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OPTIMIZATION OF CULTURAL CONDITIONS FOR THE PRODUCTION OF L-DOPA (L-3, 4 DIHYDROXY PHENYL ALANINE) FROM L-TYROSINE BY *ASPERGILLUS ORYZAE*

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Abstract

The present study is concerned with the optimization of different cultural conditions for the production of L-DOPA from L-Tyrosine by surface culture technique. Of the 12 different isolates of *Aspergillus oryzae* tested, for production of mycelium, strain ISB-9 was found to give maximum yield of L-DOPA (0.34 mg/ml). L-DOPA production was maximum at pH 3.0 of the reaction mixture, 50°C, 50 minutes and 2.5 mg/ml, optimum temperature, time and L-Tyrosine concentration, respectively

Introduction

L-DOPA (3, 4-dihydroxy phenyl-L-alanine), an amino-acid isolated from various plant sources is used in the treatment of Parkinson's disease (Lee *et al.*, 1996; Isaac *et al.*, 1997) and for controlling the changes in enzymes of energy metabolism following neurogenic injury (Raju *et al.*, 1993). L-DOPA occurs naturally in seedlings, pods and beans of *Vicia faba* and in the seeds of *Mucuna pruriens* but not found in animal body (Michael & Tsvetelina, 1997; Loganathan, 1998). The mycelium of different species of *Aspergillus* show more frequency of catalysing activity for the formation of L-DOPA from L-Tyrosine than the mushrooms (Toshiteru *et al.*, 1991). Haneda *et al.*, (1973) used *Aspergillus oryzae* for the conversion of L-Tyrosine to L-DOPA. It is produced from L-Tyrosine by the one step oxidation reaction catalyzed by enzyme tyrosinase. Rosazza *et al.*, (1995) studied the microbial synthesis of L-DOPA by surface culture and submerged fermentation. L-Tyrosine derivatives were incubated with *Aspergillus* species and the resulting L-DOPA products were isolated and characterized. Young & Luying (1998) made a study of novel biological process for L-DOPA production from L-Tyrosine.

The present study is concerned about the optimization of different cultural conditions for the production of L-DOPA from L-Tyrosine by *Aspergillus oryzae*. Since the enzyme is intracellular, thus mould mycelium was used for the biochemical transformation of L-Tyrosine to L-DOPA.

Materials and Methods

Cultures of *Aspergillus oryzae* were isolated from soil by serial dilution method (Clark *et al.*, 1958) and maintained on potato dextrose agar slants, identified after reference to Raper & Fennel (1965).

Ten ml of sterilized monoxal O.T. (Dioctyle ester of sulpho succinic acid) solution was added to 3-7 day old slant having profuse conidial growth on its surface. Clumps of conidia were separated with an inoculum needle.

Surface culture technique was employed for L-DOPA fermentation. Twenty five ml of fermentation medium containing glucose 20g, polypeptone 10g, NH_4Cl 3g, KH_2PO_4 3g, $MgSO_4$ $7H_2O$ 0.2g, Yeast extract 10g adjusted at pH 5.0 was transferred to 250 ml conical flask, 25ml/per flask. The medium was autoclaved at 15 p.s.i for 15 minutes. The flasks were then cooled at room temperature and 1ml of spore suspension was added in each treatment. The flasks were incubated at 30°C for 72 hours. There were 3 replicates of each treatment. The mycelium harvested by filtering through a funnel, washed free of adhering medium with ice cold water, was dried in filter paper folds and stored at 5°C till further use.

The reaction for L-DOPA production from L-Tyrosine was carried out in a suspension of intact mycelium (Haneda *et al.*, 1973). Fifteen ml of acetate buffer (pH 3.5, 50 mM) containing L-Tyrosine 2.5 mg/ml, L-ascorbic acid 5.0 mg/ml and intact mycelium 75 mg/ml was taken in 250 ml flask. The reaction was carried out aerobically at 50°C for 60 minutes in a hot plate with magnetic stirrer. The sample was withdrawn, centrifuged and supernatant used for estimation of L-DOPA. L-DOPA and L-Tyrosine were determined by the method of Arnow (1937).

Results and Discussion

Of the 12 isolates of *Aspergillus oryzae* tested, cultures were found to produce L-DOPA ranging from 0.125 to 0.34 mg/ml. Strain ISB-9 was found to be the best producer of L-DOPA (0.34 mg/ml) presumably because of its high tyrosinase activity. Of the different nitrogen sources (NH_4Cl, $NaNO_2$, $(NH_2)2CO$, $NaNO_3$), NH_4Cl gave maximum L-DOPA production of 0.37 mg/ml (Table 1). Raju *et al.*, (1993) obtained maximum production of L-DOPA (0.46 mg/ml) using NH_4Cl as the nitrogen source by submerged fermentation. Where glucose, surcrose, xylose, lactose were used as carbon sources, maximum production of L-DOPA (0.35 mg/ml) was recorded in the medium containing glucose (Table 1) as also observed by Raju *et al.*, (1993). Sarin *et al.*, (1980) obtained maximum production of L-DOPA (0.39 mg/ml) by using glucose as the carbon source in surface culture technique. Maximum conversion of L-Tyrosine to L-DOPA (0.39 mg/ml) was found at pH 3.0 of the reaction mixture (Table 1). At lower or higher pH, the growth of mycelium was affected, and tyrosinase activity was inhibited, resulting in decreased production of L-DOPA. This is in accordance with the findings of Haneda *et al.*, (1971) and Evans & Raper (1996). Olsen (1981) obtained maximum production of L-DOPA (0.375 ug/ml) at pH 4.0 of the reaction mixture.

Incubating the culture at (45-60°C), maximum production of L-DOPA (0.372 mg/ml) was achieved when the temperature of the reaction mixture was kept at 50°C, with the increase of temperature the conversion of L-Tyrosine to L-DOPA decreased (Table 1). Temperature is a major factor that has a great influence on the activity of enzyme. At low temperature the activity of enzyme was low and that is why production of L-DOPA was not enough. With the increase of temperature, the enzyme activity also increased and hence L-DOPA production increased. Enzyme production was maximum at 50°C of the reaction mixture, beyond which L-DOPA production greatly reduced. L-Tyrosine and L-DOPA were decomposed to metabolites such as melanin etc. Ellosiah & Rama (1989) also reported maximum L-DOPA production (0.375 mg/ml) at 50°C of the reaction mixture. Haq *et al.*, (1998) obtained maximum production of L-DOPA (0.50 mg/ml) at 60°C of the reaction mixture by submerged fermentation.

Table 1. Effect of different nitrogen, carbon sources, pH, temperature and incubation period on the production of L-DOPA by *Aspergillus oryzae* ISB-9.

Fermentation Conditions	L-Tyrosine (mg/ml) Added	Residual	Used	L-DOPA (mg/ml)
Nitrogen Sources				
NH_4Cl	2.5	1.7	0.80	0.37
$NaNO_2$	"	1.95	0.55	0.215
$(NH_2)_2CO$	"	2.14	0.36	0.33
$NaNO_3$	"	2.37	0.13	0.112
Carbon Sources				
Glucose	2.5	1.74	0.76	0.35
Sucrose	"	2.11	0.39	0.272
Xylose	"	2.24	0.26	0.16
Lactose	"	2.06	0.44	0.21
pH of Reaction Mixture				
2.5	2.5	1.97	0.53	0.186
3.0	"	1.60	0.95	0.39
3.5	"	1.82	0.68	0.315
4.0	"	2.11	0.39	0.162
Temperature (°C)				
45	2.5	1.79	0.71	0.27
50	"	1.45	1.05	0.372
55	"	1.52	0.98	0.345
60	"	2.04	0.46	0.124
Incubation Period (Minutes)				
40	2.5	1.60	0.90	0.26
50	"	1.35	1.15	0.361
60	"	1.46	1.04	0.228
70	"	1.69	0.81	0.125

Keeping the culture grown in medium containing glucose and NH_4Cl at pH 3.0 for 40-70 minutes, maximum conversion of L-Tyrosine to L-DOPA (0.361 mg/ml) was achieved after 50 minutes of the reaction which greatly reduced when the incubation period was increased beyond 50 minutes. It might be due to the fact that after 50 minutes of incubation period, L-DOPA changed into dopamine and melanin. Mason (1948) also reported the same results, with maximum (0.38 mg/ml) L-DOPA production when incubation period was increased upto 50 minutes. L-DOPA production can thus be increased by improving the cultural conditions of the reaction mixture.

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