Lysine, Methionine, and Tryptophan Content of Microorganisms

II. Yeasts


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As part of a survey to determine the suitability of microorganisms as sources of protein and amino acids, the lysine, methionine, and tryptophan in the cells of 271 strains of yeasts were determined. Yeast cells are an established source of proteins and vitamins in feeds. As a group, yeast possess certain advantages for large-scale propagation of cells. They can be grown easily on a variety of carbohydrate substrates and the cells can be separated conveniently from the culture liquor and dried. The lysine, methionine, and tryptophan contents of bacteria were reported in a previous publication (Anderson et al., 1958).

Methods

Production of cells. The representative yeasts studied during this work were selected from the Agricultural Research Service (ARS) Culture Collection maintained by the Fermentation Laboratory at the Northern Utilization Research and Development Division. The yeasts first were grown on slant cultures on a medium composed of glucose, 1 per cent; peptone, 0.5 per cent; yeast extract, 0.3 per cent; malt extract, 0.3 per cent; and agar, 2.0 per cent. Cells washed from 24- to 48-hr-old slant cultures of the yeasts grown on this medium were used as inocula for flask cultures. The cells used for analysis were obtained from cultures of the yeasts in a medium containing (per L): commercial glucose, 33 g; yeast extract, 2.5 g; urea, 2.0 g; KH₂PO₄, 1.5 g; and MgSO₄·7H₂O, 0.5 g. Portions (140 ml) of this medium, contained in 500-ml Erlenmeyer flasks, were inoculated with 10 ml cell suspensions and incubated for 66 hr at 28 C on a rotary shaker. At the end of the fermentation period, the cultures were adjusted with distilled water to their original volumes (150 ml) and the cells were harvested by filtration of the cultures under vacuum through fine texture filter paper² in Buchner funnels. The clear filtrates were removed and refrigerated after determination of the pH. Yeast cells retained on the filter paper were washed twice in situ with liberal quantities of distilled water and then were removed from the paper with tared crucibles with the aid of a spatula. The harvested cells were dried for 120 hr at 80 C in an air oven and were subsequently weighed to determine the yield of cells. The dried cell mass was finely ground in a Wiley mill (60 mesh screen), and the resultant cell material was stored in screw-capped vials at room temperature until analyzed. Ordinarily, enough cells were obtained from the harvest of duplicate 150-ml quantities of the fermentation broths to provide sufficient dried material on which to conduct the analyses.

Methods of analysis. The sugar content of culture filtrates was determined by the method of Shaffer and Somogyi (1933). The nitrogen content of the cells was measured by a micro-Kjeldahl technique. Lysine and methionine were determined by microbiological assay of acid hydrolyzates of the cells. Five-ml quantities of 2.5 x HCl were added to 200-mg samples of the dried cells in small test tubes, the tubes were sealed, and the hydrolyses were carried out in an autoclave at 121 C for 16 hr. The crude hydrolyzates obtained were adjusted to pH 4.0 with NaOH and were immediately filtered through sintered glass filters to remove the residual particulate cell material and humin. Humin, which is formed by the interaction of amino acids and other cell material during acid hydrolysis, causes still further loss in amino acids upon continued contact and seriously interferes with the microbiological determination (Horn et al., 1953, 1955). Accordingly, the hydrolyzates were adjusted to pH 4.0 before filtration to insure maximal precipitation of the humin. The pH of the filtered hydrolyzates was further adjusted with NaOH to pH 6.8. Hydrolyzates then were made to an appropriate volume and aliquots of the diluted material were utilized for microbiological assay employing modifications of the procedures and media of Steele et al. (1949). Leuconostoc mesenteroides P-60 (strain NRRL 1153) was used for the measurement of both lysine and methionine in the hydrolyzed cell material; growth of the assay organism was determined turbidimetrically.

The tryptophan content of yeast cells was estimated colorimetrically according to the method of Spies and Chambers (1948, 1949). This method, which is based on the color reaction of tryptophan with p-dimethyl-
aminobenzaldehyde, was developed for the analysis of unhydrolyzed protein and employs 19 x H₂SO₄ to effect solution of proteinaceous material. The concentrated acid employed made possible application of this reaction to the direct analysis of intact microbial cell material. Previous attempts to utilize a variety of alkaline hydrolysis procedures on the cells had proved unsatisfactory, either because solubilization of the cell material was incomplete or because tryptophan was destroyed during the procedure. To estimate tryptophan by the Spies and Chambers procedure, weighed samples of the dried, ground cells were placed in test tubes equipped with ground glass stoppers, p-dimethylaminobenzaldehyde in 19 x H₂SO₄ was added, and the mixture of cells and reagent was shaken for 16 hr at 28 C. Because residual insoluble cell material sometimes remained after the acid treatment and because the action of the H₂SO₄ alone on the cells often gave color, determinations employing 19 x H₂SO₄ without p-dimethylaminobenzaldehyde were made and the results of the analysis were corrected accordingly. Sodium nitrite was added to develop the color; the color reaction mixtures were filtered when necessary through sintered glass filters to remove particulate material and the color was measured spectrophotometrically. The amount of tryptophan was calculated by reference to a standard curve prepared with pure DL-tryptophan.

RESULTS AND DISCUSSION

Lysine, methionine, and tryptophan contents of the cells of 271 strains of yeast were determined. Most of these strains occur among 196 species falling in 32 genera. A miscellaneous group of yeasts also was examined; among these were hybrid yeasts, a yeast-like mold, several yeasts unidentified as to genera, and a colorless alga. The data obtained by the analysis of these yeasts are summarized according to genera in table 1.

The amino acid content of the cells was calculated as "g amino acid per 16 g of nitrogen" and thus approximates the percentage of a given amino acid in the cell protein. The amount of nitrogen in the cells is included in table 1 to allow appraisal of the protein content of the yeast cells and also to permit calculation of the amount of the amino acids per g of dry cell material. Differences in the amino acid and protein content of species within a genus can be ascertained by inspection of the data, which show the range of values obtained, as well as by noting standard deviations. Standard deviations are included only for those genera in which six or more strains were examined.

It is apparent from the data in table 1 that there is a considerable degree of uniformity in the range of amino acid composition of the yeast strains. The average values for a given amino acid in the cells of organisms of different genera occur within a relatively narrow range. The relative abundance of the three amino acids concerned is generally the same; lysine is about 5 to 6 times as abundant as is methionine, which in turn is present in about 1½ times greater concentration than is tryptophan. Average values and standard deviations for these amino acids for the total number of yeasts examined illustrate this point. The amounts of cellular methionine, lysine, and tryptophan determined in this work agree with the values obtained from the limited literature reports (Anderson and Jackson, 1958). For example, the average of the eight analyses for the lysine content of yeasts cited by these authors is 7.8 g lysine per 16 g nitrogen. There is probably no significant difference between this value and that reported in table 1 (7.2 ± 1.1). The average lysine content of three yeasts (6.8 g per 16 g N) reported by Reusser et al. (1957) agrees with the over-all average obtained in our work. In comparison to our results, a somewhat greater amount of methionine (2.0 g per 16 g N) and a markedly greater amount of tryptophan (3.8 g per 16 g N) were reported by these workers. They used a method for tryptophan analysis based on a xanthopropeic reaction. Similar differences in the reported tryptophan content of bacteria were noted earlier (Anderson et al., 1958).

The amount of lysine, methionine, and tryptophan in the cell protein of the yeasts could not be correlated with their classification. Differences in the amounts of these amino acids in the cells of different species or between strains of the same species were as great as were the variations determined for organisms grouped in different genera. For example, Brettanomyces schanderlii had the greatest amount of lysine (10.2 g per 16 g nitrogen) of any organism examined, whereas another species of the genus, Brettanomyces italicus, had the least (3.8 g per 16 g nitrogen). The variation encountered between strains of the same species is illustrated by the results obtained during the examination of 4 strains of Candida robusta (9.8, 7.9, 7.3, and 7.8 g lysine per 16 g nitrogen) and of 5 strains of Saccharomyces lactis (7.4, 7.1, 7.3, 8.6, and 7.9 g lysine per 16 g nitrogen). Furthermore, the 12 strains of yeasts which had the highest content of lysine in their cell protein were classified in 10 different genera. Similarly, the 15 strains of yeasts which contained the most methionine or tryptophan were classified in 10 genera and 9 genera, respectively. No organism was found whose cell protein contained much more than the average amount of all three amino acids; a few yeasts had a relatively large amount of two of the three amino acids determined. Values obtained for the miscellaneous group listed in table 1 do not deviate greatly from those obtained for the yeasts as a whole. The colorless alga, Prototheca sp., presumably the organism most unlike the others phylogenetically, had a comparatively low content of
the nitrogen in the cells (2.0 per cent) but the amino acid to nitrogen ratios for lysine, methionine, and tryptophan were within the ranges determined for the yeasts.

No individual strain of yeast was found which contained in its cell protein a marked disparity of lysine, methionine, or tryptophan. Neither was any obvious correlation found between the amount of protein in the cell and the proportion of lysine, methionine, or tryptophan in the cell protein. Yeast cells undoubtedly contain a variety of different cellular proteins. Presumably each of the protein components of the cell has a definite function in the cell metabolism; it can be further assumed that the amino acid composition of a given cell protein is rather rigidly established in order for that protein to fulfill its proper function. Therefore, variation in the amount of amino acids such as lysine, methionine, or tryptophan could most likely come about by variation in the relative amounts of the different proteins rather than by variations in the composition of individual proteins. Although the proteins of the living cell must suffice for all the diverse functions of cellular metabolism, it must be concluded that the compositions for different yeasts, like those for bacterial cells previously studied (Anderson et al., 1958), do not show marked variation in amino acid concentration. The diversity in form, structure, and physiology which causes these yeasts to be classified

### TABLE 1

<table>
<thead>
<tr>
<th>Genus</th>
<th>No. of Strains</th>
<th>No. of Species</th>
<th>Cell Nitrogen (%)</th>
<th>Lysine</th>
<th>Methionine</th>
<th>Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Avg</td>
<td>Avg</td>
<td>Range</td>
</tr>
<tr>
<td>Total</td>
<td>271</td>
<td>207</td>
<td></td>
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<td>Range</td>
<td>Avg</td>
<td>Avg</td>
<td>Range</td>
</tr>
</tbody>
</table>

* Thirty-two for tryptophan analysis.
† Including 2 hybrids, several unidentified yeasts, a yeast-like mold and a colorless alga.
in 32 genera and 196 species is not sufficient to be reflected in corresponding differences in the amounts of amino acids.

SUMMARY
The nitrogen, lysine, methionine, and tryptophan content of the cells of 271 strains of yeasts was determined. The results obtained from the analysis of individual strains are summarized according to the 32 genera and 1 miscellaneous group into which the yeasts were classified. The yeasts examined were found to contain from 3.8 to 10.2 g lysine per 16 g nitrogen (average 7.2), from 0.4 to 1.7 g methionine per 16 g nitrogen (average 1.2), and from 0.3 to 1.6 g tryptophan per 16 g nitrogen (average 0.8). The cells contained an average of 6.4 per cent nitrogen. No correlation was found between the classification of the yeasts and the amount of the amino acids in the cell protein.

REFERENCES
SHAFFER, P. A. AND SOMOGYI, M. 1933 Copper-iodometric reagents for sugar determination. J. Biol. Chem., 100, 695-713.

Chromatographic Study of the Oligomycin Complex Produced under Various Conditions of Fermentation

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...It seems to be a general rule that if a microorganism produces an antibiotic it makes more than one such compound. Usually these products are closely related compounds, for example, bacitracins, penicillins, tetracyclines, and actinomycins. This interrelationship of compounds applies also to pigments, acids, and other metabolic products of microorganisms (Stickings and Raistrick, 1956).

The strain of microorganism is an important factor in determining the ratio of the products, as is well known in the commercial production of antibiotics. Nutrition is a second important factor. The relationship is very obvious in the effect of precursors for production of different types of penicillin and the requirement for adequate amounts of chlorides in the production of 7-chlortetracycline (Aureomycin) (Doerschuk et al., 1959).

In other fermentations the effect of nutrients does not seem so direct. More nitrogen in the form of soya peptone favored the production of the antifungal antibiotic, fradicin, and reduced the proportion of the antibacterial compound, neomycin (Swart et al., 1950). High nitrogen (2.5 mg or more per ml), either organic or inorganic, stimulated the production of the B component and low nitrogen (1.2 mg or less per ml) increased the production of the A component in erythromycin fermentation (Denison et al., 1958; Friedland et al., 1958). Katz et al. 1958 reported that individual amino acids markedly affected the proportion of certain components of the actinomycin complex.

In the early work on the antifungal antibiotic, oligomycin, paper chromatography showed that two components were produced, one of which was isolated and designated oligomycin A (Smith et al., 1954). Later, after the yields were greatly increased by means of a better medium, three components called A, B, and C were found even in a well-crystallized product (Visser, 1955). Paper chromatography showed that A was the major component.