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POLYPHENOL OXIDASE ACTIVITY AND DIFFERENTIATION OF RHIZOMORPH IN *ARMILLARIA MELLEA* (VAHL. EX. FR.) KARST

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Abstract

Polyphenol oxidase (PPO) activity varied in differentiation from hyphae to rhizomorph and croute in *A. mellea*. In undifferentiated hyphae there was not any PPO activity, while croute showed the highest activity. Electrophoresis in the presence of SDS allows the detection of intensive enzyme forms of PPO, and can also convert slower migrating enzyme forms to faster ones. Some fluorescent compounds, as intense activators of PPO, show an intense band of PPO in electrophoresis but not in the migrating band.

Introduction

Tyrosinase (monophenol, dihydroxyalanine, oxygen oxidoreductase, EC 1.14.18.1) is found in all plant and fungal species [1-3]. This enzyme can utilize catechol, dihydroxy phenylalanine (DOPA) or tyrosine as substrate. The enzyme from mushrooms has received considerable attention in the last ten years. Most of the studies have relied on commercial sources. Representative topics in these studies have included the examination of inhibitory effects using methimazole [4], mimosine [5], tropolone [6], ascorbic acid and its derivatives [7, 8], cysteine [9] and 3-amino L-tyrosine [10]. The enzyme has also been studied during aging and post harvest storage periods [11].

Investigations have also been carried out to examine ways to increase the shelf life and quality of mushrooms [12]. Tyrosinase activity is present in the pileus (cap) and stipe (stalk) of mushrooms at three developmental stages [13]. Recently, it has been reported that the skin contains more enzyme than the flesh, based on either fresh weight or on protein weight [14]. According to some investigations, PPO inhibitors can be grouped into two classes: compounds that interact with copper in the enzyme and those that affect the active site for the phenolic substrates. Among the second type of inhibitors, aromatic carboxylic acids of the benzoic and cinnamic series have been widely studied [15, 16]. The enzyme can be activated by treatment with proteolytic enzymes [17], fatty acids [18], denaturants [19], and detergents [20]. Various investigators have shown that PPO can be activated by treatment with the denaturant sodium dodecylsulfate (SDS) although the mode of its activation has not been explained [20]. In this report, PPO activity in hyphae, rhizomorph and croute, three developmental and differentiation stages of *A. mellea*, were examined.

Keywords: *Armillaria mellea*; Polyphenol oxidase; Fluorescent compounds

Materials and Methods

A. mellea (Vahl ex. Fr.) Karst was obtained from Quercus from the Karasang forest in the north of Iran. An explant of fungi was grown in stock medium containing malt agar (Difco. Co.). The colonies were maintained on malt agar at 4°C and later transferred to other media: Knop macroelements - Heller microelements containing NO_3 (MN medium) or NH_4 (MA medium), or prune juice medium [21].

The fresh extracts of hyphae, rhizomorph or croute were prepared by homogenization in 0.1 M sodium phosphate buffer (pH 6.2) containing 0.05 M NaCl for 1 min, then centrifuged for 10 min at 9000 g. The supernatants were stored at -20°C until use. Electrophoresis was performed according to the method mentioned in reference [2] with or without the addition of SDS, F^1_1 , F^2_3 and MF^3_3 [23]. Electrophoresis was carried out for 4 h at 120 v. After electrophoresis, one gel (A: without SDS in the separating and stacking gel) was incubated in 0.05 M sodium phosphate (pH 6.5) for 30 min, then stained for PPO with 0.5 m DL-DOPA and 2 mM catechol in 0.05 M sodium phosphate buffer (pH 6.2) containing 0.05 M NaCl for 1 min, then centrifuged for 10 min at 9000g. The supernatants were stored at -20°C until use. Electrophoresis was performed according to the method mentioned in reference [22] with or without the addition of SDS, F_1 , F_3 and MF_3 [23]. Electrophoresis was carried out for 4 h at 120 v. After electrophoresis, one gel (A: without SDS in the separating and stacking gel) was incubated in 0.05 M sodium phosphate buffer (pH 6.5) for 30 min, then stained for PPO with 0.5 m DL-DOPA and 2 mM catechol in 0.05 M sodium phosphate buffer (pH 6.5). F_1 , F_3 and MF_3 were added to the crude extract of PPO in doses of 15-30 mg/ml. The second gel (B: without SDS in the separating and stacking gel) was incubated in 0.05 M sodium phosphate buffer (pH 6.5) containing 0.1 SDS(W/V) for 30 min, then stained for PPO in the above buffer containing 2 mM catechol. PPO activity was determined by observing the increase in absorbance at 41 nm. One unit of activity was defined as change of one absorbance unit per min at 25°C. Enzyme activity spectra were recorded on a Shimadzu- 160 UV-VS spectrophotometer. Reflection spectra of TLC and transmission spectra of gel were recorded at 41 nm by Shimadzu CS-9000 densitometer.

Results and Discussion

Specific activity of PPO in undifferentiated hyphae is zero, but croute has more activity than rhizomorph (Table 1).

With the TLC of chloroform extract of fluorescent compounds of croute, some yellow bands appeared. Reflection mode densitometry with λ = 410 nm showed nine peaks (Fig. 1). In a previous study [22], we defined that the percentage of F_3 and MF_3 compounds is greater in croute than rhizomorph and F_1 is less than them. In this work, we indicate that in the presence of F_3 and MF_3, PPO activity is greater than in control, and F_1 is fewer (Table 2).

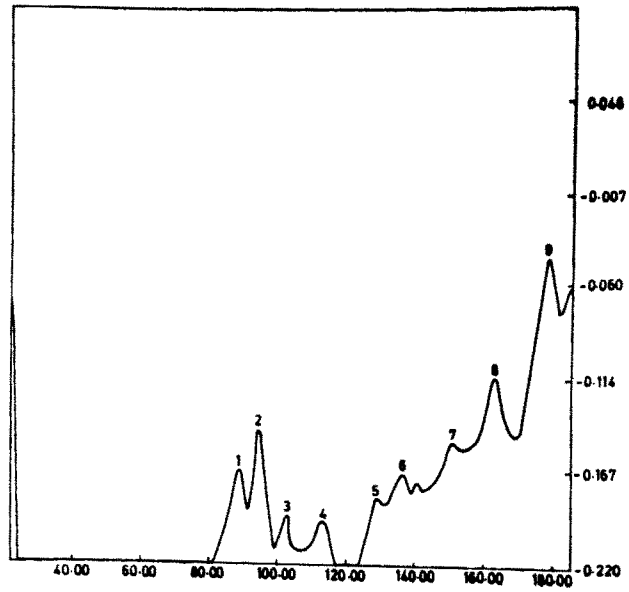

Figure 1. Reflection densitometry of fluorescent compounds of crude extract of croute at λ = 410 nm. Nine bands are indicated.

Table 1. PPO activity of crude extracts of hyphae, rizomorph and croute

Organ	Specific activity dA/min/mg
Undifferentiated hyphae	0
Differentiated hyphae	0.02
Rhizomorph	0.11
Croute	0.24

Table 2. The effect of fluorescent compounds on the activity of PPO

Experiment No.	Activity of PPO dA/min	Activity of PPO in the presence of fluorescent compounds		
		F_1	F_3	MF_3
1	0.033	0.003		
2	0.045		0.064	
3	0.019			0.026

[1] F_1: Phenol, 2,6- Bis (1,1 dimethyl ethyl) -4-ethyl

[2] F_3: 1,2- Benzendicarboxylic acid, butyl 2-methyl hexyl ester

[3] MF_3: 1,2- Benzendicarboxylic acid, butyl decyl ester

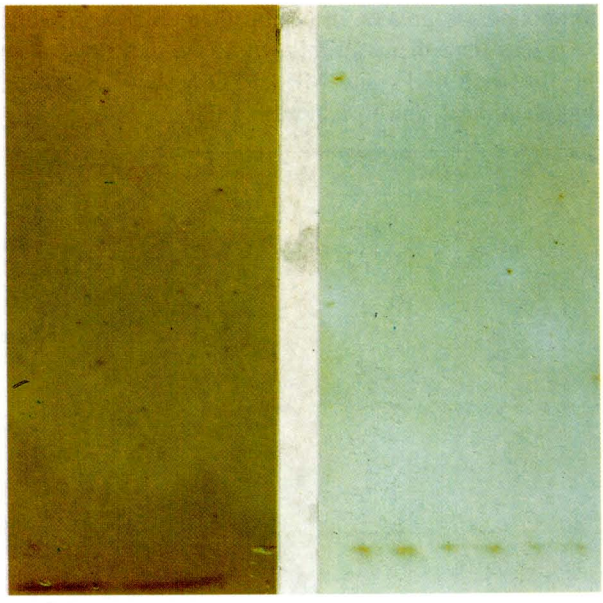

Figure 2. Electrophoresis enzyme profile of PPO
- Intensive bands in the presence of F_3 and MF_3
- Weak band in the presence of F_1
- Intensive and migrating band in the presence of SDS

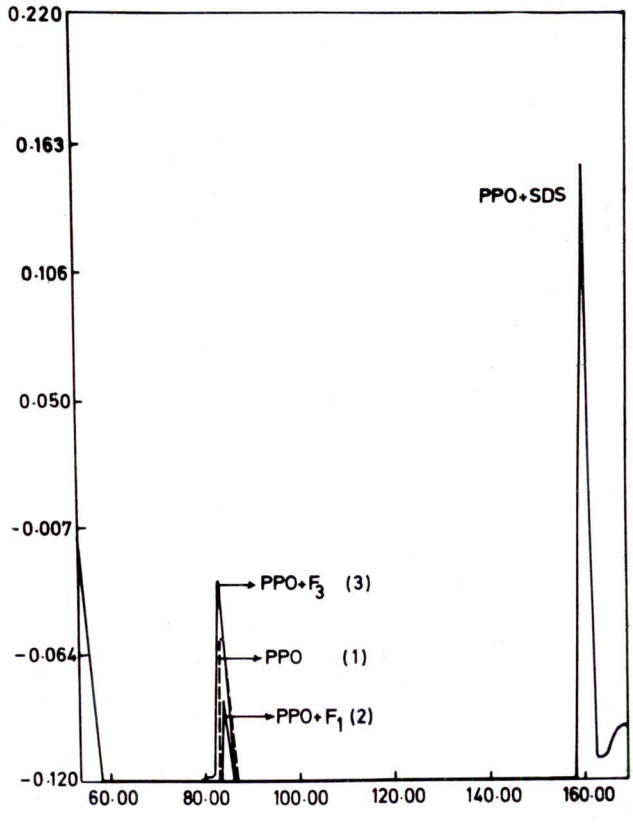

Figure 3. Scanning densitometry of gel of PPO in the presence of F_1, F_3 and SDS

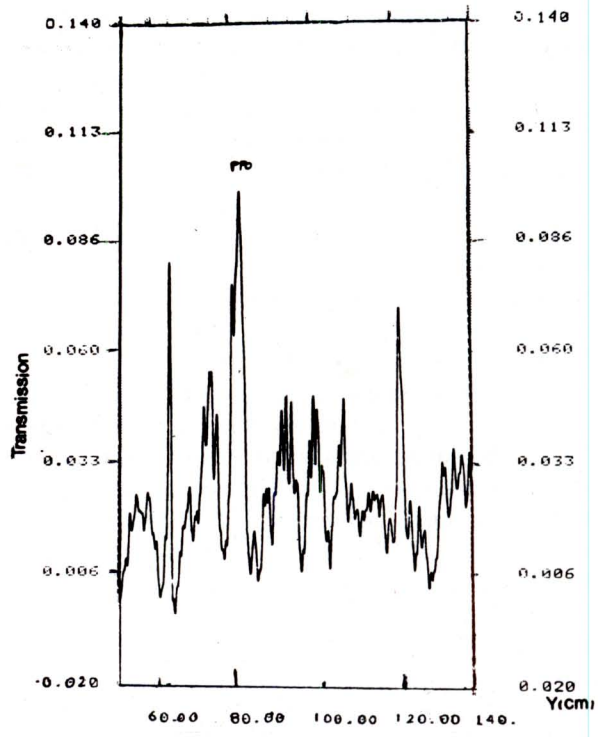

Figure 4. Transmission scanning densitometry of gel (soluble proteins) at $\lambda = 600$ nm

The electrophoretic enzyme profile of PPO showed intensive bands in the presence of F_3 and MF_3 and a weak band for F_1 (Figs. 2,3). The extracts were subjected to the presence of SDS and showed a more intensive and faster moving band (Figs. 2,3); Rm changed from 0.47 to 0.97.

By comparing Rm of PPO with the scanning densitometry of gel of the soluble proteins of crude extract, the position of PPO is indicated (Fig. 4).

The F_3 and MF_4 compounds probably change the conformation of PPO into a favorable form for more activity, and F_1 with phenolic group probably plays a role as a competitive inhibitor [15, 16]. The effect of SDS may be to produce a few subunits with more PPO activity, whereas F_3 and MF_3 do not [20].

Oxidation of polyphenols in the croute of *Armillaria mellea* plays a special role in this parasitic fungus and while maintaining hyphae in survival conditions, increases the attacking power of rhizomorph.

Meanwhile, remarkable increases in F_3 and MF_3 in croute and rhizomorph indicate that these compounds, contrary to ascorbic acid and its derivatives [7, 8] and cysteine [9], act as activatory agents increasing the shelf life and quality of mushrooms [12].

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