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Synthesis of L-Tyrosine or 3,4-Dihydroxyphenyl-L-alanine from DL-Serine and Phenol or Pyrocathechol†

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Reaction conditions for the synthesis of L-tyrosine or L-dopa from DL-serine and phenol or pyrocatechol were studied with intact cells of *Erwinia herbicola* (ATCC 21434) containing high tyrosine phenol lyase activity. The optimum pH for this reaction was around 8.0, and the optimum temperature range was between 37～40°C for the synthesis of L-tyrosine and between 15～25°C for that of L-dopa. Sodium sulfite and EDTA were added to protect the synthesized L-dopa from decomposition. As high concentrations of phenol or pyrocatechol denatured the enzyme, each substrate was fed to maintain the optimum concentration during incubation.

The reaction mixture (100 ml) containing 4.0 g of DL-serine, 1.0 g of phenol or 0.7 g of pyrocatechol, 0.5 g of ammonium acetate and the cells, was incubated. During incubation, phenol or pyrocatechol was fed at intervals to maintain the substrate at the initial concentration. 5.35 g of L-tyrosine or 5.10 g of L-dopa was synthesized in 100 ml of the reaction mixture.

In the previous paper of this series, we reported the occurrence of tyrosine phenol lyase in bacteria and cultural conditions to prepare cells of high enzyme activity with *Erwinia herbicola* ATCC 21434 as a likely strain. The enzyme of *Erwinia herbicola* has been crystallized and its properties were investigated.[1～4]

This paper deals with the reaction conditions for synthesizing L-tyrosine and L-dopa with intact cells of *Erwinia herbicola* ATCC 21434.

EXPERIMENTAL

Chemicals. All chemicals used in this work were commercial products. L-Dopa was purchased from Daiichi Kagaku Company, Ltd., Tokyo.

Microorganism. *Erwinia herbicola* ATCC 21434 was used as the enzyme source.

Preparation of intact cells. Intact cells containing high tyrosine phenol lyase activity were prepared as previously described.[3]

Enzyme assay. Tyrosine phenol lyase activity in the intact cells was assayed by measuring the amount of L-dopa synthesized from DL-serine and pyrocatechol under the conditions described in the previous paper.[3]

Synthesis of L-tyrosine or L-dopa. A reaction mixture (10 ml) containing 200 mg of DL-serine, 100 mg of phenol or 70 mg of pyrocatechol, 50 mg of ammonium acetate and cells (about 100 mg as wet cell) harvested from 10 ml of culture broth, was incubated at 15～25°C to synthesize L-dopa, or 37～40°C to synthesize L-tyrosine, to which phenol or pyrocatechol was fed at intervals. To synthesize L-dopa, 20 mg of sodium sulfite and 10 mg of EDTA were added to protect the L-dopa from decomposition.

Analytical method. (1) Amino acids synthesized by this method were qualitatively detected by the ninhydrin reaction on a paper chromatogram developed with a solvent system of butanol: acetic acid: water (4: 1: 2).

(2) L-Tyrosine and L-dopa were determined as previously described.[2]

(3) Pyruvic acid was measured using the method of Friedeman and Haugen.[5]

(4) Serine was analyzed with a Yanagimoto model LC–5S automatic amino acid analyzer.

† Microbiological Synthesis of L-Tyrosine and 3,4-Dihydroxyphenyl-L-alanine. Part IV.

(5) Phenol and pyrocatechol were determined by the modified method of Porteous and Williams.[6]

RESULTS

1) Optimum pH. The relative activity for L-tyrosine or L-dopa synthesis at various pH values was determined by adjusting the pH with ammonia. The optimum pH for L-tyrosine synthesis was at about 8.0 and that for L-dopa was around 8.4, as shown in Fig. 1.

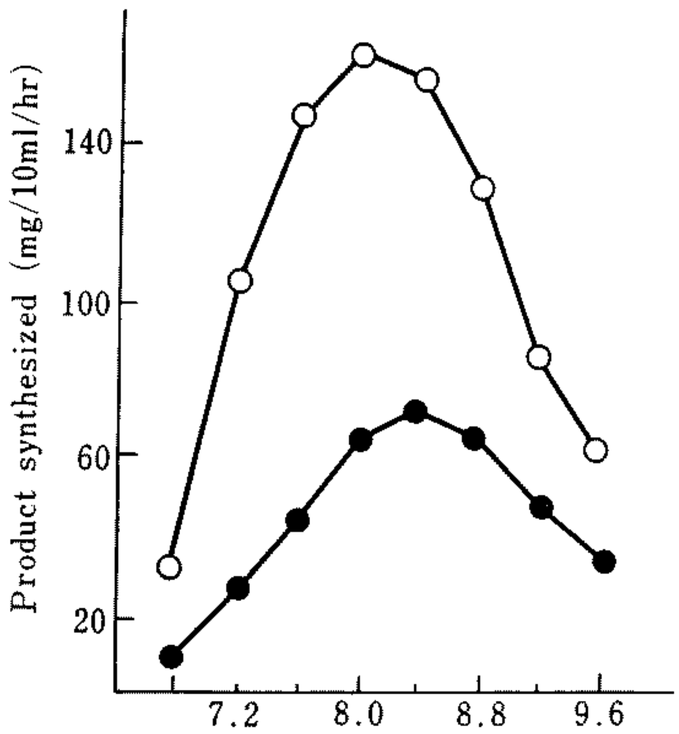


FIG. 1. Effect of pH on L-Tyrosine or L-Dopa Synthesis.
The synthesis of L-tyrosine (○—○) or L-dopa (●—●) was carried out at 37°C or 30°C for 1 hr in the same reaction mixture as described in the text. The pH of the mixtures was adjusted as indicated, by adding ammonia.

2) Optimum temperature. The effect of temperature on L-tyrosine or L-dopa synthesis was examined. The optimal temperature for L-tyrosine synthesis was between 35°C and 40°C. The optimum temperature for L-dopa synthesis, on the other hand, was around 25°C, as shown in Fig. 2.

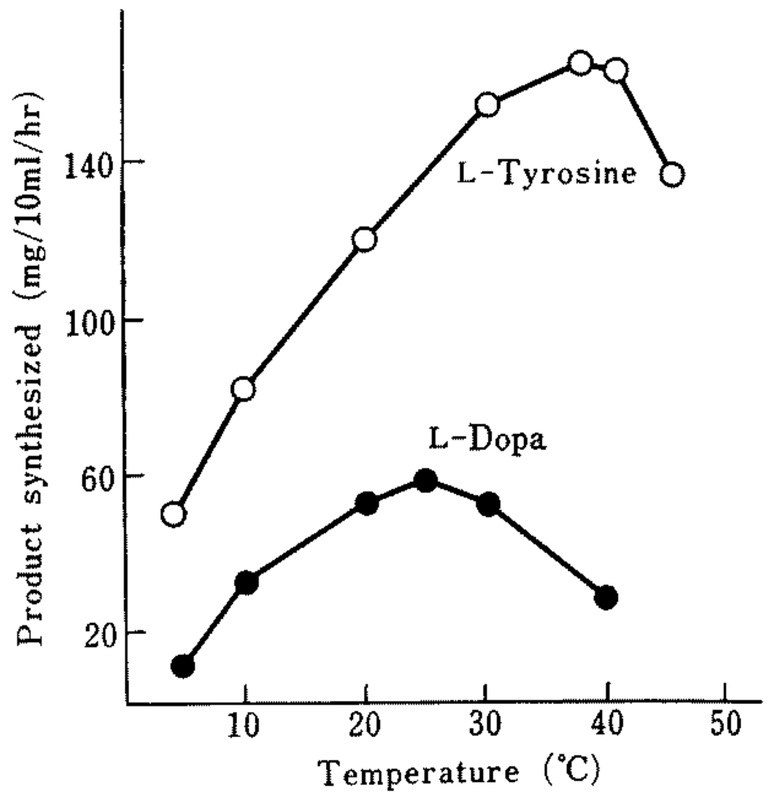


FIG. 2. Effect of Temperature on L-Tyrosine or L-Dopa Synthesis.
The synthesis of L-tyrosine (○—○) or L-dopa (●—●) was carried out at various temperatures as indicated, for 1 hr in the same reaction mixture (pH 8.0) as described in the text.

3) Effects of salts and neutralizing agents on L-dopa synthesis. Ichihara *et al.*, with a cell free extract prepared from *Bacterium coli phenologenes*, examined the cation requirements for tyrosine phenol lyase.[7] Kumagai *et al.*[8] reported that the crystalline enzyme obtained from *E. intermedia* A–21 also required K^+ and NH_4^+ for the maximum activity of pyruvate formation from L-tyrosine. The requirement of various ions for the synthesis of L-dopa by tyrosine phenol lyase was examined here using a variety of salts and neutralizing agents. As shown in Table I, ammonium salts; ammonium chloride, am-

TABLE I. EFFECT OF SALTS AND NEUTRALIZING AGENTS ON L-DOPA SYNTHESIS.
The reactions were carried out at 22°C for 1 hr in 10 ml of the reaction mixture (pH 8.0) described in the text. Various salts and neutralizing agents were added at the concentrations of 0.2 (○—○) and 0.5% (●—●).

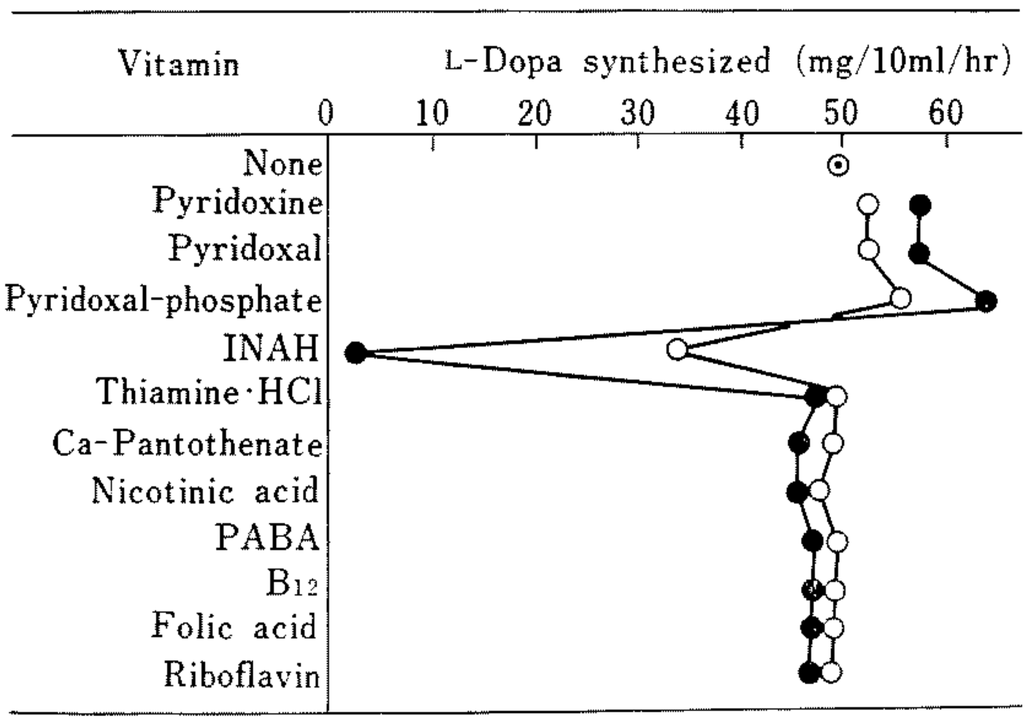
Neutralizing agent	Salt	L-Dopa synthesized (mg/10ml/hr) 0 10 20 30 40 50
KOH	None	⊙
	KCl	
	KH_2PO_4	
	NH_4Cl	
	$(NH_4)_2HPO_4$	
	NH_4NO_3	
	CH_3COONH_4	
NaOH	None	⊙
	KCl	
	KH_2PO_4	
	NH_4Cl	
	$(NH_4)_2HPO_4$	
	NH_4NO_3	
	CH_3COONH_4	
NH_4OH	None	⊙
	KCl	
	KH_2PO_4	
	NH_4Cl	
	$(NH_4)_2HPO_4$	
	NH_4NO_3	
	CH_3COONH_4	

monium phosphate, ammonium nitrate and ammonium acetate stimulated L-dopa synthesis, but potassium salts; potassium chloride and potassium phosphate did not. Of the neutralizing agents tested, ammonia was the best to accelerate this reaction.

4) Effects of vitamins on L-dopa synthesis. Tyrosine phenol lyase required pyridoxal phosphate as a cofactor.[9~11] The effects of various vitamins added to a reaction mixture on L-dopa synthesis were examined. The result is shown in Table II. The B_6-vitamins;

TABLE II. EFFECT OF VITAMINS ON L-DOPA SYNTHESIS.

The reactions were carried out under the same reaction conditions as described in Table I. Various vitamins were added at the concentrations of 10 (○—○) and 1000 μg/liter (●—●).



pyridoxine, pyridoxal and pyridoxal-phosphate, slightly accelerated the L-dopa synthesis. This enzyme activity was remarkably inhibited isonicotinic acid hydrazide which is known to be a inhibitor of pyridoxal-phosphate dependent enzymes. Other vitamins were not effective.

5) Effects of reducing and chelating agents on L-dopa synthesis. To prevent the decomposition of synthesized L-dopa during the reaction, the effects of reducing or chelating agents on L-dopa synthesis was examined. The results are shown in Tables III and IV. Sodium sulfite,potassium sulfite or ammonium sulfite increased the amount of L-dopa synthesized, but no effect was found with thiosulfates, ascorbic acid or cysteine. Since

TABLE III. EFFECT OF REDUCING AGENTS ON L-DOPA SYNTHESIS.

The reactions were carried out under the same reaction conditions as described in Table I, except that sodium sulfite was replaced by various reducing agent indicated. Concentrations of reducing agents were 0.2 (○—○) and 0.5% (●—●).

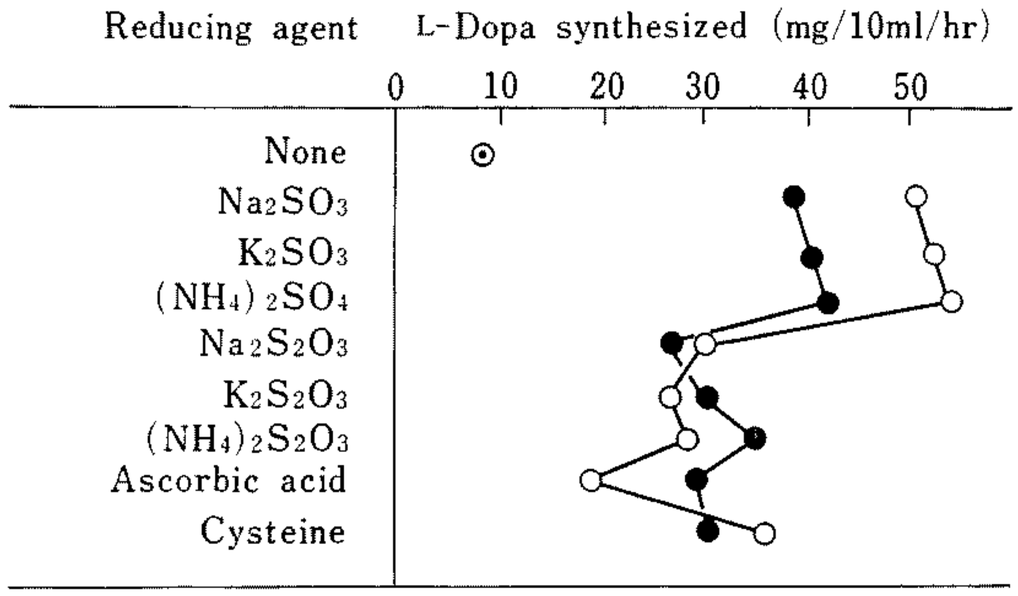
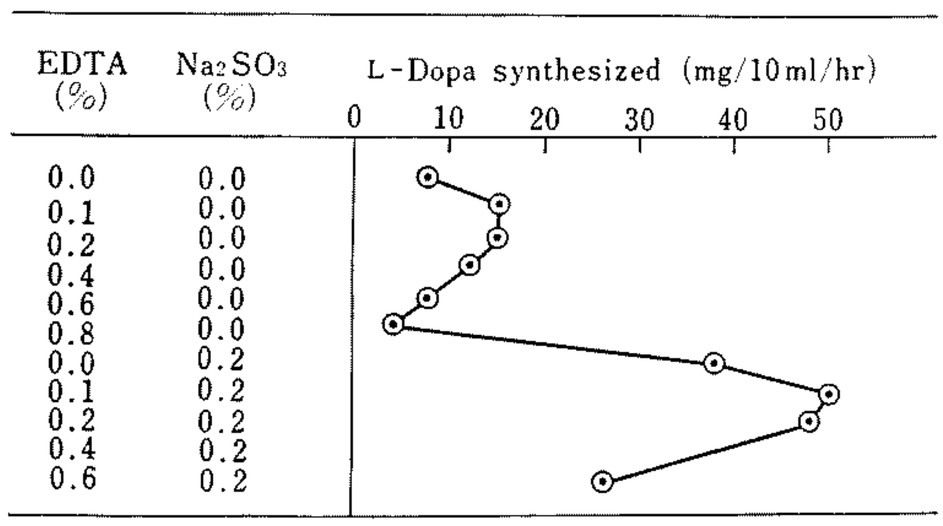


TABLE IV. EFFECT OF EDTA ON L-DOPA SYNTHESIS.

The reactions were carried out in the same reaction conditions described in Table I, except that the concentrations of EDTA and sodium sulfite were varied as indicated.



potassium sulfite and ammonium sulfite were very deliquescencible and unstable to change in potassium sulfate and ammonium sulfate, sodium sulfite was used thereafter to prevent the decomposition of L-dopa. Table IV shows that the addition of EDTA as a chelating agent accelerated L-dopa synthesis at the concentration of 0.1~0.2%. Synthesis of L-dopa was further accelerated in the presence of EDTA together with Na_2SO_3.

6) *Effect of substrate concentration on L-tyrosine or L-dopa synthesis.* Since phenol and pyrocatechol are protein denaturating agents, the optimum concentration of phenol or pyrocatechol in a reaction mixture was examined to obtain the maximum yield of

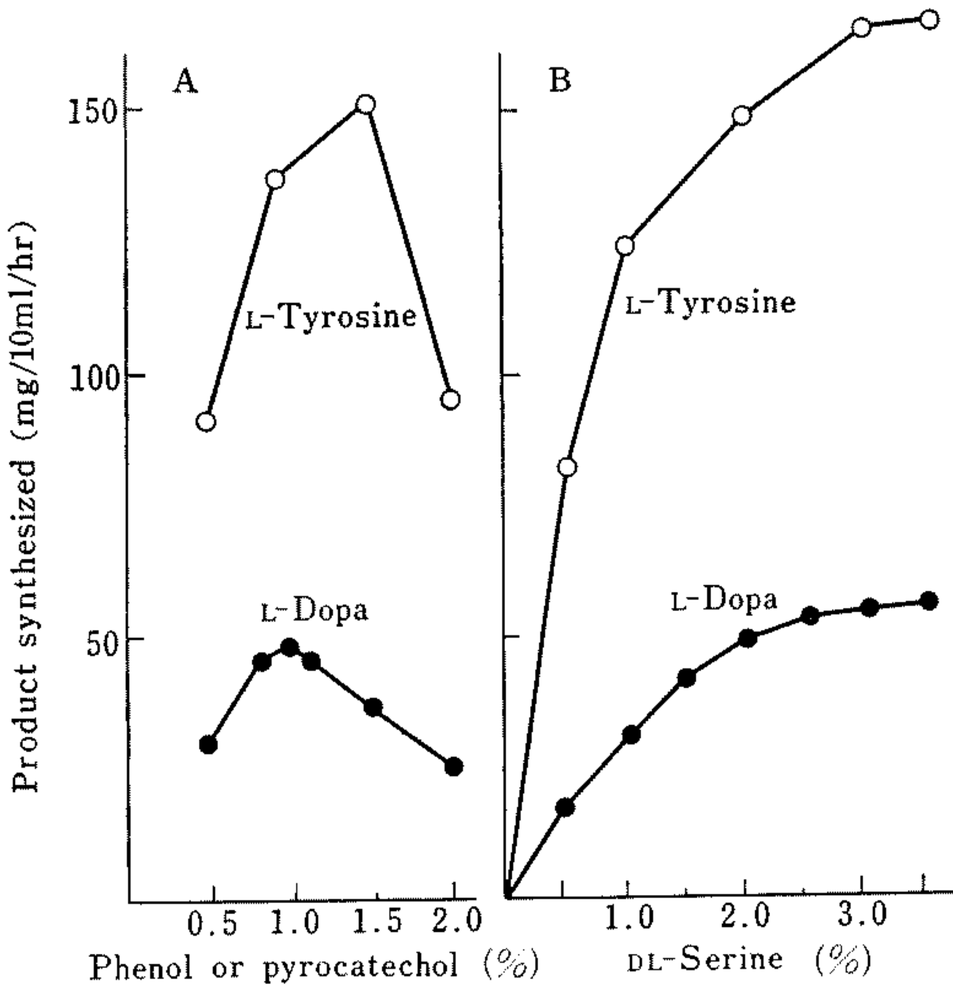


FIG. 3. Effect of Substrate Concentrations on L-Tyrosine or L-Dopa Synthesis.

In Fig. 3A, the synthesis of L-tyrosine (○—○) or L-dopa (●—●) was carried out at 37°C or 22°C for 1 hr in the same reaction mixture (pH 8.0) was described in the text, except that the concentration of phenol or pyrocatechol was varied as indicated. In Fig. 3B, the synthesis of L-tyrosine (○—○) or L-dopa (●—●) was done at 37° or 22°C for 1 hr in the same reaction mixture as described in the text, except that the concentration of DL-serine was varied.

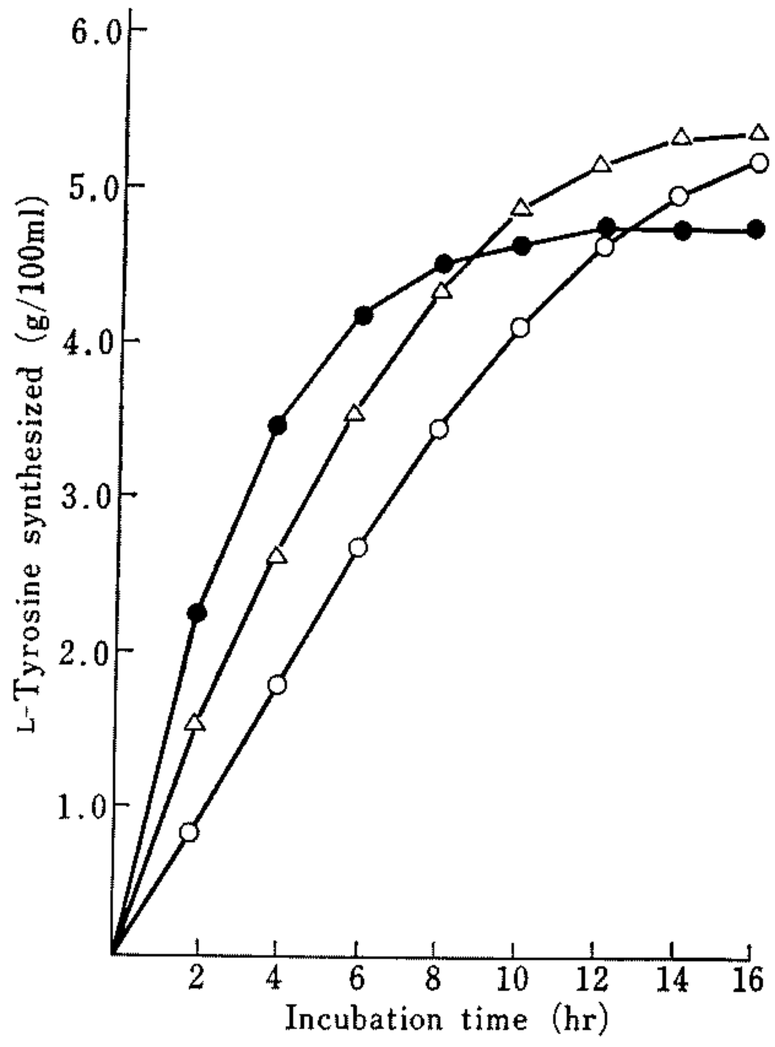


FIG. 4. Effect of Phenol Concentrations on L-Tyrosine Synthesis.

A reaction mixture containing 4 g of DL-serine, 1 g of ammonium acetate, various concentrations of phenol and cells harvested from 100 ml of culture broth in a total volume of 100 ml (pH 8.0), was incubated at 37°C for 16 hr. At 2 hr intervals, phenol was fed to maintain the concentration at 0.8 (○—○), 1.0 (△—△) or 1.2% (●—●).

L-tyrosine or L-dopa. The optimum concentration of DL-serine was studied at the same time. The results are shown in Fig. 3. For L-tyrosine synthesis, the optimum concentration of phenol was around 1.5%, and the sufficient amount of DL-serine was above 3.0%. The optimum concentration of pyrocatechol for L-dopa synthesis was around 1.0% and the sufficient amount of DL-serine was more than 2.0%. Substrate inhibition was found above 1.5% of phenol, or above 1.0% of pyrocatechol in each reaction mixture.

7) Production of L-tyrosine through successive feeding of phenol. As amounts of phenol larger than 1.5% inhibited L-tyrosine synthesis, the reaction was carried out at 37°C for 16 hr by keeping the concentration of phenol at 0.8, 1.0 or 1.2% through successive feeding of phenol at 2 hr intervals. As shown in Fig. 4, when the concentration of phenol was kept at 1.0% during incubation, the maximum accumulation of L-tyrosine (5.35 g/dl) was obtained. The time course for L-tyrosine synthesis is shown in Fig. 5. With

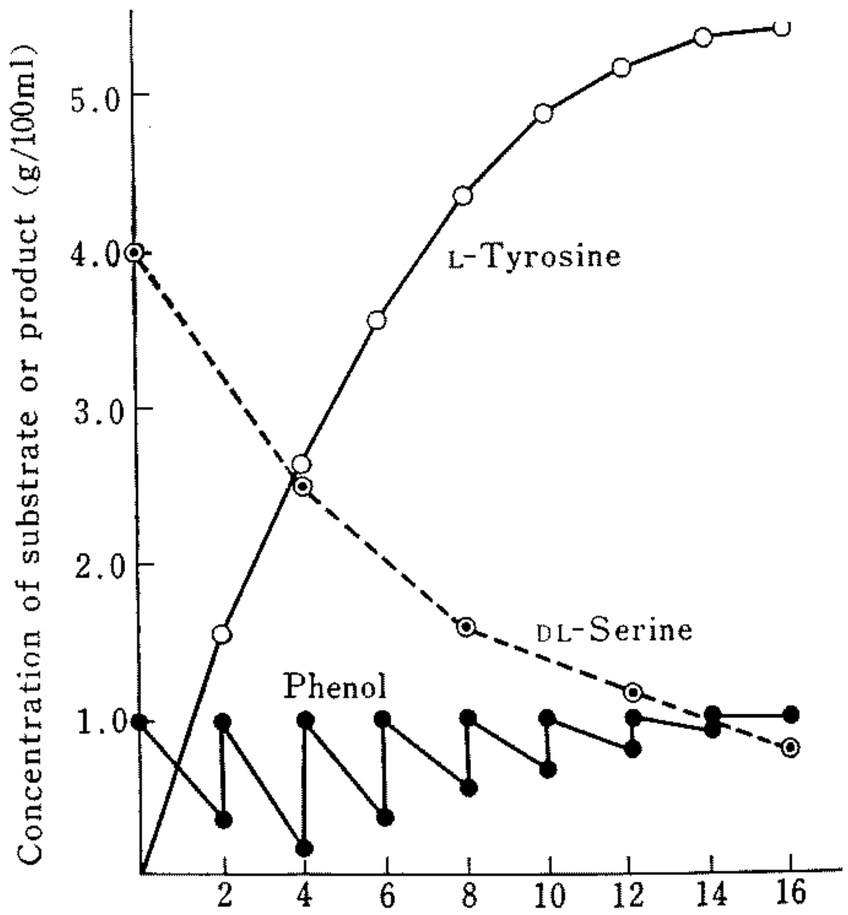


FIG. 5. Time Course of L-Tyrosine Synthesis through the Feedings of Phenol.

The synthesis of L-tyrosine was examined by maintaining the phenol concentration at 1.0% as described in Fig. 4. Substrates are DL-serine (⊙—⊙) and phenol (●—●); the product is L-tyrosine (○—○).

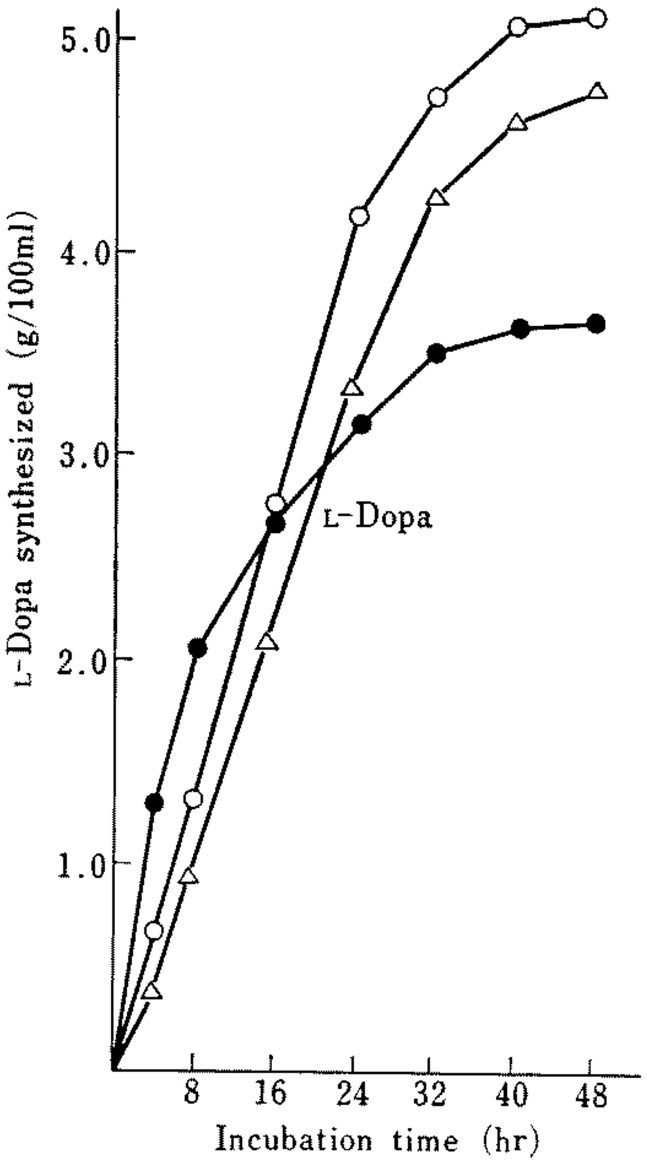


FIG. 6. Effect of Pyrocatechol Concentrations on L-Dopa Synthesis.

A reaction mixture containing 2 g of DL-serine, 1 g of ammonium acetate, various concentrations of pyrocatechol, 0.2 g of sodium sulfite, 0.1 g of EDTA and cells harvested from 100 ml of culture broth in a total volume of 100 ml (pH 8.0), was incubated at 22°C for 48 hr. At 4 hr intervals, pyrocatechol was fed to maintain the concentration at 0.5 (△—△), 0.7 (○—○) or 1.0% (●—●). After 8 hr of incubation, 2 g of DL-serine was added.

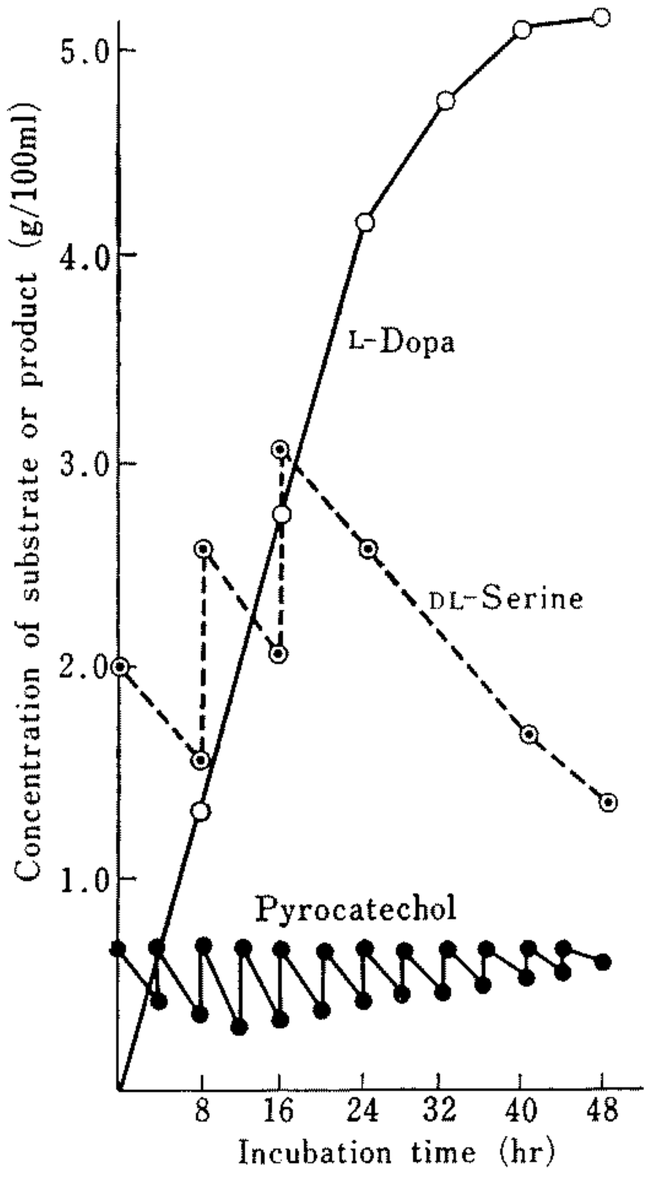


FIG. 7. Time Course of L-Dopa Synthesis through the Feedings of Pyrocatechol and DL-Serine.

The synthesis of L-dopa was examined by maintaining the pyrocatechol concentration at 0.7% as described in Fig. 6. Substrates are DL-serine (⊙—⊙) and pyrocatechol (●—●); the product is L-dopa (○—○). After 8 and 16 hr of incubation, 1 g of DL-serine was added.

the phenol concentration was kept at 1.2%, the initial velocity of L-tyrosine formation was largest, but after 6 hr of incubation, L-tyrosine synthesis was deteriorated by phenol.

8) Production of L-dopa through successive feeding of pyrocatechol and DL-serine. As amounts of pyrocatechol larger than 1.0% inhibited L-dopa synthesis, the reaction was carried out at 22°C for 48 hr by keeping the concentration of pyrocatechol at 0.5, 0.7 or 1.0% through feeding pyrocatechol at 4 hr intervals. One gram of DL-serine was also added after 8 and 16 hr of incubation. As shown in Fig. 6, when the concentration of pyrocatechol was kept at 0.7% during incubation, the maximum accumulation of L-dopa (5.15 g/dl) was obtained. The time course of this reaction is shown in Fig. 7. When pyrocatechol concentration was maintained at 1.0% during incubation, the initial velocity of L-dopa synthesis was maximum but after 8 hr of incubation, L-dopa synthesis was gradually deteriorated by pyrocatechol. As the velocity of L-dopa synthesis was slower than that of L-tyrosine, it was better to feed DL-serine three times during reaction for L-dopa synthesis.

DISCUSSION

The fermentative production of L-tyrosine or L-dopa using microorganisms has been extensively studied. Kinoshita *et al.*[12] reported extracellular accumulation of L-tyrosine with an L-phenylalanine requiring-mutant of *Brevibacterium* in a medium containing glucose as a carbon source. N-Substituted tyrosine (N-formyl or N-carbobenzoxy) was transformed into the corresponding L-dopa derivative with *Aspergillus ochraceus* or *Gliodaclium deliquescens*, by C. J. Sih.[13] And *m*-L-tyrosine

was reported to be hydroxylated to form L-dopa in growing cells of *Bacillus cereus* by J. N. Aroson *et al.*[14] In these fermentative methods, however, yields are not high enough to be useful for industrial production. Thus, a new method for the production of these amino acids has been under investigation for practical application in industry.

We investigated the enzymatic synthesis of L-tyrosine or L-dopa with intact cells of *Erwinia herbicola* ATCC 21434, containing high tyrosine phenol lyase activity, to improve and develop a practical process. As described, the amount of accumulated L-tyrosine or L-dopa was remarkably high (5.35 g/dl of L-tyrosine, 5.15 g/dl of L-dopa). Thus, this enzymatic method seems to be the simplest and most economical process to date.

The reaction conditions for synthesizing L-tyrosine or L-dopa are as follows. The optimum pH for this reaction was around 8.0, and its optimum temperature range was from 37～40°C for the synthesis of L-tyrosine and from 15～25°C for the synthesis of L-dopa. L-Dopa synthesized was decomposed when the reaction was carried out at high temperature. These reactions were accelerated in the presence of large amounts of ammonium ions, as described by Ichihara *et al.*[7] and Kumagai *et al.*,[8] but were not accelerated with potassium ion. The lack of effect of potassium ions seems to be due to the fact that the intact cells used as enzyme accumulates in their cells sufficient amounts of potassium ion during cultivation. With intact cells, therefore, it is not necessary to add potassium ion to the reaction mixture. The addition of pyridoxal-phosphate to the reaction mixture had no remarkable effect on L-dopa synthesis. This seems to be due to pyridoxal-phosphate being accumulated in intact cells during cultivation. Moreover, as L-dopa is very unstable in the reaction mixture, additions of sodium sulfite and EDTA were necessary to keep the synthesized L-dopa stable. Since phenol and pyrocatechol are protein denaturating agents, it was important to keep the optimum concentration of phenol or pyrocatechol through successive feeding.

Thus, the conditions for L-tyrosine or L-dopa synthesis were established as follows. For L-tyrosine synthesis, the enzymatic reaction was carried out at 37°C for 16 hr in a reaction mixture containing 4 g of DL-serine, 1 g of phenol, 1.0 g of ammonium acetate and intact cells harvested from 100 ml of cultured broth in a total volume of 100 ml at pH 8.0 (by ammonia). At 2 hr intervals, phenol was fed to maintain the initial concentration. For L-dopa synthesis, the reaction was carried out at 22°C for 48 hr in a reaction mixture containing 2 g of DL-serine, 0.7 g of pyrocatechol, 1.0 g of ammonium acetate, 0.2 g of sodium sulfite, 0.1 g of EDTA and cells harvested from 100 ml of cultured broth in a total volume of 100 ml at pH 8.0 (by ammonia). At 4 hr intervals, pyrocatechol was fed to maintain the initial concentration, then after 8 and 16 hr, 1 g of DL-serine was added. Under these conditions, 5.35 g of L-tyrosine or 5.10 g of L-dopa was synthesized in the reaction mixture.

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REFERENCES

1) H. Enei, H. Matsui, S. Okumura and H. Yamada, *Agr. Biol. Chem.*, in press
2) H. Enei, H. Matsui, K. Yamashita, S. Okumura and H. Yamada, *ibid.*, in press.
3) H. Enei, K. Yamashita, S. Okumura and H. Yamada, *ibid.*, in press.
4) H. Kumagai, N. Kashima, H. Torii, H. Yamada, H. Enei and S. Okumura, *ibid.*, in press.
5) T. E. Friedemann and G. E. Haugen, *J. Biol. Chem.*, **147**, 415 (1943).
6) J. W. Porteous and R. T. Williams, *Biochem. J.*, **44**, 46 (1949).
7) K. Ichihara, H. Yoshimatsu and Y. Sakamoto, *J. Biochem.*, **43**, 803 (1956).
8) H. Kumagai, H. Yamada, H. Matsui, H. Ohgishi and K. Ogata, *J. Biol. Chem.*, **245**, 1767 (1970).
9) H. Yoshimatsu, *Med. J. Osaka Univ.* (*Osaka*), **9**, 727 (1957).

10) N. Brot, Z. Smit and H. Weissbach, *Arch. Biochem. Biophys.*, **112**, 1 (1965).
11) H. Kumagai, H. Yamada, H. Matsui, H. Ohgishi and K. Ogata, *J. Biol. Chem.*, **245**, 1773 (1970).
12) K. Nakayama, Z. Sato and S. Kinoshita, *Nippon Nogeikagaku Kaishi*, **35**, 146 (1961).
13) C. J. Sih, P. Foss, J. Rosazza and M. Lemberger, *J. Am. Chem. Soc.*, **91**, 6204 (1969).
14) J. N. Aronson and G. R. Wermus, *J. Bacteriol.*, **90**, 38 (1965).