Journal of Food Technology 2 (1): 04-07, 2004

Study of Cultural Conditions for the Conversion of L-Tyrosine to L-DOPA by the Strain of *Aspergillus oryzae* ISB-9

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Abstract: The present study is concerned with the optimization of cultural conditions for maximum conversion of L-Tyrosine to L-DOPA by strain of *Aspergillus oryzae* ISB-9. The effect of time course, temperature and pH on the production of L-DOPA was checked. Surface culture method was employed for L-DOPA fermentation. Optimum temperature and time for L-DOPA production is 50 °C and 60 minutes respectively. However, the optimum pH of mycelium development was 5.0 because maximum production of L-DOPA (0.95 mg ml^{-1}) was observed at this pH. The acidic pH (3.0) of reaction mixture was found to be optimum for higher L-DOPA production (1.1mg ml^{-1}).

Key words: Cultural condition, conversion, L-tyrosine, strain, *Aspergillus orzae*, L-DOPA

Introduction

L-DOPA (3,4-dihydroxyphenyl-L-alanine) is a drug of choice of Parkinson's disease and for the control of changes in enzymes of energy metabolism of myocardium following neurogenic injury (Ali *et al.*, 2002). L-DOPA (3,4-dihydroxyphenyl-L-alanine) occurs naturally in seedlings, pods and beans of *Vicia faba* (Velvet beans) and in the seeds of *Mucana puriens*. L-DOPA is converted from L- tyrosine by one-step oxidation reaction catalyzed by Tyrosinase or Tyrosine hydroxylase in living organisms (Dean and Rob, 2001). Sih *et al.* (1969) was first to report the production of L-DOPA from L-tyrosine by mould cultures. The enzyme Tyrosinase is known to exist in several fungal microorganisms such as mushrooms *Agaricus bispora* (Smith and Krueger, 1961 and Bouchillouxeval *et al.*, 1963). Katsuji and Isao (1974) worked on the activation of enzyme of *Aspergillus oryzae* catalyzing conversion of L-Tyrosine to L-DOPA. The enzyme catalyzing the conversion of L-Tyrosine to 3,4-dihydroxyphenyl-L-alanine having Tyrosinase activity was well extracted from intact mycelia of *Aspergillus oryzae*. Tyrosine was presumed to be catalyzed by Tyrosinase or Tyrosinase like enzyme (Yuri Arita *et al.*, 2002). The activation of both enzymatic activities occurred in the acidic range from 2.0 to 5.0 (Haneda and Takeda, 1974). Found maximum conversion of L-Tyrosine to L-DOPA at 50 °C with mycelium in 60 minutes reaction time. In the present study, the effect of cultural conditions including time course, temperature, pH of cultivation medium as well as reaction mixture were studied for the optimum production of L-DOPA by using strain of *Aspergillus oryzae* ISB-9. For this purpose, surface culture technique was employed as the method of choice.

Materials and Methods

Organism and Culture Maintenance: *Aspergillus oryzae* strain ISB-9 was taken from Biotechnology laboratory, Department of Botany, Govt. College University, Lahore and was maintained on potato dextrose agar medium (Merck, Germany), pH 4.5, stored at 4°C in a refrigerator. Sub culturing of mould was carried out after 15 days on solidified potato dextrose agar slants (PDA).

Inoculum Preparation: The spore inoculum was used in the present study. The spores of 3-5 days old cultures were used for inoculation. The conidial suspension was prepared in sterilized 0.005% monoxal O.T (Di-octyl ester of sulpho-succinic acid). 10 ml of solution of Monoxal O.T. was added to each slant having the profused conidial growth on its surface. The test tubes were shaken vigorously for breaking the clumps of conidia. 1.0 ml of conidial suspension will be used for inoculation of 25 ml of fermentation medium in 250 ml flasks.

Fermentation Technique: Surface culture method was employed for L-DOPA fermentation. Twenty-five ml of cultivation medium (pH 5.0) containing (% w/v); glucose 2.0, polypeptone 1.0, NH_4Cl 0.3, KH_2PO_4 0.3, $MgSO_4.7H_2O$ 0.02, yeast extract 1.0 was transferred to 250 ml cotton plugged conical flask and sterilized in an autoclave at 15-lbs $inch^{-2}$ pressure (121°C) for 15 minutes. The flasks were then cooled at room temperature. The sterilized medium was seeded with one ml of spore suspension. The fungi were cultivated by incubating the flaks in an incubator at 30 °C for the period of 72 hours. The mycelium containing enzyme tyrosinase was harvested by filtration through a buchner funnel and washed free of adhering medium with ice-cold water.

Reaction Conditions: The reaction of L-DOPA production from L-Tyrosine was carried out in a suspension of intact mycelia. The mycelia were suspended in a reaction mixture (Haneda *et al.* 1973). Fifteen ml of acetate buffer (pH 3.5, 50 mM) with L-Tyrosine (2.5 mg ml^{-1}), L-Ascorbic acid (5.0 mg ml^{-1}) and intact mycelia (75.0 mg ml^{-1}) were taken in to 250 ml flask. The reaction was carried out aerobically at 50± 2°C or as otherwise stated under vigorous shaking for one hour in a hot plate with magnetic stirrer. The samples were withdrawn, centrifuged and the supernatant was used for further investigation.

Analysis: The supernatant was analyzed for the estimation of L-DOPA production and residual L-tyrosine of reaction mixture.

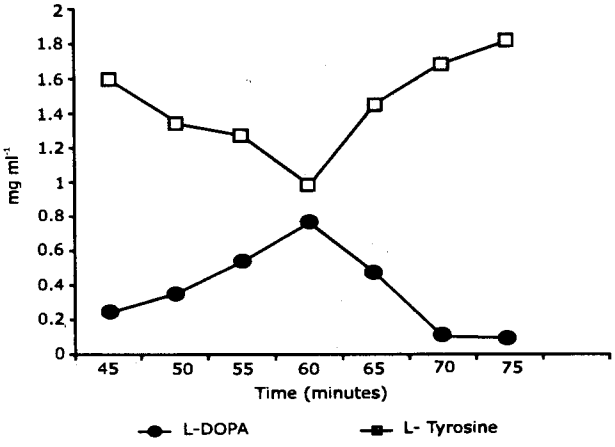
Estimation of L-DOPA: L-DOPA was estimated by the method described by Arnow (1937) by photoelectric colorimeter (AE-11) using green wratten filter of 530 nm. The amount of L-DOPA present was determined from the standard curve of L-DOPA.

Estimation of L-Tyrosine: L-Tyrosine was also determined by photoelectric colorimeter (AE-11) using green wratten filter of 530 nm. The amount of L-Tyrosine present was determined from the standard curve of L-Tyrosine.

Results and Discussion

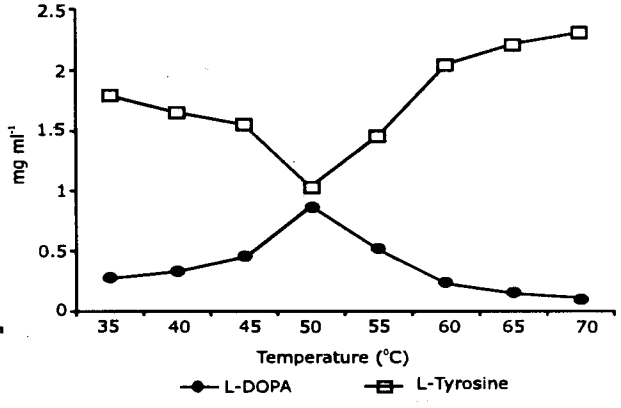
Effect of Time Course on L-DOPA Production: The effect of time course on L-DOPA production in the reaction mixture is shown in the Fig. 1. The reaction mixture was incubated for different time periods ranging from 45 to 75 minutes. Samples were estimated for L-DOPA content after each interval of 5 minutes. There was a gradual increase in L-DOPA production from 45 to 60 minutes. Maximum conversion of L-Tyrosine to L-DOPA (0.78 mg ml^{-1}) was observed in 60 minutes of reaction. After 60 minutes the conversion rate was declined. Less L-DOPA was produced below 60 minutes is due to the fact that enzyme gets insufficient time for oxidation of L-Tyrosine to L-DOPA. However, beyond 60 minutes of reaction time the L-DOPA changed in to other metabolites. This was indicated by change in colour from red to black suggesting the formation of melanin like substances.

Effect of Temperature on L-DOPA Production: The temperature of the reaction mixture is one of the critical factors that have a profound effect on L-DOPA production. The enzyme production is high at low temperature and at high temperature enzyme denatures (Ali *et al.* 2002). The production of L-DOPA from L-Tyrosine using mould mycelium as source of enzymes at different temperatures (35 -70 °C) was carried out (Fig. 2). The amount of L-DOPA produced at 35°C was 0.27 mg ml^{-1} and it was increased with increase in temperature of reaction mixture. L-DOPA production was highest (0.85 mg ml^{-1}) at 50 °C. Further increase in temperature greatly reduced the production



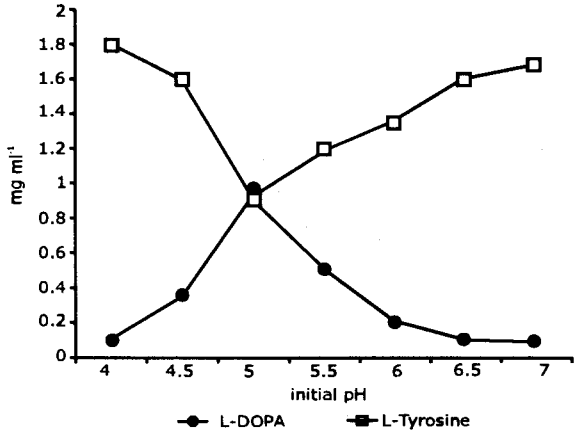
Temperature = 50 °C pH =3.0

Fig. 1: Effect of time course of the reaction mixture on the production of L-DOPA by strain of *Aspergillus oryzae* ISB-9



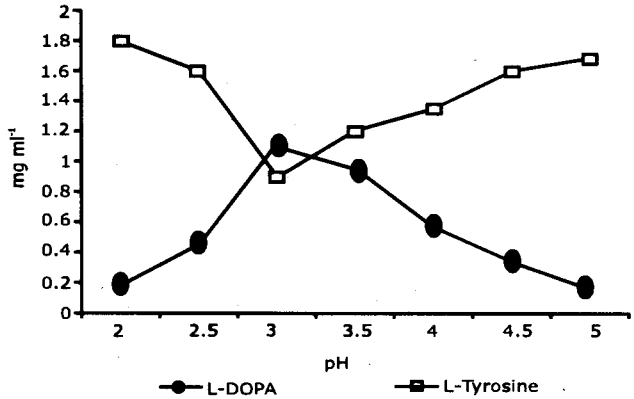
Time = 60 minutes pH =3.0

Fig. 2: Effect of temperature of the reaction mixture on the production of L-DOPA by strain of *Aspergillus oryzae* ISB-9



Time = 60 minutes Temperature = 50 °C
pH = 3.0

Fig. 3: Effect of different pH of the cultivation medium on the production of L-DOPA by strain of *Aspergillus oryzae* ISB-9



Time = 60 minutes Temperature = 50°C

Fig. 4: Effect of different pH of the reaction mixture on the production of L-DOPA by strain of *Aspergillus oryzae* ISB-9

of L-DOPA which is due to the decomposition of L-Tyrosine and L-DOPA to other metabolites as melanin. Slow rate of reaction at low temperature was due to the fact that activity of enzyme is directly related to the temperature. So at low temperature, enzyme activity is also low resulting in low L-DOPA formation. The study is related to with Haneda *et al.* (1973).

Effect of different pH of Cultivation Medium on L-DOPA Production: The effect of different initial pH was investigated on the production of L-DOPA. The pH of cultivation medium was adjusted in the range of 4.0-7.0 (Fig. 3). The maximum production (0.95 mg ml^{-1}) was obtained when pH of the cultivation medium waš adjusted at 5.0. This is due to the fact that at this pH all the metabolic pathways were operating at the optimum level and the production of enzymes including Tyrosinase, Tyrosine hydroxylase and β Tyrosinase were also very high. Further increase in pH (5.0-7.0), resulted in the decrease of L-DOPA production because all the metabolic pathways slow down resulting in low yields of L-DOPA. So the pH 5.0 is optimized for further studies. Raju *et al.*(1993) obtained maximum production of L-DOPA (0.54 mg ml^{-1}) when the pH of culture medium was 5.0.

Effect of Different pH of Reaction Mixture on L-DOPA Production: The pH of the reaction mixture greatly affects the conversion of L-Tyrosine to L-DOPA (Rosazza *et al.*1995). Different pH (2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0) was checked for the production of L-DOPA (Fig. 4). The amount of L-DOPA produced at pH 2.0 was 0.186mg ml^{-1} and it was increased with the increase of the pH. L-DOPA formation, however found to be maximum (1.1 mg ml^{-1}) at pH 3.0. Further increase in the pH of the reaction mixture greatly decreased the conversion of L-Tyrosine to L-DOPA. So the result showed that acidic pH favored the dissolution of L-Tyrosine as a substrate in the aqueous solution as well as stabilizing the L-DOPA formed. But below pH 3.0 the enzyme Tyrosinase was also inhibited, hence production of L-DOPA was decreased. In the basic range decreased production of L-DOPA was due to the fact that L-DOPA, which was obtained from L-tyrosine, immediately changed in to melanin. This work is in accordance with the finding of Loganathan, (1998) and Sharmilla *et al.*(1994).

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