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MICROBIAL PRODUCTION OF L-DOPA FROM L-TYROSINE

BY

PONTY ROY AND ARATI DAS *

Department of Microbiology
Bose Institute, Calcutta-700 009.

This paper reports a strain of *Aspergillus oryzae* capable of producing L-dopa from L-tyrosine by the activity of tyrosinase. The L-dopa was identified by chromatography. This oxidation activity was most effective when mycelia were 48-hrs old. The maximum of 75% conversion rate was observed with 0.5% tyrosine as the substrate.

INTRODUCTION

Microbial conversion of L-tyrosine to L-dopa was first observed by Sih *et al* (1969). Later, Singh *et al* (1973) reported the different factors effecting the production of L-dopa from L-tyrosine by a *Pseudomonad* mutant. Haneda *et al* (1973) also studied a strain of *Aspergillus oryzae* for the production of L-dopa by conversion of L-tyrosine. Shibata *et al* (1974) reported the parameters for L-dopa production by a strain of *Streptomyces*.

In view of the pharmaceutical importance of L-dopa, an investigation was carried out in this laboratory to find out a fungal strain for producing L-dopa, and the present paper describes the results observed so far.

MATERIALS AND METHODS

Strains—During the course of screening different fungal cultures (obtained from culture stock of the Department), for the probuction of L-dopa, AR5 strain of *Aspergillus oryzae* was found to be most efficient in this respect. L-dopa formation was confirmed by chromatography. This strain was used in the present investigation. The culture was maintained in 2% malt extract agar.

* For reprint request.

Media and culture methods—The basal medium used for mycelial growth of the strain contained (g/100 ml), glucose, 2; NH_4CL, 0.3; KH_2PO_4, 0.3; $MgSO_4.7H_2O$, 0.2.

Erlenemeyer flasks (250 ml), each containing 50 ml of the medium were inoculated with 5 ml of aquous spore suspension ($4\text{-}4.5 \times 10^7$ spores/ml) and incubated at 28°C in shake condition (rotating at 250 rpm). After 48 hrs of growth (unless mentioned otherwise) the mycelial mat was harvested, washed twice with ice-cold water, dried by pressing between filter papers and then stored at 4°C till further use.

Effects of mycelial age of L-dopa production—Cultures were grown in shake condition for different days and after every 24 hrs of incubation, mycelia were harvested and used for synthesis of L-dopa.

Effects of carbon sources on L-dopa production—Glucose in the basal medium was substituted with different carbohydrates in 2% concentration and the cultures were incubated at 28°C for 48 hrs. Then the mycelia were harvested and used for L-dopa synthesis as usual.

Effects of nitrogen sources on L-dopa production—Peptone, beef extract, and yeast extract were added to the nitrogen free basal medium in different concentrations individually as also in combination. After 48 hrs of incubation mycelia were harvested and L-dopa synthesis was determined.

Effects of reaction period and substrate concentration for L-dopa synthesis—L-tyrosine was added to the reaction mixture in different concentrations of 0.05-0.2% and reaction was allowed to proceed as usual. At intervals of 15, 30, 45 and 65 mins. aliquots were removed and L-dopa formation was determined.

Assay method—The synthesis of L-dopa was studied by using L-tyrosine as tne substrate. Seven grams of wet mycelia (equivalent to 1.3 g of dry weight) were suspended in 100 ml acetate buffer (pH 3.5) to which 100 mg of L-tyrosine and 200 mg of ascorbic acid were added. The reaction mixture was incubated at 45°C under vigorous shaking for 15 minutes. The supernatant was then tested for L-dopa.

Quantitative estimation of L-dopa—L-dopa was quantitatively determined by colorimetric method of Arnow (1937). One ml of the supernatant was added to 3 ml of a solution containing 1 ml of nitrite molybdate reagent, 1 ml of 0.5N HC1 and 1 ml of N NaOH. The mixture was finally made up to 5 ml with dist. water and the colour was read at 540 $m\mu$.

Chromatography—L-dopa was detected by TLC, using butanol : acetic acid : water (4 : 1 : 10) as solvent. Spots were detected after spraying with ninhydrin.

Biomass—The culture was filtered through Buchner funnel over weighed filter paper, washed with distilled water and then dried at 80°C to constant weight. The weight of dry mycelial mat was expressed as biomass in g/100 ml.

RESULTS AND DISCUSSION

Effects of mycelial age on L-dopa production—A. oryzae 5 was grown for different days and Table 1 reveals that the synthesis was maximum with mycelia taken from 48 hrs-old culture. So in all succeeding experiments cultures were grown for 48 hrs and the mycelia were harvested for L-dopa synthesis.

Effects of carbon sources on L-dopa. production—When cultures were grown in media with different carbon sources results (Table 2) indicate that glucose was the best carbohydrate for inducing the enzyme necessary for conversion of L-tyrosine to L-dopa. Maltose was also favourable for the conversion, while fructose, arabinose, xylose and sucrose were moderately effective in this respect. In similar experiments with a strain of *Streptomyces roseochromogenes*, Shibata *et al* (1974) observed maximum synthesis with mycelia growing in fructose,

Table 1. *Effects of mycelial age on L-dopa production by* A. oryzae AR5.

Age (hrs)	L-dopa (mg/100ml)
0	—
24	10.5
48	25.5
72	20.3
96	8.1

Table 2. *Effects of carbon sources on the production of L-dopa by* A. oryzae AR5.

Carbohydrate sources	Concentration %	Mycelial weight (mg/100 ml)	L-dopa (mg/100 ml)
Glucose (control	2	280	25.0
Maltose	"	285	20.0
Fructose	"	260	18.5
Arabinose	"	180	17.6
Xylose	"	195	16.5
Sucrose	"	202	15.0

Table 3. *Effects of nitrogen sources on the production of L-dopa by* A. oryzae AR5.

Nitrogen sources	Concentration (%)	L-dopa (mg/100 ml)
NH_4Cl (control)	0.1	20.7
	0.3	26.2
	0.5	25.0
Peptone	0.25	15.35
	1.0	36.50
Yeast extract	0.25	1.2
	1.0	2.2
Beef extract	0.25	11.0
	1.0	11.5
Y. extract + B. extract + Peptone	1.0 + 0.25 + 0.25	38.8

Tahlc 4. *Effects of substrate (L-tyrosine) concentration and reaction time on the production of L-dopa*

L-tyrosine (mg/100 ml)	Reaction time (minutes)							
	15		30		45		60	
	A	B	A	B	A	B	A	B
50	14.1	28.2	22.2	44.4	30.0	60.0	37.5	75.0
100	28.5	28.5	38.5	38.5	56.2	56.2	65.7	65.7
150	30.3	20.2	42.0	28.0	78.0	52.0	93.5	62.33
200	60.5	30.3	65.2	32.6	90.0	45.0	112.2	56.0

A = L-dopa (mg/100 ml)
B = % conversion of L-dopa

followed by glucose and mannose. Maltose was favourable for vegetative growth but unfavourable for the synthesis of L-dopa.

Effects of nitrogen sources on L-dopa production—Cultures were grown in media containing different nitrogen sources and Table 3 describes that peptone was most effective for the induction of the enzyme tyrosinase. This stimulatory effect could not increase appreciably when a combination of yeast extract and beef extract was used together with peptone to serve as the nitrogen source for mycelial growth.

Effects of reaction period and substrate concentration for L-dopa synthesis—Experiments were carried out and results are presented in Table 4. It indicates

that the rate of L-dopa production increased gradually with increase in the time of reaction. However, the maximum of 75% conversion rate was obtained with 0.5% tyrosine as the substrate.

So the series of experiments carried out in the present investigation indicate that the strain *A. orgzae* AR5 is an active producer of L-dopa from L-tyrosine. Further work is in progress to improve the production level by way of mutagenesis. Results will be reported only.

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