LYSINE, METHIONINE AND TRYPTOPHAN CONTENT OF MICROORGANISMS

I. Bacteria

RALPH F. ANDERSON, ROBERT A. RHODES, GEORGE E. N. NELSON, MARGARET C. SHEKLETON, ALBERT BARRETO, JR.,[1] AND MARGIE ARNOLD

Fermentation Laboratory, Northern Utilization Research and Development Division,[2] Peoria, Illinois

Received for publication February 3, 1958

Cells of certain microorganisms, particularly yeasts of the genera *Saccharomyces* and *Torulopsis*, are used extensively as sources of essential amino acids in animal feeds. The amino acids frequently limiting in animal feed grains are lysine, methionine, and tryptophan. Accordingly, the Northern Utilization Research and Development Division has been conducting studies on the composition of microorganisms in order to indicate their usefulness as protein supplements for feeds. In the course of this work, the cells of a wide variety of aerobic bacteria were examined.

METHODS

Preparation of bacterial cells. The organisms[3] employed in these studies were cultivated in a medium composed of 4 per cent glucose, 0.5 per cent yeast extract, 0.5 per cent beef extract, and 1.0 per cent clarified corn steep liquor. Slant tubes of this medium, prepared by the inclusion of 1.5 per cent agar, were used to grow the bacteria for use as inocula. Cells washed from a 24-hr slant culture with 10 ml of sterile water were transferred into 140 ml of the broth medium in a 500 ml Erlenmeyer flask. The flask cultures were incubated at 28 C for 66 hr on a rotary shaker. The cells harvested from duplicate flasks by centrifugation were combined, washed with 0.85 per cent KCl, and recentrifuged. The washed cells, resuspended in a small amount of water, were steamed for 15 min and stored at 5 C as a thick slurry until analysis. Ordinarily, the quantity of cells obtained from two flask cultures (300 ml fermentation broth) made to a 20 ml volume gave a convenient concentration of wet cells for analysis. One ml of such a suspension of cells usually contained between 40 and 70 mg of dry cell matter. Storage of the cell suspensions for a period of one month did not result in significant variation of the quantities of cellular amino acids. Since aliquots of the whole cell suspensions (cells + liquid) were analyzed, extraction of amino acids from the cells during steaming or storage did not alter the results. For comparative purposes, all the strains of bacteria were propagated and handled in the same manner.

Analytical procedures. (1) Analysis of culture liquors—The sugar concentration of the supernatant fermentation broths was determined by the method of Shaffer and Somogyi (1933).

(2) Analysis of wet cell suspensions:—(a) The nitrogen content of the cells was determined by the use of a micro-Kjeldahl technique.

(b) The methionine and lysine contents of the wet cell material were determined by microbiological assay of acid hydrolyzates of the cells. One ml aliquots of the cell suspensions were measured into small test tubes, 1.0 ml of 2.5 N HCl added, the tubes sealed, and the hydrolysis of the cells carried out in an autoclave at 121 C for 16 hr. The hydrolyzates obtained by this procedure were adjusted with NaOH to pH 4.0 to aid precipitation of humin and filtered through glass filters to remove humin and residual cell debris. The filtrates were further adjusted to pH 6.8, and aliquots of the resultant clear hydrolyzates diluted to volumes appropriate for microbiological assay. The lysine and methionine contents of the hydrolyzates were determined at four different dilutions of each sample by turbidimetric measurement of the growth of *Leuconostoc mesenteroides* strain P-60 (NRRL B-1153), using modifications of the microbiological assay pro-

[1] Present address: Microbiological Research Department, Merck and Company, Inc., Rahway, New Jersey.

[2] Agricultural Research Service, U. S. Department of Agriculture.

[3] Selected from the bacteria maintained in the ARS Culture Collection at the Northern Utilization Research and Development Division.

cedures of Steele *et al.* (1949). Sufficient duplicates were run to insure precision.

(c) The tryptophan content of the bacterial cells was determined by the colorimetric method of Spies and Chambers (1948, 1949). In this procedure, the reaction product of tryptophan and *p*-dimethylaminobenzaldehyde is obtained without prior hydrolysis of protein by carrying out the reaction in 19 N sulfuric acid, which dissolves proteinaceous material. Accordingly, 1 ml aliquots of the cell suspensions were treated with H_2SO_4 (19 N after dilution by the cell suspension) containing *p*-dimethylaminobenzaldehyde. The reaction mixtures (10 ml in volume) were agitated in 20 by 130 mm glass-stoppered tubes on a reciprocal shaker in the dark for 16 hr. To each tube was then added 0.1 ml of 0.08 per cent $NaNO_2$ solution to cause development of the blue color. All reaction products were filtered through glass filters to remove cell material not completely dissolved by the acid. Because the reaction of H_2SO_4 and the cell material resulted in extraneous color formation, blank determinations routinely were made, using cell suspensions and H_2SO_4 without *p*-dimethylaminobenzaldehyde. The density of color was measured at 590 mμ with a Coleman spectrophotometer (model 14),[4] and the amount of tryptophan was calculated from a standard curve prepared with DL-tryptophan.

RESULTS AND DISCUSSION

Analyses of 86 cultures of bacteria, representing 81 species classified in 30 genera, are presented in table 1. The amounts of lysine, methionine, and tryptophan are tabulated as g of amino acid per 16 g of nitrogen. This designation approximates the percentage of a particular amino acid in the protein. However, 16 per cent is only the average nitrogen content of proteins, and not all of the amino acid content of the cells is incorporated into protein; furthermore, the Kjeldahl value includes nitrogen other than protein nitrogen. Hence, the term "amino acid (g per 16 g of N)" is used for tabulating our results. In the text, for the sake of brevity, the term "cell protein" is used to denote the arbitrary protein content of the cells (6.25 $\times$ per cent nitrogen).

[4] The mention of products does not imply endorsement by the Department of Agriculture over others of a similar nature not used.

The values obtained by microbiological assay of the acid hydrolyzates accurately indicate the lysine and methionine content of the cells. The assay was checked systematically by reference to the results obtained with a standard sample of dried yeast cells which was carried through the hydrolysis and assay procedures with each determination. The Spies chemical procedure for the determination of tryptophan was designed for the analysis of purified proteins; with such materials it offers accurate and reproducible results. Application of the Spies method to whole cells is less satisfactory. However, the conditions employed in this work, including the corrections obtained by the use of individual blank determinations for each culture, are believed to provide a reliable picture of the relative amounts of tryptophan in the cells of the different bacteria.

Results indicate that most bacteria have about the same ratios of the three amino acids in the protein of their cells. On the average, the cell protein of the bacteria contained 6.5 per cent lysine, 1.8 per cent methionine, and 0.3 per cent tryptophan. Values previously reported by other workers (Mondolfo and Hounie, 1951; Camien *et al.*, 1945) are about the same for lysine, slightly less for methionine (1.0 per cent), and greater for tryptophan (0.5 per cent). Although general agreement exists among the analyses of microbial cells by different workers, variant values sometimes are obtained. For example, concentrations of tryptophan up to 5.6 g per 16 g of nitrogen have been reported to occur in bacterial cells by Reusser *et al.* (1957); tryptophan was measured by a xanthoproteic colorimetric method in this work. The concentrations of lysine and methionine, as estimated by paper chromatography by these workers, were comparable to other published results. A critical evaluation of procedures for the quantitative determination of tryptophan is outside the scope of the present publication. Until such a definitive study is made, the reported quantities of tryptophan in microbial cells must be regarded as provisional.

The amount of lysine, methionine, and tryptophan in the cell protein of the bacteria was a characteristic of the species or of an individual strain of the organism and was not related to genera or other phylogenetic criteria. As much variation in the proportion of lysine in the cell protein occurred among species of the same genus

TABLE 1

Lysine, methionine, and tryptophan content of bacterial cells

NRRL Number	Organism	Amino Acid (g per 16 g N)		
		Lysine	Methionine	Tryptophan
	Achromobacter (avg)	5.7	3.0	0.4
B-1323	*A. ammoniagenes*	5.3	1.6	0.4
B-551	*A. lacticum*	6.1	4.5	0.4
	Aerobacter (avg)	6.9	2.3	0.3
B-199	*A. aerogenes*	7.2	2.6	0.3
B-126	*A. cloacae*	6.2	2.6	0.4
B-320	*A. hibernicum*	6.7	2.1	0.3
	Aeromonas (avg)	6.7	1.7	0.4
B-909	*A. hydrophila*	6.3	1.5	0.4
B-926	*A. ichthyosmia*	6.4	2.1	0.4
B-914	*A. punctata*	7.4	1.5	0.4
	Agrobacterium (avg)	6.9	2.3	0.3
B-181	*A. radiobacter*	5.8	2.0	0.3
B-193	*A. rhizogenes*	8.4	2.7	0.4
B-36	*A. tumefaciens*	6.6	2.1	0.2
	Alcaligenes (avg)	6.4	2.0	0.3
B-170	*A. faecalis*	5.9	2.9	0.3
B-182	*A. viscolactis*	7.0	1.1	0.3
B-488	*Azotobacter chroococcum*	6.7	2.4	0.3
	Bacillus (avg)	8.6	2.2	0.3
B-768	*B. coagulans*	8.0	1.7	0.4
B-349	*B. megaterium*	9.8	2.9	0.3
B-1827	*B. megaterium*	11.8	3.4	0.7
B-208	*B. pumilus*	7.1	1.9	0.2
B-544	*B. subtilis*	8.0	2.0	0.3
B-1296	*B. subtilis*	8.2	1.9	0.1
B-675	*B. technicus*	7.1	1.3	0.2
	Bacterium (avg)	6.1	1.9	0.3
B-744	*Bacterium* sp.	6.5	—	0.2
B-1022	*Bacterium* sp.	5.3	1.4	0.2
B-1030	*Bacterium* sp.	7.3	2.0	0.4
B-1091	*Bacterium* sp.	5.4	2.3	0.3
	Cellulomonas (avg)	7.4	2.1	0.4
B-401	*C. biazotea*	6.9	2.1	0.4
B-404	*C. uda*	8.0	2.1	—
B-668	*Cellvibrio vulgaris*	8.1	1.3	0.2
B-1085	*Chromobacterium violaceum*	7.8	1.9	0.2
	Corynebacterium (avg)	4.9	1.2	0.3
B-190	*C. fascians*	3.6	1.2	0.2
B-729	*C. flaccumfaciens*	3.6	0.8	0.5
B-33	*C. michiganense*	6.6	1.2	0.4
B-1379	*C. xerosis*	5.7	1.5	0.2
B-422	*Erwinia carotovora*	7.7	1.5	0.4
	Escherichia (avg)	6.3	1.6	0.3
B-210	*E. coli*	7.9	2.0	0.3
B-281	*E. coli*	7.3	1.8	0.4
B-766	*E. coli*	3.7	0.9	0.2

TABLE 1—*Continued*

NRRL Number	Organism	Amino Acid (g per 16 g N)		
		Lysine	Methionine	Tryptophan
	Flavobacterium (avg)	3.6	1.1	—
B-184	*F. aurantiacum*	4.1	1.5	—
B-185	*F. sulfureum*	3.0	0.7	0.1
B-935	*Hydrogenomonas pantotropha*	7.7	1.5	0.4
	Micrococcus (avg)	8.7	1.5	0.3
B-635	*M. conglomeratus*	8.2	1.4	0.3
B-287	*M. lysodeikticus*	11.4	2.0	0.3
B-186	*M. subcitreus*	6.6	1.1	0.2
	Mycobacterium (avg)	4.9	1.4	0.3
B-609	*M. phlei*	3.1	1.0	0.3
B-1306	*M. rhodochrous*	5.4	1.3	0.3
B-612	*M. smegmatis*	6.0	1.4	0.3
B-692	*Mycobacterium* sp.	5.0	1.7	0.4
	Mycoplana (avg)	5.2	1.9	0.4
B-1090	*M. bullata*	4.6	1.6	0.4
B-1031	*M. dimorpha*	5.9	2.2	0.4
	Nocardia (avg)	4.8	1.1	0.3
B-1365	*N. coeliaca*	6.6	0.9	0.2
B-1532	*N. erythropolis*	4.1	1.3	0.3
B-1531	*N. polychromogenes*	3.8	1.0	0.4
	Protaminobacter (avg)	6.4	2.0	0.2
B-1051	*P. alboflavus* var. α	4.3	1.6	0.2
B-1048	*P. ruber*	8.5	2.4	0.2
	Proteus (avg)	7.3	2.0	0.4
B-420	*P. ammoniae*	7.6	2.0	0.4
B-400	*P. mirabilis*	7.1	2.2	0.3
B-123	*P. vulgaris*	7.3	1.9	0.6
	Pseudomonas (avg)	6.2	1.9	0.3
B-823	*P. alliicola*	6.0	1.2	0.4
B-1543P	*P. aureofaciens*	6.4	2.3	0.3
B-1101	*P. cepacia*	4.4	1.7	0.3
B-560	*P. chlororaphis*	5.5	2.1	0.3
B-21	*P. mildenbergii*	5.6	1.5	0.2
B-6bs	*P. reptilivora*	5.4	2.9	0.3
B-311	*P. riboflavina*	9.9	1.6	0.4
	Rhizobium (avg)	5.8	2.2	0.4
B-1261	*R. meliloti*	5.1	2.1	0.4
B-327	*R. trifolii*	6.6	2.2	0.3
B-175	*Rhodospirillum rubrum*	6.0	2.2	0.4
	Sarcina (avg)	10.3	1.7	0.3
B-1262	*S. flava*	13.5	1.6	0.3
B-1018	*S. lutea*	7.1	1.3	0.2
B-286	*S. ureae*	10.2	2.1	0.3
	Serratia (avg)	6.8	1.8	0.3
B-284	*S. marcescens*	7.5	1.6	0.3
B-1481	*S. marcescens*	6.2	2.0	0.3
B-313	*Staphylococcus aureus*	11.2	1.4	0.2
	Streptomyces (avg)	4.8	1.3	0.3
B-546	*S. antibioticus*	5.8	1.1	0.3

TABLE 1—*Concluded*

NRRL Number	Organism	Amino Acid (g per 16 g N)		
		Lysine	Methionine	Tryptophan
B-1221	*S. californicus*	3.8	1.0	0.3
B-556	*S. fradiae*	7.2	2.4	0.3
B-1062	*S. griseolus*	2.6	0.7	0.2
B-150	*S. griseus*	5.0	1.2	0.3
B-1125	*S. olivaceus*	4.7	1.2	0.3
	Vibrio (avg)	5.3	1.6	0.4
B-781	*V. percolans*	4.4	1.3	0.3
B-782	*V. rubicundus*	6.2	1.8	0.4
	Xanthomonas (avg)	5.8	1.4	0.4
B-34	*X. begoniae*	5.2	0.9	0.8
B-1459	*X. campestris*	5.1	1.6	—
B-215	*X. malvacearum*	6.6	2.3	0.1
B-1460	*X. phaseoli*	5.0	1.5	0.3
B-28	*X. stewartii*	7.2	0.7	0.4

as was observed in average values obtained for different genera. Further, the twofold variation of the lysine content (3.7 to 7.9 per cent) of the cell protein found among the three strains of *Escherichia coli* analyzed was greater than the differences which were found to exist between most of the genera tested. Mondolfo and Hounie (1951) reported differences of approximately the same magnitude (3.5 to 6.0 per cent lysine in the cell protein) among the strains of *E. coli* which they studied.

There was a tendency for the organisms of the order Eubacteriales to contain greater concentrations of both lysine and methionine in the cell protein than did the organisms representative of the order Actinomycetales (Streptomyces and Nocardia). No large differences were noted in the tryptophan content of the cellular proteins of these orders. Among the Eubacteriales, more lysine occurred in the cell proteins of gram-positive than in those of gram-negative organisms, although the genus *Corynebacterium* apparently was an exception. Results reported by previous workers do not indicate that the lysine content of the cell protein is related to the Gram stain reaction of the organism (Mondolfo and Hounie, 1951; Stokes and Gunness, 1946; Garibaldi *et al.*, 1953; Camien *et al.*, 1945). Nevertheless, the average content of lysine in the cellular proteins of members of the genera *Bacillus*, *Micrococcus*, and *Sarcina* was significantly greater ($P < 0.01$) than that in the proteins of other genera tested. Differences in the analyses of gram-positive and gram-negative bacteria may reflect only differences in the ease and extent of hydrolysis of cell material, particularly the cell wall and cell membrane whose characteristics are associated with the Gram stain reaction. It is of interest that Mittwer and Bartholomew (1956) were not able to demonstrate a correlation between the sulfhydryl content of whole cells and the Gram stain behavior of the bacteria which they studied, although earlier work had indicated that sulfhydryl groups were involved in the Gram stain reaction (Bartholomew and Umbreit, 1944). The spatial arrangement of the reactive groups of amino acids in the cell material may have a greater influence on the Gram stain response of the organism than do differences in the quantity of amino acids in the cells.

SUMMARY

The cells of the bacteria studied contained 2.6 to 13.5 (average, 6.5) g of lysine, 0.7 to 4.5 (average, 1.8) g of methionine, and 0.1 to 0.8 (average, 0.3) g of tryptophan per 16 g of nitrogen.

In general, the quantities of these amino acids contained in bacterial cells were characteristic of the individual strain of the organism and were not related to its classification. The extreme differences which occur in the composition of specialized tissue cells of plants and animals were not observed among the cells of the bacteria studied.

The quantity of lysine contained in cell protein varied more among different organisms than did the quantity of either methionine or tryptophan. Organisms of the order Eubacteriales contained more lysine than did organisms classified as Actinomycetales; among the Eubacteriales, gram-positive bacteria contained more lysine than did gram-negative bacteria.

REFERENCES

BARTHOLOMEW, J. W. AND UMBREIT, W. W. 1944 Ribonucleic acid and the Gram stain. J. Bacteriol., **48**, 567–578.

CAMIEN, M. N., SALLE, A. J., AND DUNN, M. S. 1945 Investigations of amino acids, peptides and proteins. XXII. Percentages of some amino acids in lactobacilli. Arch. Biochem., **8**, 67–78.

GARIBALDI, J. A., IJICHI, K., SNELL, N. S., AND

LEWIS, J. C. 1953 *Bacillus megatherium* for biosynthesis of cobalamin. Ind. Eng. Chem., **45**, 838–846.

MITTWER, T. AND BARTHOLOMEW, J. W. 1956 Investigation of the role of sulfhydryl groups in bacteria in relation to the Gram stain. J. Bacteriol., **72**, 337–339.

MONDOLFO, H. AND HOUNIE, E. 1951 Sobre los amino ácidos que componen las proteínas bacterianas. Rev. asoc. bioquím. arg., **16**, 416–418.

REUSSER, F., SPENCER, J. F. T., AND SALLANS, H. R. 1957 Essential amino acids in microorganisms. Can. J. Microbiol., **3**, 721–728.

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

SPIES, J. R. AND CHAMBERS, D. C. 1948 Chemical determination of tryptophan. Anal. Chem., **20**, 30–39.

SPIES, J. R. AND CHAMBERS, D. C. 1949 Chemical determination of tryptophan in proteins. Anal. Chem., **21**, 1249–1266.

STEELE, B. F., SAUBERLICH, H. E., REYNOLDS, M. S., AND BAUMANN, C. A. 1949 Media for *Leuconostoc mesenteroides* P-60 and *Leuconostoc citrovorum* 8081. J. Biol. Chem., **177**, 533–544.

STOKES, J. L. AND GUNNESS, M. 1946 The amino acid composition of microorganisms. J. Bacteriol., **52**, 195–207.