ASPARTIC ACID DECARBOXYLATION BY RHIZOBIUM TRIFOLII

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Although Virtanen and Laine (Enzymologia, **3**, 226, 1937) have claimed that *Rhizobium leguminosarium* decarboxylates aspartic acid with the formation of *beta*-alanine, as determined by the isolation and chemical identification of this product from a 47-day fermentation mash containing aspartic acid, actual proof of the conversion of aspartic acid to *beta*-alanine is lacking.

The decarboxylation of a dicarboxylic amino acid, aspartic acid, could yield either *alpha*-alanine or *beta*-alanine depending on which carboxyl group is attacked. We have been studying the enzymatic decarboxylation of aspartic acid

Production of beta-alanine from aspartic acid by Rhizobium trifolii

BACTERIAL N PER TUBE	*Beta*-ALANINE PRODUCED	
	No aspartic acid	Plus aspartic acid
mg	μg	μg
0.19	0	1.0
0.38	0	2.0
0.94	0	4.8
1.87	0	9.0
2.80	1.2	>13.3

Washed cells were incubated 1 hour at 30 C in 0.1 M phosphate buffer, pH 5, with 0.005 M L-aspartic acid as the final concentration. Total volume was 2 ml. Controls contained no aspartic acid. Tubes were shaken in a water bath during the experiment. The reaction was stopped by boiling 5 minutes, and an aliquot of the supernatant, after centrifugation, was assayed for *beta*-alanine as described in the text.

by utilizing a microbiological assay for *beta*-alanine as an index of decarboxylation.

The medium employed was composed of 1 per cent each of tryptone, yeast extract, and glucose and 0.1 per cent DL-aspartic acid. After incubation for 18 hours at 27 to 30 C on a mechanical shaker (final pH 4.4 to 4.7), the cells were harvested by centrifugation, washed twice with equal volumes of distilled water, and resuspended in distilled water. The concentration of cell suspension was determined by the measurement of turbidity in a Klett-Summerson photoelectric colorimeter and converted to bacterial nitrogen by the use of previously standardized tables.

A microbiological assay for *beta*-alanine was made by using a modification of the medium suggested by Snell *et al.* (J. Am. Chem. Soc., **62**, 175, 1940) with *Saccharomyces fragilis* as the assay organism. The modifications consisted of the following additions per 100 ml: 5 ml acid-hydrolyzed vitamin-free casein, 10 μg nicotinic acid, and 2 μg *para*-aminobenzoic acid. The range of *beta*-alanine

stimulation of the growth of *S. fragilis* as determined by turbidity measurement was approximately 0.5 to 4.0 μg.

That *beta*-alanine is actually produced under the conditions of the experiment, although in small quantities, may be seen from the data in the table. The production of *beta*-alanine from aspartic acid appears to be a linear function and is indicative of enzymatic action. The small quantities produced suggest the reason for the negative results of many investigators utilizing the measurement of CO_2 production by conventional Warburg techniques.

Further studies are in progress on this problem.