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Aspartic Acid as a Partial Substitute for the Growth-Stimulating Effect of Biotin on *Torula cremoris.*

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In recent work in this laboratory with *Torula cremoris* it was found that aspartic acid partially substituted for the growth-stimulating effect of biotin. Studies on the accessory factor requirements of this yeast, which will be published in detail elsewhere, have shown that nicotinamide, pantothenic acid, thiamin, and biotin are all needed for rapid growth with development of marked turbidity in 24 hr. Omission of any one of these 4 factors produced either a slight delay in the rate of growth, a marked delay, or no appreciable growth, depending upon the factor omitted.

When biotin was omitted the results varied with the basal medium employed in the tests. In an ammonium phosphate-glucose-inorganic salt medium omission of biotin resulted in a very marked delay, visible growth appearing only after 4 to 7 days or longer, at 37°C. When a mixture of 19 amino acids was substituted for ammonium phosphate the omission of biotin produced only slight delay; uniformly good growth was produced at 48 hr, though only light growth or no growth at 24 hr.

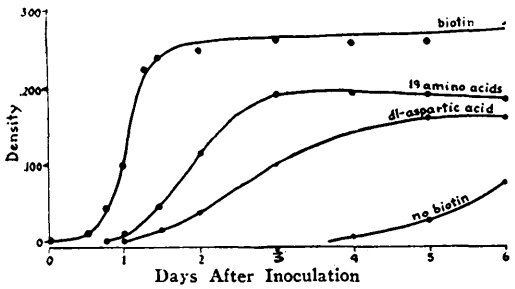

FIG. 1.

Effect of aspartic acid, amino acid mixture, and biotin on growth of *Torula cremoris* in an ammonium phosphate-glucose-inorganic salt basal medium. Nicotinamide, calcium pantothenate and thiamine were added in each instance in the amount of 0.2 μg per ml. Biotin, when present, was supplied in the amount of .002 μg per ml.

Density (L = 2-log G) was determined with the Evelyn colorimeter equipped to hold selected tubes of 15 mm diameter in place of the standard Evelyn tubes.

Incubation was at 37°C.

To determine whether this stimulation of growth in the absence of biotin could be related more definitely to certain of the amino acids, additional experiments were performed in which amino acids were supplied singly and in various combinations. It was found that much of the growth-stimulating effect of the original amino acid mixture was supplied by l(—)-aspartic acid. Two additional samples of l(—)-aspartic acid and one of synthetic dl-aspartic acid were tested with identical results.

A typical experiment is given in Fig. 1. In the presence of the other 3 accessories, but without biotin, growth of *T. cremoris* was quite slow, requiring in this instance 4 to 5 days before it became evident. In some experiments growth did not appear until 7 days or more after inoculation. When the amino acid mixture was supplied growth appeared much sooner, though not as promptly as in the presence of biotin. Much of the growth stimulation produced by the amino acid mixture was reproduced by either l(—)-aspartic acid or dl-aspartic acid. Since this effect was produced by synthetic dl-aspartic acid it seems unlikely that the stimulation caused by this compound is due to the presence of biotin as an impurity.

Rather large amounts of aspartic acid were necessary to produce stimulation such as that shown in Fig. 1. Usually 100 or 200 μg per ml of medium were used, however 40 μg gave growth almost equal to 100 μg while 20 and 10 μg produced successively less stimulation.

The other 18 amino acids of our original mixture were also tried singly. With the exception of l(+)-glutamic acid no noteworthy stimulation of growth was observed.

This amino acid produced some stimulation in the absence of biotin but it was not as marked as that caused by aspartic acid. The use of dl-glutamic acid resulted in less stimulation than that produced by the natural amino acid, so that the presence of biotin as an impurity may have been partially responsible for the results with the l(+)-compound.

Alanine, which along with aspartic and glutamic acids has been found to play a prominent role in transamination reactions,[1] produced no stimulation under the conditions of our tests.

Asparagine and glutamine produced marked stimulation but the presence of biotin as impurity appears likely and thus far no samples have been secured which could be regarded as definitely free of biotin.

In view of the recent work on the structure of biotin and the suggested relationship of pimelic acid to part of the biotin molecule[2,3] it seemed of interest to determine the effect of pimelic acid on growth of *T. cremoris* in the absence of biotin. The ammonium phosphate-glucose medium was used with nicotinamide, thiamin and calcium pantothenate. To separate tubes of this were added pimelic acid and also pimelic acid and cysteine together.[4] Experiments were performed with 10 μg and 100 μg of each compound per ml of medium.

No stimulation of growth was apparent when pimelic acid and cysteine were supplied. When these two compounds were present with dl-aspartic acid there was no appreciable stimulation over that caused by the aspartic acid itself. Thus, although *T. cremoris* grows but slowly in the absence of biotin, a supply of pimelic acid and organic sulfur does not facilitate cell multiplication in the simple basal medium used here. These results are in contrast to those secured with aspartic acid.

A possible explanation of the activity of aspartic acid might be that biotin is concerned in the formation of this amino acid and that its inclusion in the medium decreases the need for biotin. Another explanation might be that this amino acid is capable to a limited extent of performing a function of biotin. However, we have no direct evidence for either of these possibilities.

Conclusion. The omission of biotin resulted in a pronounced delay of growth of a stock laboratory strain of *Torula cremoris* in an ammonium phosphate-glucose-inorganic salt medium. Under these conditions l(—)-aspartic acid or dl-aspartic acid, when supplied in place of biotin but in much larger amounts, produced a distinct stimulation of growth. A less marked stimulation was caused by glutamic acid. Seventeen other amino acids did not produce this effect.

Pimelic acid and cysteine did not stimulate growth in the absence of biotin under the conditions of these tests.

[1] Cohen, P. P., Transamination, Chapter in *A Symposium on Respiratory Enzymes*, University of Wisconsin Press, Madison, 1942.

[2] Hofmann, K., Melville, D. B., and du Vigneaud, V., *J. Am. Chem. Soc.*, 1941, **63**, 3237.

[3] du Vigneaud, V., Dittmer, K., Hague, E., and Long, B., *Science*, 1942, **96**, 186.

[4] Eakin, R. E., and Eakin, E. A., *Science*, 1942, **96**, 187.