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Microbiological Transformation of L-Tyrosine to 3,4-Dihydroxyphenyl L-Alanine (L-Dopa) by a Mutant Strain of *Aspergillus oryzae* UV-7

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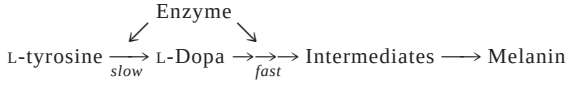
Abstract. The present study deals with the microbiological transformation of L-tyrosine to 3,4-dihydroxyphenyl L-alanine by a mutant strain of *Aspergillus oryzae* UV-7. Sixteen different mutant strains of *Aspergillus oryzae* (GCB-6) were isolated through UV-irradiation. These mutant strains were screened for the production of mold mycelia by submerged fermentation in 250-ml Erlenmeyer flasks. Of all the mutant strains examined, UV-7 gave maximum production of L-dopa (1.28 mg/ml). The reaction was carried out using mold mycelium as a source of enzyme tyrosinase in shake flasks. The maximum production of L-dopa was obtained when glucose (25 mg/ml) was used as the carbon source and NH_4Cl (3 mg/ml) was used as the nitrogen source. The optimum pH for mycelium development was 5.0; L-dopa production was maximum at pH 3.5 of the reaction mixture. The reaction by mold mycelium (75 mg/ml) was carried out under acidic conditions. Optimum temp, time, and L-tyrosine concentration were 55°C, 60 min, and 3.0 mg/ml, respectively.

L-Dopa (3,4-dihydroxyphenyl L-alanine) is an amino acid isolated from various plant sources (seedlings, pods, and beans of *Vicia faba* and seeds of *Mucana pruriens*), but not found in the animal body [6]. It is a drug of choice for controlling the changes in enzymes of energy metabolism of myocardium following neurogenic injury and also for the treatment of Parkinson's disease [7]. The production of L-dopa from L-tyrosine by fungi was first reported by Sih et al. [14]. Haneda et al. [5] used *Aspergillus oryzae* for the conversion of L-tyrosine to L-dopa. The oxidation of L-tyrosine to L-dopa is catalyzed by three different types of enzymes: tyrosine hydroxylase, tyrosinase, and β-tyrosinase [4]. Tyrosinases are also known to exist in several fungal microorganisms such as *Neurospora crassa* and *Agaricus bispora*, which were used for production of L-dopa from L-tyrosine [12].



In microorganisms, tyrosinase activity is generally very weak and L-tyrosine and L-dopa are rapidly decomposed to other metabolites. Thus, stoichiometric formation of L-dopa from L-tyrosine was difficult to achieve [1]. The present work is concerned with the microbiological transformation of L-tyrosine to L-dopa (3,4-dihydroxyphenyl L-alanine) by mutant strains of *Aspergillus oryzae*. The enzyme was intracellular; thus, mold mycelium after harvesting from the fermented broth in shake flasks was used for biochemical conversion of L-tyrosine to L-dopa.

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Materials and Methods

Organism and cultivation of mycelium. *Aspergillus oryzae* strain GCB-6 was used for present study. The submerged culture method [11] was employed for cultivation of the mycelium.

Improvement of strain after UV-irradiation. The *Aspergillus oryzae* strain GCB-6 was improved after UV-irradiation.

a) Conidia from 4- to 7-day-old cultures were harvested in phosphate buffer containing (g/L): K_2HPO_4 3.5; KH_2PO_4 1.5 at pH 7.2. The spore suspension was exposed to UV-irradiation for different time intervals (15–120 min), aseptically. The irradiated spore suspension was poured on agar-malt extract with tyrosine plates [3].

b) *Aspergillus oryzae* strain UV-3, a hyper producer of L-dopa obtained after UV-irradiation was used for mycelial mutation. Vogel medium, 100 ml, containing (g/L): trisodium citrate, 2.5; NH_4NO_3, 2.0; KH_2PO_4, 5.0; $(NH_4)_2SO_4$, 4.0; $MgSO_4 . 7H_2O$, 0.2; peptone, 2.0; yeast extract, 1.0 at pH 5.5 with glass beads in a 1-L cotton wool-plugged conical flask sterilized at 15 lb/inch^2 (121°C) for 15 min. A small amount of spores from the slant (3–5 days old) was aseptically transferred with the help of an inoculating needle to the flask. The flask was

Table 1. Screening of mold cultures after UV-irradiation for the production of L-dopa in shake flasks

Aspergillus oryzae/Mutant Strains	L-Dopa (mg/ml)
UV-1	0.92
UV-2	1.045
UV-3	1.015
UV-4	0.94
UV-5	0.98
UV-6	0.80
UV-7	1.28
UV-8	1.115
UV-9	1.13
UV-10	1.00
UV-11	1.04
UV-12	1.08
UV-13	1.06
UV-14	1.18
UV-15	0.992
UV-16	1.095

Incubation time = 60 min.
Temperature = 50°C.
pH = 3.5.

incubated at 30°C in an incubator shaker at 200 rpm for 24 h. The inoculum was kept homogeneous, and the optical density was maintained at 1.0 with the help of a photoelectric colorimeter, with a 530-nm filter. Five milliliters of the vegetative inoculum was placed in a petri plate, and then UV treatment was given from 15 to 25 min intervals, following the method of Pontecarvo et al. [10]. The mutant cultures were incubated at 30°C for 3–4 days for maximum sporulation.

Reaction procedure and estimation method. The reaction for L-dopa production was carried out in a suspension of intact mycelium [5]. L-Dopa and L-tyrosine were determined by the methods of Arnow [2].

Results and Discussion

Screening of mutant strains of *Aspergillus oryzae*. The data of Table 1 show the production of L-dopa by 16-different mutant strains of *Aspergillus oryzae* GCB-6 isolated through UV-irradiation. These mutant strains were screened for the production of mold mycelium by submerged fermentation in 250-ml Erlenmeyer flasks. The mutant strains of *Aspergillus oryzae* were found to produce L-dopa ranging from 0.80 to 1.28 mg/ml. Of all the mutant strains examined, *Aspergillus oryzae* UV-7 gave maximum production of L-dopa (1.28 mg/ml), while UV-14 and UV-9 gave 1.18 mg/ml and 1.13 mg/ml production of L-dopa, respectively. The other mutant strains produced relatively a smaller amount of L-dopa. The mutant strain of *A. oryzae* UV-7 was found to be the best producer of L-dopa from L-tyrosine and it was selected for further investigations.

Effects of different carbon sources. The effect of different carbon sources (glucose, sucrose, maltose, xylose, lactose, glycerol) on the production of L-dopa from L-tyrosine by fungal mycelium is shown in Fig. 1. The best yield of L-dopa (1.32 mg/ml) was obtained when glucose was used as a carbon source. However, there was decrease in the production of L-dopa when sucrose, maltose, lactose, or glycerol was used as a carbon source. Minimum production was obtained with glycerol, i.e., 0.26 mg/ml. Glucose is the best source of carbon because by using this carbon source in the cultivation medium, the tyrosinase activity increased, resulting in greater production of L-dopa. The conversion of L-tyrosine to L-dopa declined with glycerol or lactose as the sole carbon sources. The study is in agreement with the report by Sarin et al. [13]. However, our finding is different from that of Lee et al. [7], who reported sucrose as the best source for L-dopa production.

Effect of initial pH on cultivation of *Aspergillus oryzae*. The effect of different initial pH (3.0–7.0) of the cultivation medium on the production of L-dopa was studied (Fig. 2). There was a gradual increase in L-dopa production from pH 3.0 to 5.0 of the cultivation medium. Maximum production (1.29 mg/ml) was obtained when the pH of the cultivation medium was adjusted at 5.0. Further increase in pH (6.0 and 7.0) resulted in decreased production of L-dopa. The optimum pH for the growth of fungi is 5.0–5.5. At this pH, the growth of the *Aspergillus oryzae* was maximum and all the metabolic pathways were operating at an optimum. The production of enzymes including tyrosinase, tyrosine hydroxylase, and β-tyrosinase was also very high. These enzymes catalyzed the oxidation of L-tyrosine to a greater extent, and more L-dopa was produced. Increase or decrease in pH disturbed fungal physiology; enzyme production was decreased and hence L-dopa was also decreased. Haneda et al. [5] obtained maximum production of L-dopa (0.84 mg/ml) when the pH of the cultivation medium was 5.0.

Effect of incubation period. The effect of the incubation period on L-dopa formation in the reaction mixture from L-tyrosine with mold mycelium as the source of enzyme is shown in Fig. 3. The reaction mixture was incubated at 55°C for different time periods ranging from 45 to 75 min. Samples were estimated for L-dopa content after each interval of 5 min. There was a gradual increase in L-dopa production from 45 to 60 min. Maximum conversion of L-tyrosine to L-dopa was observed during 60 min of reaction (1.26 mg/ml). After 60 min the conversion rate declined. At an incubation period below 60 min, the lower L-dopa production was due to the fact that the enzyme has insufficient time for oxidation of L-tyrosine to L-dopa. At an incubation period beyond 60 min, the production decreased because L-dopa was changed into other metabolites. This was indicated by a

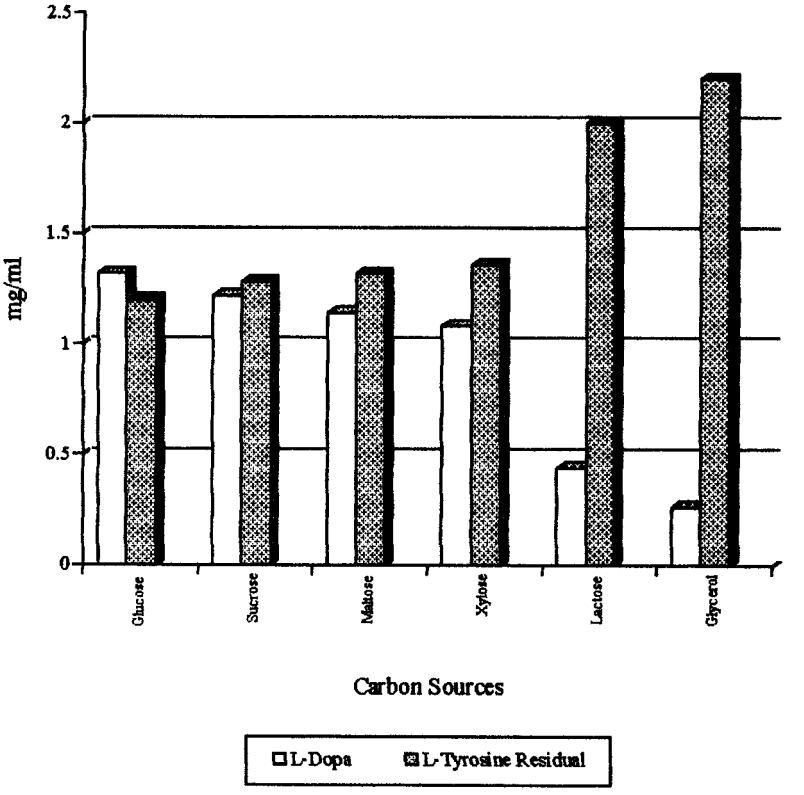

Fig. 1. Effect of the addition of different carbon sources on the production of L-dopa by *Aspergillus oryzae* UV-7.

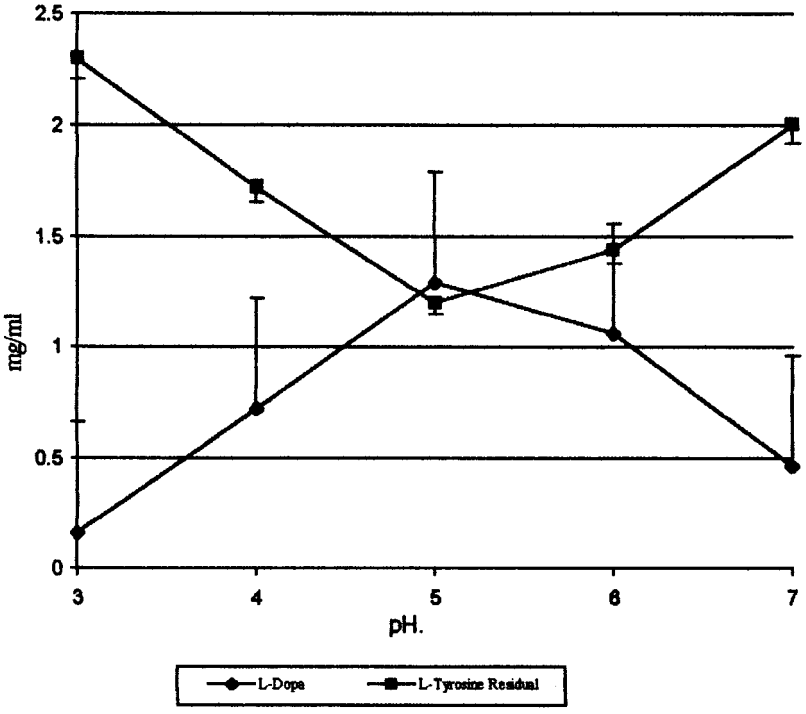

Fig. 2. Effect of the initial pH of culture medium on the production of L-dopa by *Aspergillus oryzae* UV-7.

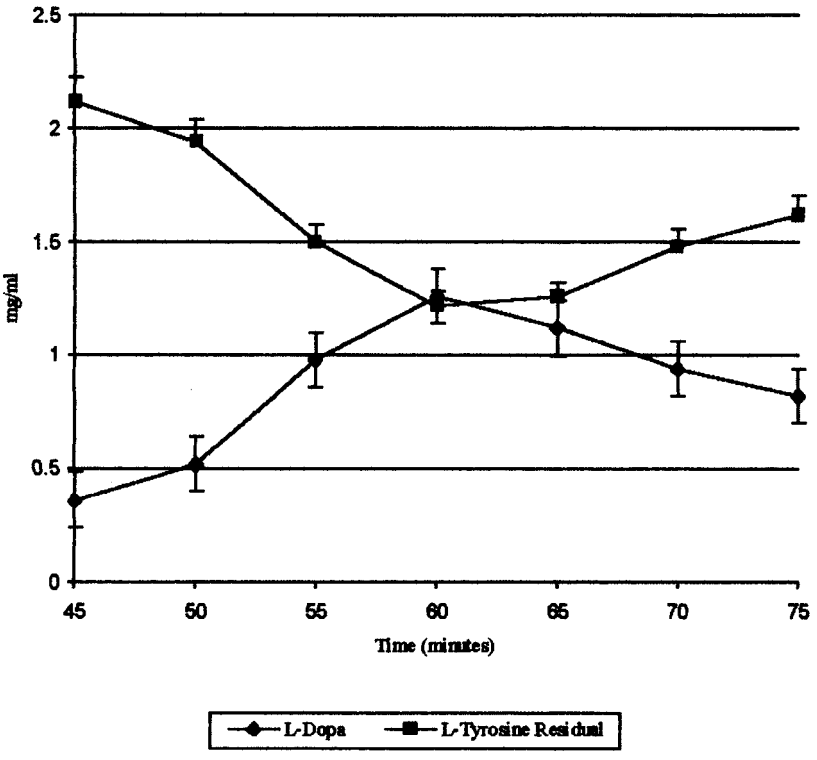

Fig. 3. Effect of time on rate of production of L-dopa by *Aspergillus oryzae* UV-7.

change in color from red to black, suggesting the formation of a melanin-like substance. Mason [8] reported that L-dopa production was low in a short incubation period (30 min). When the incubation period was increased to 60 min, L-dopa production was highest (1.30 mg/ml). By further increase in the incubation period (60–70 min), L-dopa production decreased.

Effect of incubation temperature. The production of L-dopa from L-tyrosine with mold mycelium as the source of enzymes at different temperatures (35–70°C) was carried out (Fig. 4). The amount of L-dopa produced at 35°C was 0.31 mg/ml, and it increased with increase in the temperature of the reaction mixture. L-Dopa production was maximum at 55°C (1.28 mg/ml). Further increase in the temperature of the reaction mixture greatly reduced the production of L-dopa from L-tyrosine. Thus, the optimum temperature for L-dopa production was found to be 55°C. The slow rate of reaction at lower temperatures was due to the fact that the activity of enzyme is directly related to the temperature. At low temperature, the activity was low, hence the production was minimal. Decrease in production above 55°C temperature may be due to the decomposition of L-tyrosine and L-dopa at higher temperatures to other metabolites such as melanin.

Effect of L-tyrosine concentration. The effect of different concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mg/ml) of L-tyrosine on the production of L-dopa by mold cultures in the reaction mixture was studied (Fig. 5). There was a gradual increase in L-dopa formation from a concentration of 1.0 mg/ml to 3.0 mg/ml. Further increase in the concentration of L-tyrosine resulted in a decrease of the metabolite. The optimum L-tyrosine concentration for L-dopa production (1.32 mg/ml) was 3.0 mg/ml. The maximum production of L-dopa at 3.0 mg/ml of L-tyrosine may be due to an increase in the ratio between the active sites of an enzyme and the substrate concentration. With a higher ratio the competition of substrate (L-tyrosine) for active sites of the enzyme was increased, hence production of L-dopa was decreased. L-Dopa formation proceeded with the best yield when the concentration of L-tyrosine as a substrate in the reaction mixture was 2.0 to 3.0 mg/ml. Lee [7] described the maximum production of L-dopa at 2.5 mg/ml concentration of L-tyrosine in the reaction mixture. Hence, our finding is more encouraging than that of Lee [7].

Effect of pH on biochemical conversion. The production of L-dopa from L-tyrosine with mold mycelium as the source of enzyme at different pHs (2.0, 2.5, 3.0, 4.0, 4.5, 5.0) was carried out (Fig. 6). The amount of L-dopa produced at pH 2.0 was 0.18 mg/ml, and it increased with increase in the pH. L-Dopa formation, however, was found maximum at pH 3.5, i.e., 1.24

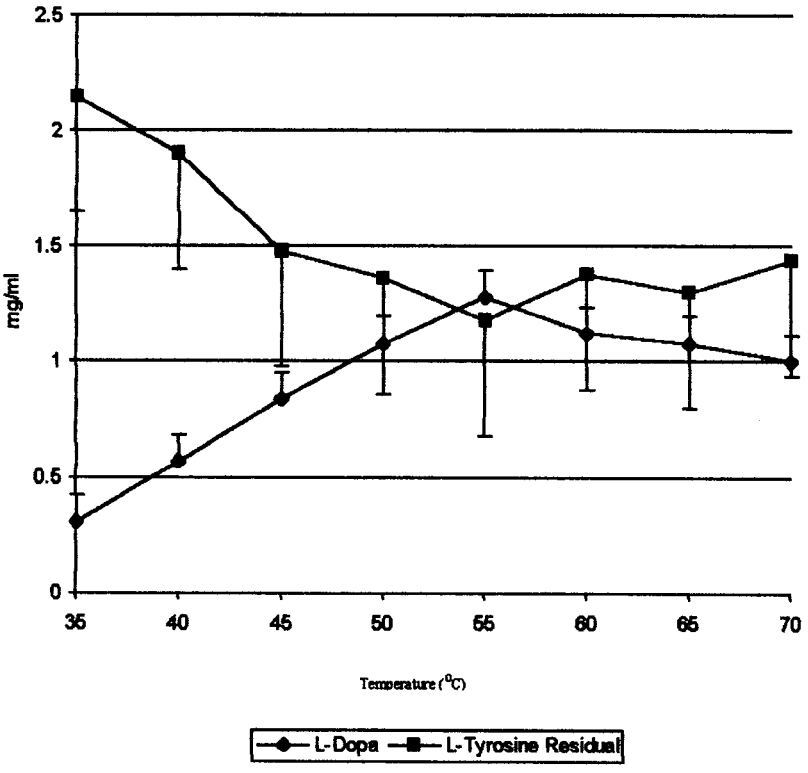

Fig. 4. Effect of different temperatures of the reaction mixture on the production of L-dopa by *Aspergillus oryzae* UV-7.

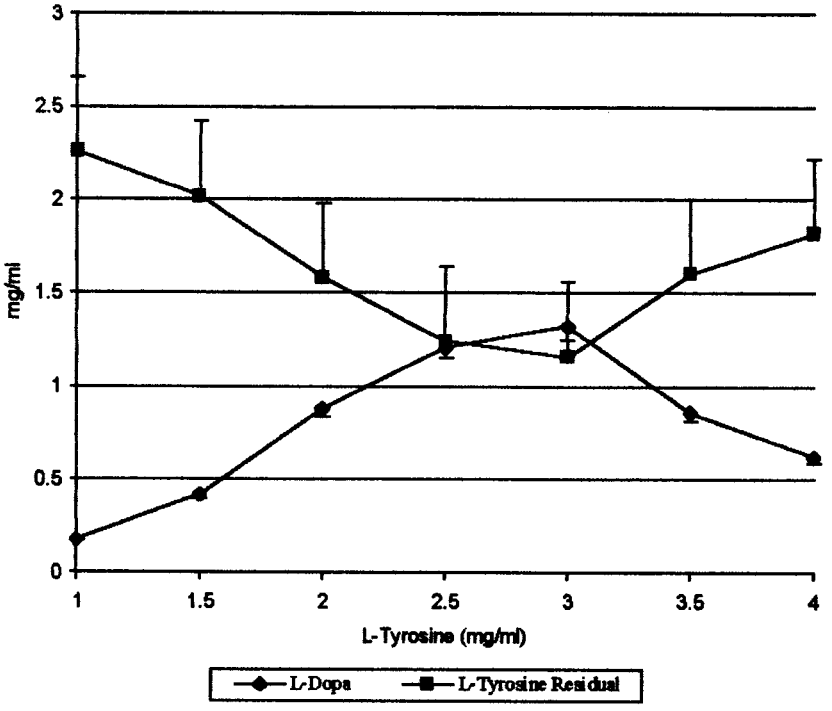

Fig. 5. Effect of the addition of different concentrations of L-tyrosine on the production of L-dopa by *Aspergillus oryzae* UV-7.

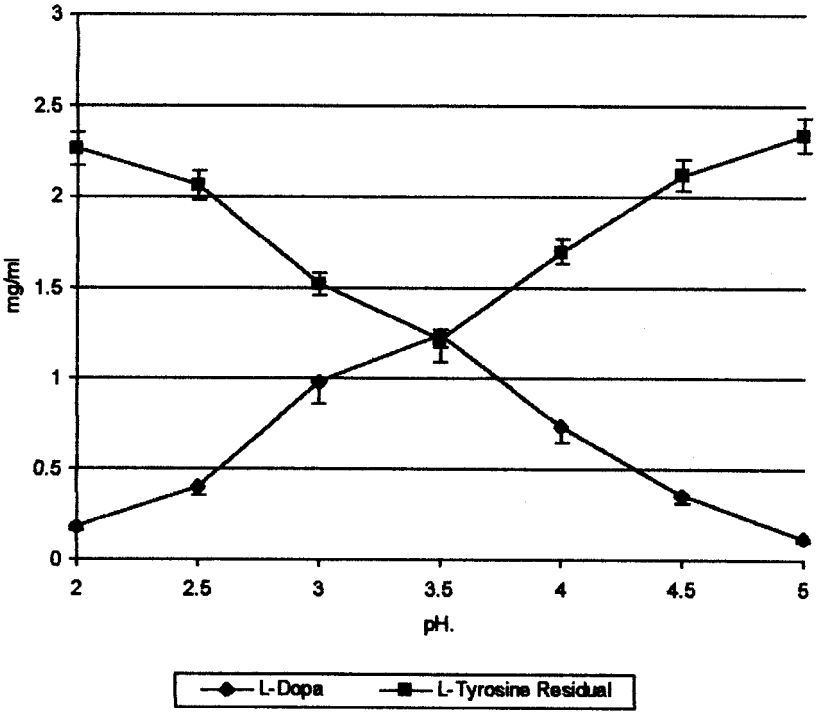

Fig. 6. Effect of different pHs of the reaction mixture on the production of L-dopa by *Aspergillus oryzae* UV-7.

mg/ml. Further increase in pH resulted in decrease of L-dopa formation. Thus, the optimum pH was found to be 3.5. The results showed that acidic pH favored the dissolution of L-tyrosine as a substrate in the aqueous solution as well as stabilization of the L-dopa formed. However, with increase in the pH beyond 5.0, very little or no activity was observed. In the basic pH range, decreased production of L-dopa was due to the fact that the L-dopa obtained from L-tyrosine immediately changed into melanin. Below pH 3.5, the enzyme tyrosinase was also inhibited, hence production of L-dopa was decreased. This work is in accordance with the findings of Haneda et al. [5]. However, Olsen [9] obtained maximum production of L-dopa (375 μg/ml) at pH 4.0 of the reaction mixture.

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