PREPARATION OF L-ASPARTIC ACID BY BACTERIAL ASPARTASE (Preliminary Report)

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Since the discovery of aspartase in *Escherichia coli* by J. H. QUASTEL and B. WOOLF in 1926 (1), bacterial aspartase has been investigated by many workers (2-5). Most work has been carried out from the stand point of nitrogen metabolism exclusively, and it is hard to find any report dealing with attempts to apply this enzyme to the commercial production of L-aspartic acid.

Recently, the production of glutamic acid and some other amino acids has become available by fermenting process. The present authors have searched for an enzymic process applicable for L-aspartic acid synthesis in industrial manufacture. In this paper, the selection of a suitable micro-organism and its reaction conditions are presented.

Selection of Strains

For the purpose of obtaining suitable strains the following method was employed. Each strain tested was incubated in a malt-bouillon medium¹ adjusted near its optimum temperature. After 24 hours the substrate solution containing same amount of Na-fumarate and ammonium chloride was added to the culture broth so as to make the final concentration of fumaric acid to 2%, and incubation was continued further for 1 day at 37°, the optimum temperature for aspartase reaction.

Fumaric acid in the reaction mixture was determined by permanganometry (6) and aspartic acid was roughly estimated by paperchromatography.

As summarized in Table 1, it is found that *Escherichia coli* No. 2, *Serratia marcescens* and *Bacterium succinicum* appear to be strains suitable for our purpose.

Enzymic Synthesis of L-Aspartic Acid

Enzyme preparation: Cells of *Escherichia coli* No. 2, propagated in a

¹ Malt-bouillon medium: an equivolume mixture of malt extract and nutrient bouillon.

Table 1. Selection of Aspartase Forming Strain.

Organism	Fumaric acid consumed. %	Spot of aspartic acid*
<i>Pseudomonas ovalis</i>	87.4	+++
<i>Pseudomonas xanth</i>	56.1	
<i>Achromobacter cycloclaster</i>	53.3	
<i>Alcaligenes faecalis</i>	3.8	
<i>Flavobacterium lactis</i>	42.3	
<i>Serratia marcescens</i>	90.2	+++++
<i>Erwinia carotovora</i>	23.6	++
<i>Aerobacter aerogenes</i>	49.5	+++
<i>Aeromonas hydrophila</i>	91.3	+++
<i>Escherichia coli</i> No. 2	91.7	+++++
<i>Escherichia coli</i> K ₁₂	1.1	
<i>Agrobacter tumefaciens</i>	37.4	+
<i>Aerobacter cloacae</i>	8.3	+
<i>Micrococcus lysodeikticus</i>	9.3	
<i>Micrococcus luteus</i>	4.9	
<i>Lactobacillus casei</i>	0	
<i>Propionibacterium arabinosum</i>	59.9	
<i>Bacillus subtilis</i>	75.3	
<i>Bacillus coagulans</i>	0	
<i>Bacterium succinicum</i>	90.1	++++
<i>Streptomyces griseus</i>	7.7	
<i>Nocardia asteroides</i>	7.7	+
Beer yeast 1	11.5	+
Beer yeast 2	25.3	
Wine yeast	15.9	
<i>Willia saturnus</i>	67.2	

* Paper chromatography is carried on as follows: Tōyō filter paper No. 50; solvent system, n-butyl alcohol-acetic acid-water (4:1:1).

medium for 2 days at 37°, were harvested by centrifugation, washed once with distilled water and lyophilized. The yield of the dried cells was approximately 0.7 g per 1 liter culture broth. This dried preparation was used as the enzyme material throughout this work.

Composition of reaction media:

Medium 1,	Sodium-fumarate	10 g (free acid basis)
	Ammonium chloride	10 g
	Dried cells (enzyme material)	0.25 g
	Distilled water	100 ml
Medium 2,	Ammonium-fumarate	20 g (free acid basis)
	Dried cells	0.5 g
	Distilled water	100 ml

Medium 3, Ammonium-fumarate	50 g (free acid basis)
Dried cells	1 g
Distilled water	100 ml

Note: The ammonium-fumarate in Medium 3 was prepared as a saturated solution containing an abundant amount of crystals. These crystals were resolved into the solution accompanied with the proceeding of enzyme reaction.

Reaction was carried on with slow agitation at 37° and pH 7.2-7.4. After 24 hours, almost all of the fumarate disappeared and was converted into L-aspartic acid. By simple adjustment of the hydrogen ion concentration to the isoelectric point, pH 2.77, the majority of the product was crystallized and could be readily separated from the reaction mixture by filtration. The experimental results are presented in Table 2.

Table 2. Enzymic Synthesis of L-Aspartic Acid.

		Medium 1		Medium 2		Medium 3	
		Na-salt		NH ₄ -salt			
		Fumaric g	Aspartic g	Fumaric g	Aspartic g	Fumaric g	Aspartic g
Initial							
	Substrate	10.0		20.0		50.0	
	Loss of determination	0.21		0.52		0.59	
	Difference	9.79		19.48		49.41	
Final*							
	Crystals	0	8.72	0	19.69	0.2	51.20
	Mother liquid	0.32	1.50	0.07	1.15	0.1	2.61
	Loss of determination		0.31		1.40		2.21
	Total aspartic		10.53		22.19		56.02
Yield							
	Formed aspartic per consumed fumaric (mol./mol.)	93.9%		99.3%		99.4%	
	Recovered aspartic as crystals (mol./mol.)	77.8%		88.1%		90.4%	

Determination of L-aspartic acid: The colorimetric method(7) was employed.

* After the reaction and pH adjustment

The recrystallized product from water showed a melting point of 270°.

Anal. Calcd. for C₄H₇NO₄

C 36.13% H 5.27% N 10.46%

Found C 36.09% H 5.30% N 10.52%

Specific rotation, $[\alpha]_D^{20} = +24.0^\circ$ (C=1 in 1.91% HCl)

In conclusion the present authors wish to state that, if a suitable enzyme

preparation and its reaction condition can be selected, some of the enzymes other than hydrolase will also be promising for industrial manufacture. Bacterial aspartase, for example, will be the most advantageous tool for the production of L-aspartic acid because it shows not only full activity in the saturated ammonium-fumarate solution but also shows an equilibrium constant shifting to the direction of aspartic acid formation.

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