ON THE ENZYMATIC PREPARATION OF L-PHENYLALANINE. (Preliminary Report)

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Considerable interest has been directed to the production of amino acids by microbial processes. Especially, it is worth to note that L-glutamic acid and L-lysine are now being manufactured in this country, and attempts to produce nutritional essential amino acids such as L-valine, L-tryptophan, L-threonine and L-isoleucine have been made by many workers.

In this paper, the authors devised a new practical method for the preparation of L-phenylalanine, one of the essential amino acids by bacterial transaminase.

The selection of suitable strains capable of producing L-phenylalanine from phenylpyruvic acid and amino donors was carried out. The following

Table 1. Transaminase activities of various bacteria.

Organism	L-Phenylalanine		L-Tyrosine*
	mg/1.5ml	%	
<i>Alcaligenes faecalis</i> B-141-1	9.08	63.5	—
<i>Pseudomonas cruciviae</i> 1-2	8.46	59.2	+
<i>Pseudomonas aeruginosa</i> MT	8.00	55.9	++
<i>Aerobacter aerogenes</i> 9	7.58	53.0	++
<i>Escherichia coli</i> 10-7	7.58	53.0	++
<i>Achromobacter cycloastes</i> B 101-1	4.34	30.3	++
<i>Sarcina lutea</i>	3.98	27.8	+
<i>Kluyvera citrophila</i>	3.95	27.6	+++
<i>Pseudomonas fluorescens</i> 5-6	3.50	24.5	+
<i>Micrococcus lysodeikticus</i> 61-2	3.25	22.7	+

Reaction mixture:

30 mg of dried cells,
14.3 mg of phenylpyruvic acid (or p-hydroxyphenylpyruvate),
16.1 mg of L-glutamate,
12.3 mg of L-aspartate,
total volume 1.5 ml with M/15 phosphate buffer (pH 7.6).
incubated for 5 hrs. at 37°

* measured by paper chromatography.

medium was used for bacterial growth: glucose, 10 g; NH_4Cl , 2 g; poly-peptone, 2 g; meat-extract, 2 g; yeast extract, 2 g; KH_2PO_4 , 1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; NaCl , 1 g. per 1,000 ml. PH 7.0. One hundred ml. of this medium was dispensed in a 500 ml. shaker flask and inoculated. The culture was incubated for 24 hours at 30° on a reciprocal shaker at the rate of 120 r.p.m. The cells were collected by centrifugation, washed twice and dried up in vacuo. The cell preparation obtained was used for L-phenylalanine production. A large number of the bacteria tested could produce L-phenylalanine. Examples of transaminase activities of bacteria belonging to various genera are shown in Table 1 with that of p-hydroxyphenylpyruvic acid-amino acid transaminase. Yields of L-phenylalanine formed were 63.5 per cent and 59.2 per cent with *A. faecalis* B-141-1 and *Ps. cruciviae* 1-2, respectively. The activities of phenylpyruvic acid-amino acid transaminase of these bacteria did not agree with that of p-hydroxyphenylpyruvic acid-amino acid transaminase, which was tested at the same time. For instance, *K. citrophila* which was most suitable for p-hydroxyphenylpyruvic acid-amino acid transamination was not found to be so good in the case of phenylpyruvic acid-amino acid transamination. Then, these two kinds of transaminase seem to be different kinds of enzymes.

Table 2. Transaminase activities in *Alcaligenes faecalis* B-141-1.

Amino donor	L-Phenylalanine	
	mg/1.5 ml	%
L-Glutamic acid	3.80	36.4
L-Aspartic acid	4.90	46.9
L-Leucine	4.03	38.6
L-Isoleucine	4.20	40.2
L-Valine	2.50	23.9
L-Alanine	1.13	10.8
L-Methionine	2.80	26.8
L-Threonine	0.55	5.3
L-Lysine	0.68	6.6
L-Tyrosine	2.53	24.2
L-Arginine	0.78	7.5
L-Tryptophan	1.27	12.2
L-Histidine	1.48	14.1
L-Serine	1.18	11.3

Reaction mixture:

30 mg of dried cells,
 65.3 μ M of phenylpyruvic acid,
 100 μ M of amino acid,
 total volume 1.5 ml with M/15 phosphate buffer (pH 7.6).
 incubated for 5 hrs. at 37°

The effects of amino donors are shown in Table 2. Among the donors L-aspartate was found to be most favourable. The optimal incubation time and PH were about 24 hours and 7.5-8.0, respectively. Transaminase activity was more active at 37° than in cases of 30° and 45°.

The insoluble matter of the incubation mixture was removed and the pH was adjusted to 6.1 with dilute hydrochloric acid. The clear solution was passed through a column of Permutit DR and then the solution was followed by water. The effluent was concentrated in vacuo to dryness and the residue was recrystallized from water to crystals of d.p. 276-7°.

Anal. Calcd. for $C_9H_{11}O_2N$:

C 65.43% H 6.71% N 8.48%

Found C 65.36% H 6.72% N 8.41%

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