181. THE IMPORTANCE OF AMINO-ACIDS AS YEAST NUTRIENTS

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At the time of the earliest chemical studies of yeast propagation it was supposed that protein material could serve effectively for yeast nutrition. The formation of higher alcohols was later traced to the presence of specific amino-acids in the culture medium.

The discovery of "bios" in Ide's laboratory threw doubt on the efficacy of material strictly of protein origin, but the existence of "bios" was doubted and Pringsheim [1906], thinking that material of protein origin constituted the alleged mysterious substance, published an extensive article on the nitrogen nutrition of yeast [Pringsheim, 1907] without so much as referring to "bios" investigations. His attitude no doubt had much to do with the abandonment of "bios" studies from 1906 to 1919, when one of us postulated a relationship between "bios" and vitamin B [Williams, 1919].

Recognizing the existence of specific vitamin-like nutritional factors, quite distinct from amino-acids, Williams and co-workers have pointed out the importance of asparagine [Williams, 1920] and aspartic acid [Williams & Rohrman, 1936] as yeast nutrients and Miller and co-workers have found leucine [Miller, 1936] to be a nutrient factor of importance for certain yeasts. Still more recently, Nielsen & Hartelius [1937] have studied the effect of adding amino-acids to a medium containing a small amount of beerwort, and have shown that for the yeast under investigation a number of amino-acids have individually toxic effects under these conditions but collectively have a beneficial action, increasing the yeast crops by as much as 84 % under the experimental conditions used.

In all of these studies the investigation of the effects of amino-acids could not be made adequately because sources of the nutrient factors, free from amino-acids, were not obtainable. In the latter study one strain of yeast only was investigated.

Since the isolation of pantothenic acid, biotin, aneurin and vitamin B₆ (as well as inositol), all of which are yeast nutrient factors, these substances have all been available in our laboratory [Williams *et al.* 1940] and it becomes possible to determine more definitely how important amino-acids are for the nutrition of yeast and especially to investigate the differences which exist between different strains of *Saccharomyces cerevisiae*. Our experience led us to expect striking differences and to the opinion that a study of a single strain of yeast could not be taken as generally significant.

Materials

The twenty amino-acids used were Eastman products: asparagine, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, *isoleucine*, leucine, lysine, methionine, phenylalanine, proline, serine, threonine,

tryptophan, tyrosine and valine. Solutions of each amino-acid containing 0.5 mg. per ml. were prepared and used in the individual tests. Solutions were also prepared containing equal amounts of the above twenty amino-acids, one with 5 mg. total per ml. (250 μ g. of each) and one with 0.5 mg. total per ml. (25 μ g. of each).

The casein hydrolysate was prepared as follows: 10 g. of "B"-free casein (Eimer and Amend) was autoclaved 6 hr. with 100 ml. of 6N H_2SO_4 . After removal of the sulphate with barium hydroxide, 200 mg. tryptophan were added to replace that destroyed in hydrolysis. Dilutions were then made to yield solutions equivalent to 5 and 0.5 mg. of the original casein per ml.

The basal medium was that of Williams & Saunders [1934], containing inositol but excluding asparagine (and aspartic acid). In addition a supplement of nutrient factors was added. These were as follows with the amounts of each added per culture: aneurin 1 μ g., vitamin B_6 2 μ g., biotin¹ 0.0005 μ g., calcium pantothenate 0.5 μ g., riboflavin 2 μ g., uracil 2 μ g., choline 3 μ g., acetylcholine 2 μ g., ethanolamine 10 μ g., nicotinamide 10 μ g. The effects of the first four of these on certain strains of yeast have been studied by Williams *et al.* [1940]. The last six compounds have been found to be somewhat stimulating for certain yeasts in unpublished work in this laboratory.

Yeast seedings were in most cases obtained from cultures maintained in this laboratory for several years. Five are strains of *Saccharomyces cerevisiae*:

F.B.—Fleischmann Bakers'.

2190—British Type Culture Collection (Narayanan).

H. 1—Wildiers' yeast (Hansen 1).

G.M.—Gebrüder Mayer.

O.P.—"Old Process" Fleischmann.

A strain of *Saccharomyces ellipsoides* (no. 4098 American Type Culture Collection) was also included in the tests.

Procedure

The general method followed was similar to that of Williams *et al.* [1940]. In these experiments, however, the amino-acid samples were added in test tubes in a volume of 1 ml. to which were added 3 ml. of medium containing the appropriate seeding and nutrient factor supplement. After 15 hr. at 30° the cultures were diluted with 5 ml. of saturated chlorothymol solution and the yeast crops determined by the thermocouple method in use in this laboratory [Williams *et al.* 1929].

RESULTS

The results from several experiments are summarized in Table 1. The seedings and control crops are given in mg. per culture, while the effects of the amino-acids are given as percentage increase over the control crop. For the sake of brevity, only the individual amino-acids giving greatest stimulation are included. No more than a trace of toxicity was observed in any case. A maximum stimulating effect was usually obtained with the 0.5 mg. dosage of the individual amino-acids, but the 5.0 mg. dosage of the amino-acid mixtures usually caused the greatest response.

¹ This was 75 % pure biotin methyl ester obtained through the generosity of Prof. F. Kögl. Biochem. 1940, 34

Table 1. *Effects of amino-acids on yeasts*

Yeast strain and seeding (moist) F.B. (0.01 mg.)

Control crop	1.80 mg.
0.5 mg. mixture	+ 53 %
5.0 mg. mixture	+ 170 %
0.5 mg. casein digest	+ 43 %
5.0 mg. casein digest	+ 120 %

Amino-acids showing greatest individual effects: (56–18 %) glutamic acid, asparagine, aspartic acid, leucine, lysine, arginine.

No. 2190 (Narayanan) (0.06 mg.)

Control crop	0.50 mg.
0.5 mg. mixture	+ 500 %
5.0 mg. mixture	+ 1200 %
0.5 mg. casein digest	+ 420 %
5.0 mg. casein digest	+ 1000 %

Amino-acids showing greatest individual effects: (530–30 %) aspartic acid, glutamic acid, asparagine, leucine, serine, methionine, glycine, histidine, tryptophan, arginine, lysine, valine, phenylalanine, cystine, alanine, hydroxyproline, tyrosine.

G.M. (0.01 mg.)

Control crop	1.53 mg.
0.5 mg. mixture	+ 110 %
5.0 mg. mixture	+ 240 %
0.5 mg. casein digest	+ 90 %
5.0 mg. casein digest	+ 170 %

Amino-acids showing greatest individual effects: (150–32 %) glutamic acid, aspartic acid, arginine, proline, asparagine, phenylalanine, serine, tryptophan, leucine, histidine, glycine, lysine.

H. 1 (0.03 mg.)

Control crop	1.6 mg.
0.5 mg. mixture	+ 104 %
5.0 mg. mixture	+ 170 %
0.5 mg. casein digest	+ 93 %
5.0 mg. casein digest	+ 116 %

Amino-acids showing greatest individual effects: (81–32 %) glutamic acid, arginine, aspartic acid, asparagine, histidine, proline, cystine, leucine, phenylalanine, serine, tryptophan, tyrosine.

O.P. (0.02 mg.)

Control crop	1.35 mg.
0.5 mg. mixture	+ 91 %
5.0 mg. mixture	+ 290 %
0.5 mg. casein digest	+ 110 %
5.0 mg. casein digest	+ 190 %

Amino-acids showing greatest individual effects: (84–15 %) glutamic acid, aspartic acid, arginine, asparagine, histidine, leucine, lysine.

S. ellipsoides, No. 4098 (0.01 mg.)

Control crop	0.65 mg.
0.5 mg. mixture	+ 200 %
5.0 mg. mixture	+ 250 %
0.5 mg. casein digest	+ 180 %
5.0 mg. casein digest	+ 280 %

Amino-acids showing greatest individual effects: (130–35 %) glutamic acid, asparagine, aspartic acid, leucine, arginine, serine, phenylalanine, proline, alanine.

DISCUSSION

A study of the table reveals a similarity in the various strains of yeast in that all six are considerably affected by five of the amino-acids; glutamic acid, asparagine, aspartic acid, arginine and leucine. Serine, lysine, histidine and

phenylalanine are effective on four organisms, and tryptophan and proline show considerable stimulation on three out of the six strains. In all cases the mixture and the casein digest show marked stimulating actions far above those of individual amino-acids.

In addition to the above similarities between the various strains, marked differences occur. The number of amino-acids that give marked stimulation on each yeast are as follows: No. 2190, 17; H. 1, 12; G.M., 12; No. 4098, 9; O.P., 7; and F.B., 6. Parallel with this there are differences in the extent to which individual amino-acids and amino-acid mixtures are capable of increasing growth, and the maximum effect of the amino-acids in turn depends in part upon the extent to which the control cultures grow. Thus, in the two extreme cases, yeast No. 2190 was affected by seventeen amino-acids, and the crop was increased 530 % by an individual amino-acid (aspartic acid) and 1400 % by a casein digest. Its multiplication in the control cultures was less than ten-fold. "F.B." yeast, on the other hand, was markedly affected by only six amino-acids; the maximum effect of a single amino-acid was 56 % and the greatest effect of a mixture was 170 %. The control cultures, however, multiplied 180-fold during the incubation period. It appears, therefore, that there is a considerable variability in the amino-acid requirements and the synthetic abilities of various strains of yeast.

All the yeasts which we have studied extensively are capable of synthesizing amino-acids from ammonia-nitrogen; nevertheless, growth is greatly increased when amino-acids are furnished in the culture medium along with the proper nutrient factors. Without these factors we have in general observed amino-acid mixtures, such as represented by a casein digest, to be at least slightly inhibitory. It appears that the amino-acids function in yeast nutrition as does arginine in animal nutrition. The latter is not an obligatory requirement, but growth is increased when it is furnished [Rose, 1938].

It appears probable that amino-acid combinations contribute to an appreciable extent to "bios" activity. In the cases where marked stimulation occurred at the 500 μ g. level, as indicated in the above table, a measurable stimulation also occurred down to 50 μ g. dosages or less. The designation of "bios" fractions by numbers and letters without definite evidence that each represents an individual compound is unwarranted and the influence of amino-acids in yeast nutrition cannot be overlooked in future "bios" studies.

Experiments with certain strains of yeast not reported here in detail indicated that the nutritional interrelationships between the different amino-acids are complex and may compare strikingly with those observed in this laboratory in connexion with the nutrition of the mould *Trichophyton interdigitale* [Mosher *et al.* 1936].

SUMMARY

The effects of twenty individual amino-acids, a mixture of the same, and a casein digest on five strains of *Saccharomyces cerevisiae* and one strain of *S. ellipsoides* have been studied with the following conclusions:

1. All six strains of yeast were markedly stimulated by the addition of individual amino-acids and of mixtures when the control medium contained an abundant supply of nutrient factors. These amino-acids are not required for growth but growth is enhanced when they are furnished.

2. The five most important amino-acids were glutamic acid, aspartic acid, asparagine, arginine and leucine. These were strongly stimulating on all of the yeast strains tested.

3. A distinct supplementary effect was shown by a combination of all twenty amino-acids.

4. Amino-acids may, depending on the test conditions and the yeast strain used, contribute materially to the "bios" effects of tissue extracts.

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