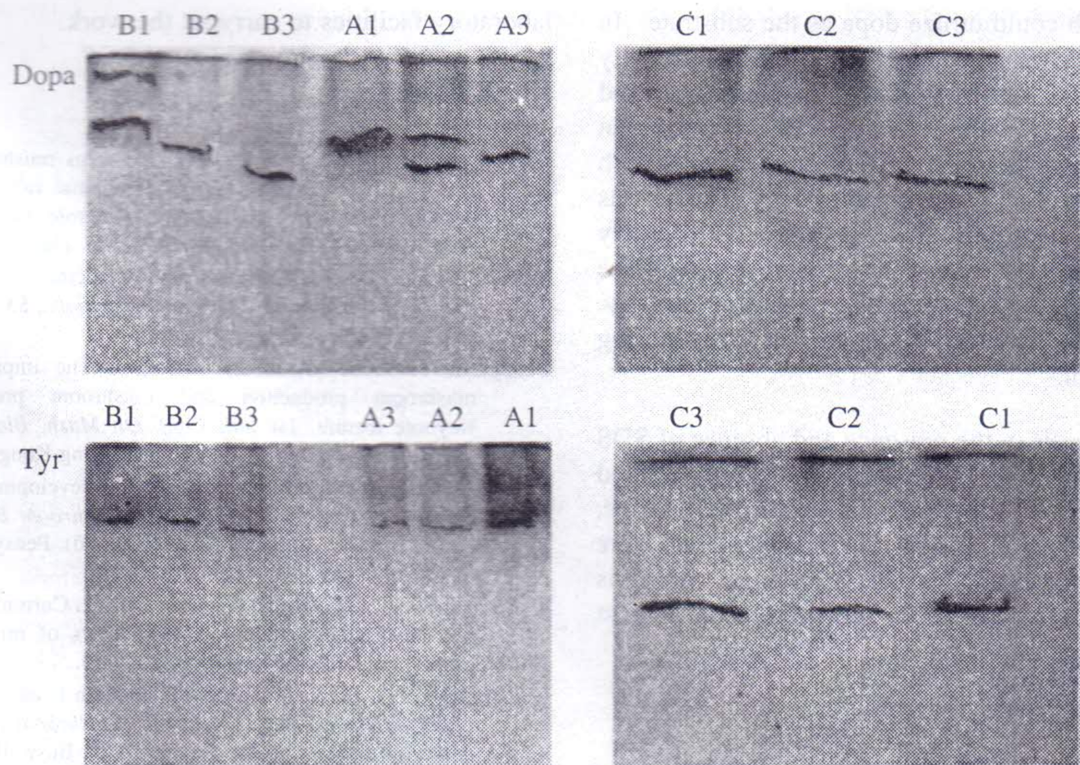

Fig. 2 : Tyrosinase activity in Mushroom (Pleurotus sp) Separated by PAGE and tested against Dopa and Tyrosine. (incubated and stained with .1% SDS)

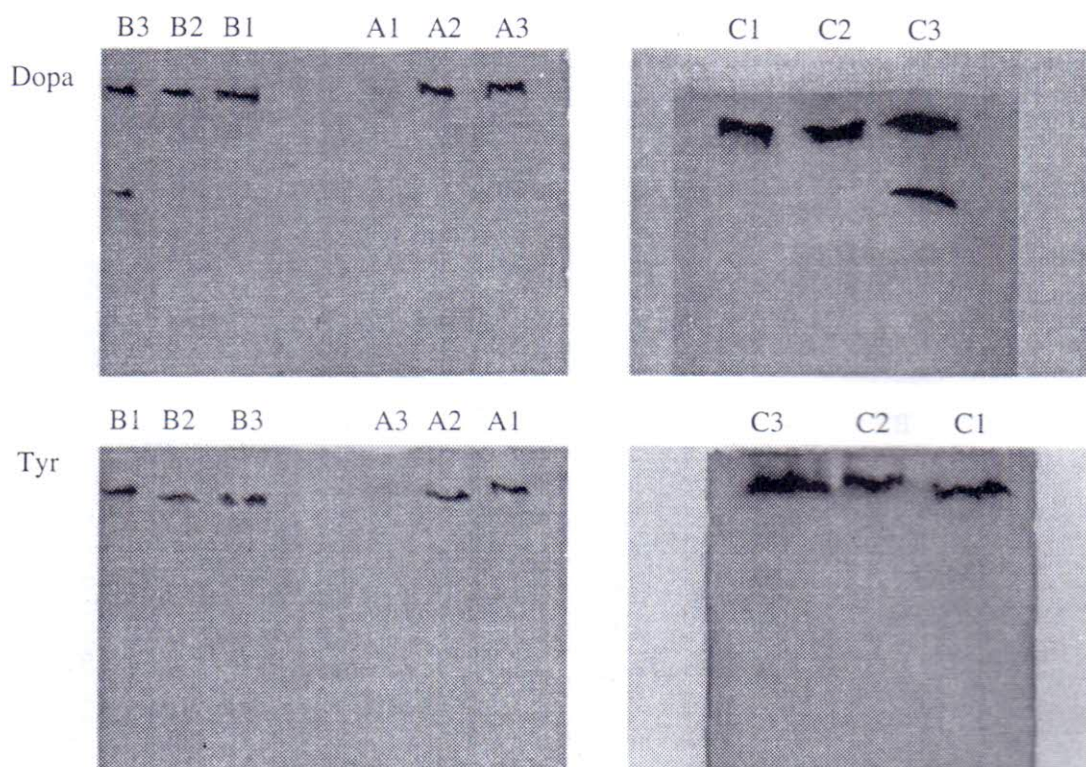

Fig. 3 : Tyrosinase activity in Mushroom (Pleurotus sp) Separated by PAGE and tested against Dopa and Tyrosine. (Gel run, incubated and stained with .1% SDS)

forms which could utilize dopa as the substrate. In addition, the upper band of dopa oxidase activity appeared to be similar in Rm to the bands observed when tyrosine was the substrate. This difference in patterns suggests multiple enzyme forms which have different substrate specificities. The results are in agreement with those of Moore and Flurkey (1989). Dopa oxidase and tyrosine hydroxylase might be better indicators of tyrosinase activity. Since they showed similar trends during development.

Electrophoresis in the presence and absence of SDS indicated that isoenzyme forms were not affected by SDS. With the combination of SDS used, isoenzyme forms varied in intensity but were relatively permanent. The findings of this experiment can be used for further research in post harvest technology.

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