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Production of L-DOPA by banana leaf polyphenol oxidase

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Abstract. An economical process to produce L-DOPA by using polyphenol oxidase (PPO) from banana leaf was developed. The yield of L-DOPA was affected by the temperature, pH, buffer type and source of PPO. The yield of L-DOPA by using enzyme from different sources was in the following order: infected leaf > healthy leaf > infected stem > healthy stem. The PPO from infected banana leaf can produce L-DOPA up to 67% under the optimum condition.

Key words: Banana leaf; Isozyme; L-DOPA; Polyphenol oxidase.

Introduction

Polyphenol oxidase (PPO, E. C. 1. 14. 18. 1) is widely distributed in the plant kingdom. It has been suggested that the enzyme might be associated with many important function such as defense and senscence (Bouthyette *et al.*, 1987; Tremolieres and Bieth, 1984; Mayer and Harel, 1979; Shaw *et al.*, 1991).

It is also useful in enzyme technology. Mushroom PPO was used for the production of polymers and L-DOPA (L-3, 4-dihydroxy-phenylalanine) (Kazandjian and Klibanov, 1985). L-DOPA is widely used for the treatment of Parkinson's disease, and it can also inhibit selectively the growth of pigmented human melanoma (Wick, 1977). Banana is an important fruit crop in tropical countries such as Taiwan. So far, there have been very few reports on PPO of banana. Galeazzi *et al.* (1981) has purified and characterized PPO from banana fruit (*Musa cavendishii* L). However, there have been no report on PPO in other tissues. The leaf and stem are agrowastes which might be a rich source of PPO. In the present work, high activities of PPO were found in the banana leaf and stem. These enzymes were used for the production of L-DOPA.

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Material and Methods

Materials

The leaves and stems of both healthy and fungi (*Phyllosticta musarm*) infected banana of cultivar "Giant Cavendishii, AAA" were obtained from Taiwan Banana Research Institute, Chiouzu, Pintong. Samples were taken from banana plants which were grown in an open field for six months. The infected samples were from the banana plants which was infected for one month. The infection was localized only to the leaf.

Chemicals

L-tyrosine, ascorbic acid, L-DOPA were obtained from E. Merck Chemical Co., Germany. All other chemicals were of reagent grade.

Preparation and Extraction of Enzyme

All samples were frozen in liquid nitrogen and powdered in a prechilled porcelain mortar with pestle. To the frozen powdered tissue, six volumes of sodium phosphate buffer (25 mM, pH 6.8) were added and macerated for 5 min. The macerate was centrifuged at 10,000 xg and 4°C for 30 min. The supernatant was then filtered through Whatman No. 4 filter paper. The filtrate was used as a crude enzyme for L-DOPA synthe-

sis.

Enzyme Assay

The enzyme activity was routinely assayed by measuring the initial rate of dopachrome formation from the oxidation of L-DOPA, as indicated by the change in absorbance at 475 nm (Pomeranz and Li, 1970). Freshly prepared 0.98 ml of 3.8 mM L-DOPA in 25 mM phosphate buffer (pH 6.8) was mixed with 0.02 ml of PPO solution and incubated at 25°C. The absorbance change at 475 nm was measured by a Beckman DU-50 spectrophotometer. The rate of reaction was calculated from the initial linear portion of the curve. One unit caused a △OD (absorbance change) at 475 nm of 0.001/min under the specified conditions.

Enzymatic Production of L-DOPA

Enzyme reactions were initiated by addition of 1 ml PPO to a mixture of L-tyrosine (0.9 mM in various buffer as indicated in different experiments) and ascorbic acid (10 mM). The reaction mixture (10 ml) was placed in a glass vial and shaken on an orbital shaker at 250 rpm and 28°C. Periodically, 0.05 ml was withdrawn and analyzed for the production of L-DOPA.

HPLC Analysis

The concentration of L-DOPA were measured by high-performance liquid chromatography (a JASCO TRIROTAR SR-2 HPLC system equipped with JASCO UVIDEC-100-V UV detector). Using a C18 reverse-phase column (Merck, 250×4 mm), the mobile phase was a 60 mM citrate/100 mM phosphate buffer (pH 4.7) isocratic elution system. The elutate was monitored by the absorbance at 254 nm.

Results and Discussion

As shown in Fig. 1, L-DOPA and L-tyrosine were seperated by HPLC with retention times of 5.61 and 7.64 min, respectively.

Polyphenol oxidase activity from the stem and leaf of both healthy and fungi-infected banana plants were compared in Table 1. Both total activity and specific activity of PPO were in the following order: infected leaf > healthy leaf > infected stem > healthy stem. Therefore, leaf contained much higher PPO activity than stem and infected tissues generally showed increased PPO activities.

Table 2 shows the maximum yield of L-DOPA produced from L-tyrosine catalyzed by banana PPO from various tissues in 25 mM potassium phosphate buffer (pH 7.5) at 28°C. It appeared that PPO from infected tissues showed higher L-DOPA yield than the healthy one. The maximum yield of 15.9% at pH 7.5 and 28°C was obtained with the diseased leaf enzyme.

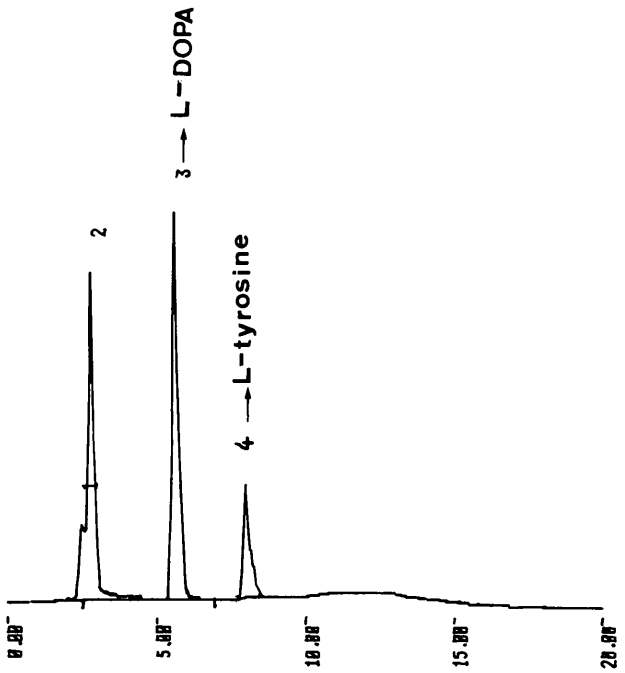

Fig. 1. The HPLC profile of L-DOPA and L-tyrosine.

Table 1. Comparison of polyphenol oxidase activity from various tissues of banana

Table 2. *L-DOPA production by different enzyme sources*

Enzyme sources	Time required for max. DOPA production (hr)	Yield of DOPA (%)
Infected leaf	36.1	15.9
Healthy leaf	25.5	9.5
Infected stem	30.8	8.1
Healthy stem	7.5	2.7

Table 3. *Temperature and pH effects on L-DOPA production by banana leaf PPO*

pH	Yield of DOPA (%)			
	28°C		40°C	
	Infected leaf	Healthy leaf	Infected leaf	Healthy leaf
4.5	16.4	9.2	8.3	4.0
5.5	36.1	16.8	–	–
6.5	17.4	9.8	–	–
7.5	15.9	9.5	–	–
9.0	–	–	–	–

– : Not detectable by the HPLC method.

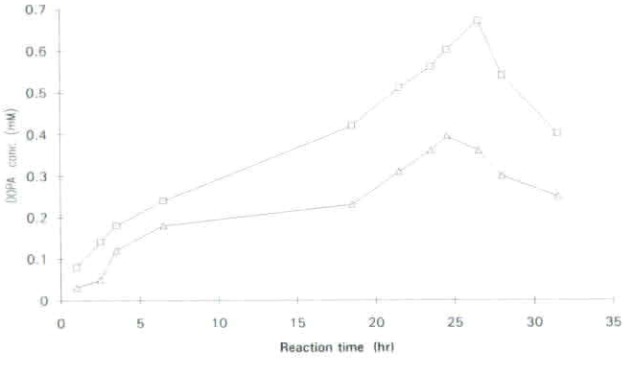

Fig. 2. Time course of L-DOPA production by banana leaf PPO in 600 mM citrate/100 mM phosphate buffer (pH 5.5); infected leaf (□-□); healthy leaf (△-△).

The maximum yield of L-DOPA were affected by the reaction temperature and pH. As shown in Table 3, the best condition for L-DOPA production is at pH 5.5 and 28°C. The maximum L-DOPA yield by PPO from infected leaf and healthy leaf were 36.1% and 16.8%, respectively.

The enzymatic production of L-DOPA was also affected by the buffer type and concentration in the reaction system (Fig. 2). When 25 mM phosphate buffer

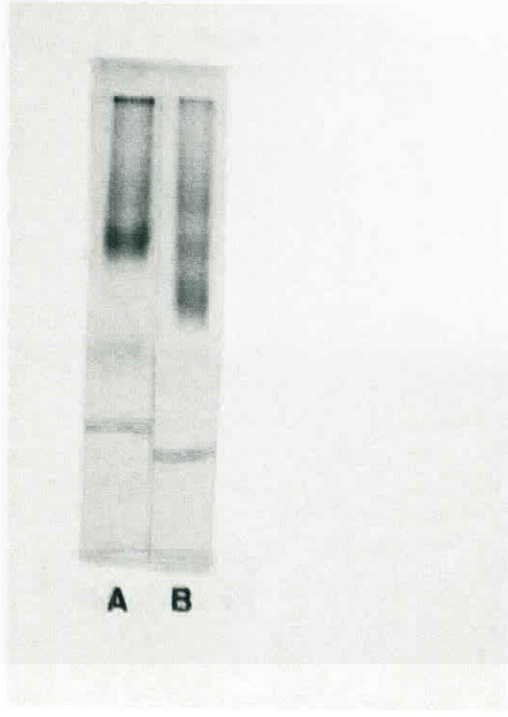

Fig. 3. PPO activity staining following electrophoresis in slab 5-20% gradient polyacrylamide gel. (A) PPO from healthy leaf; (B) PPO from infected leaf.

(pH 5.5) was replaced by 60 mM citrate/100 mM phosphate buffer (pH 5.5), we found the reaction catalyzed by PPO of infected leaf and healthy leaf can produce L-DOPA up to 67% and 39.5%, respectively. As shown in Fig. 2, the reaction time of the maximum L-DOPA production for infected leaf and healthy leaf were 26.25 h and 24.5 h, respectively. If the reaction time is futher increased, the DOPA yield decreased. It is possible that the crude enzyme preparations contained other enzymes which converted L-DOPA into other compounds and therefore decreased the yield. Synthesis of L-DOPA by purified banana leaf PPO might further increase the yield.

We have consistently found that PPO from infected leaf was much better than PPO from healthy leaf for L-DOPA production. The specific activity of infected and healthy leaf were 26,550 and 11,530 units/mg, respectively. Analysis of PPO by enzyme activity staining following electrophoresis on gradient polyacrylamide gel revealed that infected leaf contained more PPO isozymes than healthy leaf (Fig. 3). The increase of PPO activity and isozymes in the infected leaf could be due to the de novo synthesis in banana plant induced by fungi attack or the PPO secreted by

the fungi. Futher research on this phenomenon would provide insight into the role of PPO in banana defense mechanism.

In conclusion, the agrowaste banana leaf is a rich source of useful PPO, which can be used for efficient production of L-DOPA. The reaction conditions such as temperature, pH and buffer type should be optimized for the maximum production of L-DOPA. The different isozyme patterns in healthy and fungi-infected leaf would be further investigated to elucidate the possible role of PPO in banana defense mechanism.

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應用香蕉多酚氧化酶合成 L-DOPA

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農產廢棄香蕉葉與莖中之多酚氧化酶可用於生產高價值藥物 L-DOPA。於此製備方法 L-DOPA 之產率受反應溫度，pH，緩衝液種類及多酚氧化酶酵素之來源影響，四種多酚氧化酶酵素來源之 DOPA 合成率依序爲，黑星病葉＞正常葉＞黑星病假莖＞正常假莖。而於最適之狀態下，則黑星病葉之 D-DOPA 合成率可至 67%。