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The Amino-acid Composition of Certain Bacteria and Yeasts

By JOAN C. FREELAND and E. F. GALE

Medical Research Council Unit of Chemical Microbiology, Biochemical Laboratory, Cambridge

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The amino-acid composition of bacteria has hitherto received little attention; recently Stokes & Gunness (1946) have reported some analyses on a range of bacteria though detailed results are not yet available. Camien, Salle & Dunn (1945) collected values from previous literature and extended our knowledge with regard to a variety of organisms. The analysis of protein hydrolysates by the use of specific decarboxylases (Gale, 1945, 1946) enables accurate values for certain amino-acids to be obtained on comparatively small amounts of material. The present investigation was undertaken in order to determine whether the amino-acid composition of bacterial protein varies with the growth conditions of the organism and also whether there is any marked difference between the amino-acid composition of Gram + and Gram − species.

To determine the amino-acid composition of the bacterial protein it is necessary to correct values obtained with acid-hydrolysates of intact organisms for free amino-acid carried down from the growth medium. Such free amino-acid may be carried down on the surface of the organism or in the internal environment of the organism (Gale, 1947). Taylor (1947) has shown that only Gram + organisms carry down large amounts of free amino-acid in the internal environment, Gram − species containing negligible amounts of free amino-acid.

EXPERIMENTAL

Organisms. The *Esch. coli* used in these experiments was obtained from the National Collection of Type Cultures (N.C.T.C.), No. 86; a Gram − organism N.C.T.C. No. 6578 was isolated by this Unit (Gale & Epps, 1944) also the strains of *B. subtilis*, *Staph. aureus* (B) and *Strep. faecalis*; *Proteus vulgaris*, *Aerobact. aerogenes* and *Staph. aureus* (A) were given to us by the Department of Pathology, Cambridge; *B. brevis* was kindly sent by Dr R. J. Dubos; the three strains of yeast were obtained from the Carlsberg Laboratory through Prof. Winge.

Culture media. Yeasts were cultivated in nutrient salt solution containing 0·2% Difco yeast extract and 4% glucose. For the cultivation of bacteria, a tryptic digest of casein was used as basal medium with the addition of glucose and/or marmite when necessary (Taylor, 1947). *Esch. coli*, *Aerobact. aerogenes*, organism No. 6578 and *Proteus vulgaris* were grown in flasks containing 4·5 l. of medium for each experiment; the other organisms were grown aerobically in liquid media in Roux bottles lying flat. An amino-acid free medium was also used for *Esch. coli* and *Aerobact. aerogenes* and consisted of Stephenson's salt-mixture with the addition of either 1·0% sodium lactate or 1·0% glucose; this was incubated in Roux bottles.

Methods. Amino-acids were estimated by the manometric method using specific amino-acid decarboxylase preparations (Gale, 1945, 1946) and results expressed as amino-acid-N as percentage total-N determined as usual by the micro-Kjedahl method. After growth, the organisms were centrifuged out of the medium and thoroughly washed with water on the centrifuge. The washed organisms were then made up into thick suspension in water (30–50 mg. dry weight/ml.) and the suspension divided into two parts. One part was then hydrolyzed with hydrochloric acid (Gale, 1945) and total amino-acid determined on the hydrolysate. The other part was placed in a boiling-water bath for 20 min. and amino-acid and N estimations then carried out as usual; values obtained with this portion give the free amino-acid carried down on the surface and in the internal environment of the cells (Gale, 1947). The total amino-acid content of the hydrolysate was then corrected for the free amino-acid content so determined, to give the combined amino-acid liberated by acid hydrolysis.

The free and combined amino-acid content of certain micro-organisms

Table 1 gives typical results obtained for two Gram −, two Gram + organisms and two yeasts grown in casein-digest medium. The free amino-acid correction is much greater in the case of the Gram + organisms, as these possess a high concentration of certain amino-acids in the internal environment (Taylor, 1947). (Values given in Tables 2 and 3 show corrected figures only.) Since bacterial and yeast cells presumably contain carbohydrate material, there may be a small loss of arginine during hydrolysis procedures (Tristram, 1939).

Table 1. *Free and combined amino-acid content of certain micro-organisms*

(Results expressed as % total N.)

Columns: (*a*) total amino-acid estimated on acid hydrolysate of washed suspension; (*b*) free amino-acid estimated on boiled washed suspension; (*c*) combined amino-acid = (*a* − *b*).

Organism ...	*Esch. coli*			*Aerobact. aerogenes*			*Strep. faecalis*		
	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
Arginine-N	10·17	0·10	10·07	10·23	0·32	9·91	6·60	1·14	5·46
Lysine-N	7·80	0·21	7·59	7·42	0·40	7·02	10·50	2·85	7·65
Histidine-N	3·56	0·32	3·24	3·18	0·05	3·13	2·85	0·80	2·05
Tyrosine-N	1·36	0·0	1·36	1·37	0·0	1·37	0·84	0·00	0·84
Glutamic-N	5·92	0·31	5·61	5·48	0·07	5·41	5·93	1·54	4·39

Organism ...	*Staph. aureus*			Dutch top yeast			*Saccharomyces carlsbergensis*		
	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
Arginine-N	5·69	0·72	4·97	12·83	5·04	7·79	10·16	1·59	8·57
Lysine-N	11·06	1·79	9·27	15·05	7·45	7·60	12·0	3·03	8·97
Histidine-N	1·50	0·20	1·30	4·91	1·56	3·35	3·48	0·87	2·61
Tyrosine-N	1·23	0·18	1·05	1·45	0·07	1·38	1·55	0·11	1·44
Glutamic-N	7·53	3·56	3·97	8·12	2·28	5·84	7·27	1·60	5·67

Effect of growth environment on amino-acid composition of the protein of Esch. coli

The physico-chemical composition of the growth medium may have marked effects on the enzymic composition of an organism such as *Esch. coli* (Gale, 1943) and the present estimations were carried out in order to see whether these changes are reflected in any significant variation in the amino-acid composition of the bacterial protein. *Esch. coli* and *Aerobact. aerogenes* were grown in the various buffered media recorded in Table 2 (cf. Gale & Epps, 1942).

Table 2 shows the corrected figures for the amino-acid composition of the bacterial protein of the various organisms used. It can be seen that there was no significant variation with the growth media tested. These results thus confirm the general conclusion of Stokes & Gunness (1946) that the composition of bacterial protein is fixed and characteristic of the organism concerned. There is a suggestion that the lysine content increased with increasing pH at which growth occurred, but it is difficult to say with certainty whether the variation is significant. Table 2 also gives values for *Aerobact. aerogenes* grown under various conditions; again there was no significant variation.

Amino-acid composition of various bacteria and yeasts

Table 3 gives the corrected values for the amino-acid composition of the protein of a variety of organisms. Generally speaking the analyses show amino-acid contents of the same order for all the organisms tested. When the division of the bacteria into Gram + and Gram − types is considered there is, however, one significant difference, namely in the arginine content. Nine analyses of *Esch. coli* gave a mean arginine content of 10·41 % total N; five analyses of *Aerobact. aerogenes* gave a mean of 10·39 %; single analyses of *P. vulgaris*, organism No. 6578 and *B. brevis* gave 7·75, 12·18 and 9·95 % respectively; an over-all mean value of 10·3 % total N as arginine-N. Comparing these values with five analyses of *Strep. faecalis* giving a mean of 5·4 %; two with *Staph. aureus* giving 4·83 and 4·98 %; and one with *B. subtilis* giving 7·55 % (an over-all average of

Table 2. *Effect of growth environment on combined amino-acid content of the protein of* Esch. coli *and* Aerobact. aerogenes

Values represent the amino-acid content of acid hydrolysates of washed organisms corrected for free amino-acid as shown in Table 1.

Growth media: (1) salt mixture + 1·0 % sodium lactate; (2) salt mixture + 1·0 % glucose; (3) tryptic digest of casein adjusted to pH 8·5; (4), (5), (6), (7), as (3) but adjusted to 8·3, 7·4, 5·5 and 4·9 respectively; (8), (9) tryptic digest of casein + 2·0 % glucose.

(Results expressed as % total N.)

	Esch. coli								
Growth medium ...	1	2	3	4	5	6	7	8	9
Arginine-N	10·50	10·35	10·25	10·35	10·07	10·30	10·25	11·60	10·06
Lysine-N	6·74	—	8·2	7·95	7·6	7·3	6·80	8·0	7·05
Histidine-N	2·97	3·3	3·16	3·32	3·2	3·7	3·03	3·5	3·47
Tyrosine-N	1·23	1·57	1·33	1·57	1·36	1·63	1·23	1·39	1·58
Glutamic-N	5·07	5·57	5·75	5·83	5·60	5·66	5·42	5·90	5·73

	Aerobact. aerogenes				
Growth medium ...	1	3	5	7	8
Arginine-N	10·48	10·70	9·91	10·55	10·3
Lysine-N	6·8	6·48	7·02	6·38	7·2
Histidine-N	3·13	4·0	3·13	3·44	3·32
Tyrosine-N	1·35	1·73	1·37	1·37	1·37
Glutamic-N	5·57	5·80	5·41	5·35	5·85

Table 3. *Combined amino-acid content of various bacteria and yeasts*

The values represent the amino-acid content of acid hydrolysates of washed suspensions of organisms corrected for free amino-acid content as shown in Table 1.

(Results expressed as % total N.)

Gram − bacteria

Organism ...	*Esch. coli*	*Aerobact. aerogenes*	*Proteus vulgaris*	Organism No. 6578	*B. brevis*
No. of estimations ...	9	5	1	1	1
Arginine-N	10·41	10·39	7·75	12·18	9·95
Lysine-N	7·45	6·78	6·39	8·23	7·92
Histidine-N	3·29	3·40	4·39	2·04	2·49
Tyrosine-N	1·43	1·44	2·11	1·68	2·16
Glutamic-N	5·57	5·60	4·88	6·37	7·34

Gram + bacteria

Organism ...	*Strep. faecalis*	*Staph. aureus* (A)	*Staph. aureus* (B)	*B. subtilis*
No. of estimations ...	5	1	1	1
Arginine-N	5·44	4·83	4·98	7·55
Lysine-N	7·61	10·55	9·27	7·52
Histidine-N	2·05	1·15	1·30	2·47
Tyrosine-N	0·84	1·75	1·05	1·49
Glutamic-N	4·39	4·92	3·97	5·29

Yeasts

Organism ...	Yeast foam	Dutch top yeast	*Saccharomyces carlsbergensis*
Arginine-N	8·66	7·79	8·55
Lysine-N	9·07	7·60	6·39
Histidine-N	1·42	3·35	4·39
Tyrosine-N	1·11	1·38	2·11
Glutamic-N	5·50	5·84	4·88

5·57 % total N as arginine-N); it would seem that the Gram + organisms, particularly the Gram + cocci, contained about half the amount of arginine that the Gram − types did. Further confirmation of this can be obtained from the figures given by Camien, Salle & Dunn (1945). These workers used a different method for the estimation of arginine and when their figures are recalculated as a percentage of total N, they show the arginine content of *Esch. coli* as 15·6, 13·2 and 13·4 % and of *Aerobacter aerogenes* as 13·6 and 13·2 %—a mean value of 13·8 % for the five estimations. These figures can be compared with values obtained for four different *Lactobacilli* (*L. arabinosus*, *L. casei*, *L. pentosus* and *L. fermenti*) of 6·6, 7·2, 6·2 and 9·6 % respectively; the mean value of 7·4 % again represents about half the arginine content of the Gram − species investigated. Our figures would suggest that the *Bacillus* species examined have an arginine content approximately half-way between that of the Gram − coliforms on the one hand and of the Gram + cocci on the other. The yeasts again appear to occupy a similar intermediate position.

SUMMARY

1. Estimation of the combined amino-acid content of micro-organisms involves estimation of the total amino-acid content of acid hydrolysates corrected by a further estimation of the free amino-acid carried down on the surface and in the internal environment of the cells.

2. The amino-acid composition of the protein of *Esch. coli* or *Aerobact. aerogenes* was unaffected by widely varying growth conditions.

3. Values are given for the arginine, lysine, histidine, tyrosine and glutamic acid contents of various bacteria and yeasts. The Gram + cocci examined contained about half as much arginine as the Gram − bacteria.

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ADDENDUM

Since going to press, Stokes, J. L. & Gunness, M. (1946), *J. Bact.* **52**, 195, have published a paper on the amino-acid composition of micro-organisms. They find that the amino-acid composition of an organism is a stable and characteristic property of the cell although striking differences occur between different micro-organisms. They find a significantly higher value for lysine of *Staph. aureus* than *Esch. coli* but no allowance is made for the free lysine in the internal environment of the former. The same difference between the arginine-content of Gram + and Gram − bacteria, as noted in our paper, can be seen in their figures.