AMINO ACID COMPOSITION OF CULTURES OF *Salmonella typhi* AND *Salmonella paratyphi* A AND B

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The amino-acid composition of cultures of *Salmonella typhi* and *Salmonella paratyphi* varies only slightly with the species, strain, and mode of cultivation of the microorganisms. A relatively high content of alanine, glutamic acid, valine, and leucine (isoleucine) was detected.

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Several recent studies have been made of the amino-acid composition of pathogenic microorganisms and its relationship to various factors: species of the bacteria, composition of the nutrient medium, conditions of cultivation, degree of aeration, periods of growth, and so on [2, 4-6, 8]. However, only one report of an investigation of the amino-acid composition of bacteria of the typhoid-paratyphoid group could be traced [13].

As well as bound amino acids, free amino acids are also found in the bacterial cell. The presence of free intracellular amino acids has been demonstrated for many microorganisms [1, 10, 11, 14]. However, no information could be found in the accessible literature on the content of free amino acids in bacteria of the typhoid-paratyphoid group.

The object of the present investigation was to study the quantitative amino-acid composition of cultures of *Salmonella typhi* and *Salmonella paratyphi* A and B, and to determine the bound and free intracellular amino acids and their relationship to the species and strains of the bacteria and to conditions of cultivation: on liquid medium with aeration and on nutrient agar.

EXPERIMENTAL METHOD

Cultures of *S. typhi* (strains Jy_2 4446, 1203, and 0-901), *S. paratyphi* B (strains 42 and 50 602), and *S. paratyphi* A (strain 50 503) were grown under submerged conditions with aeration for 10-12 h on a synthetic medium of the following composition: 0.1% Na_2HPO_4, 0.1% KH_2PO_4, 0.5% NaCl, 0.5% glucose, 0.45% bisubstituted ammonium citrate, 0.005% tryptophan, 0.01% cysteine, 0.01% $MgSO_4$, 0.18% Na_2CO_3 (anhydrous), and 0.0005% nicotinic acid, pH 7.2. For comparative experiments the same strains of microorganisms were obtained under stationary conditions on Hottinger's nutrient agar. The bacterial mass was collected by centrifugation, washed 3 times with distilled water, and dried with acetone.

Free intracellular amino acids were first extracted from the bacterial mass with 75% ethanol followed by treatment of the solution with chloroform. After extraction of the free amino acids, the bacterial mass was hydrolyzed with 6N HCl solution for 24 h at 105° in sealed ampoules. At the end of hydrolysis the HCl was removed by repeated evaporation and the dry residue was dissolved in 10% isopropyl alcohol.

The amino-acid content was determined by quantitative chromatography on paper [3]. Tryptophan was determined chemically in individual samples by Horn's method [9]. Proline and hydroxyproline were determined qualitatively with 0.2% isathine solution in acetone [11]. The content of each amino acid was calculated as the mean of 5-8 determinations. The results were expressed in mg amino acid/g dry weight of bacterial mass.

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TABLE 1. Amino-Acid Composition of S. typhi and S. paratyphi A and B when Cultivated under Different Conditions (mg/g Dry Bacterial Mass)

Amino acid	Liquid medium with aeration					Hottinger's nutrient agar					
	S. typhi		S. paratyphi B		S. paratyphi A	S. typhi			S. paratyphi B		S. paratyphi A
	4446	1203	50 602	42	50 503	4446	1203	0-901	50 602	42	50 503
Cystine	3,35	2,55	2,88	3,09	3,08	2,46	2,30	1,82	2,98	2,45	4,88
Lysine	14,58	8,55	11,42	16,83	14,27	11,71	15,20	12,50	14,90	12,82	8,10
Histidine	14,04	6,95	8,19	7,05	7,70	8,98	8,92	4,70	6,05	8,39	9,11
Arginine	15,43	11,48	12,74	18,71	15,24	11,15	15,14	14,54	17,73	14,63	7,72
Aspartic acid	15,34	9,85	10,43	14,84	15,09	10,73	13,78	14,50	20,53	12,00	5,70
Serine	16,05	12,38	12,48	20,65	15,13	13,33	14,56	13,24	19,70	16,60	7,36
Glycocol	10,73	7,83	7,80	11,63	7,85	8,26	8,21	6,30	7,93	11,41	4,43
Glutamic acid	40,68	21,08	53,66	34,44	44,70	21,14	24,41	20,30	30,53	27,44	10,50
Threonine	18,18	13,73	13,70	22,76	15,90	10,20	15,56	13,65	17,50	18,10	2,90
α-Alanine	41,66	29,31	42,31	50,60	45,87	29,14	29,91	29,25	43,30	35,59	18,03
Tyrosine	11,29	11,53	8,00	14,15	11,58	11,93	9,99	8,70	14,25	10,40	3,10
Tryptophan	11,27	8,76	13,85	25,15	4,66	7,38	11,67	7,13	11,56	11,58	6,35
Methionine	8,16	7,28	14,35	13,91	15,67	6,43	8,89	8,01	10,89	8,44	4,36
Valine	21,96	15,80	23,30	21,23	19,20	17,51	12,35	13,11	21,51	20,45	8,50
Phenyl-alanine	10,26	5,80	15,13	10,08	19,00	7,10	8,96	7,74	12,02	9,53	5,13
Leucine (isoleucine)	34,02	21,75	29,45	38,00	33,13	23,80	32,45	24,65	35,71	31,59	9,56
Total	287,00	194,61	279,69	323,11	288,07	201,25	233,22	203,15	287,09	251,42	115,73

TABLE 2. Free Intracellular Amino Acids of S. typhi and S. paratyphi A and B when Cultivated under Different Conditions (in mg/g Dry Bacterial Mass)

Amino acid	Liquid medium with aeration					Hottinger's nutrient agar					
	S. typhi		S. paratyphi B		S. paratyphi A	S. typhi			S. paratyphi B		S. paratyphi A
	4446	1203	50 602	42	50 503	4446	1203	0-901	50 602	42	50 503
Cystine	0,72	0,89	0,99	1,35	1,27	1,61	0,96	0,90	0,76	0,58	1,40
Lysine	0,69	1,00	0,85	0,89	1,30	1,98	1,30	1,28	1,13	1,50	1,90
Histidine	0,36	0,68	0,78	0,75	2,52	1,18	1,02	0,98	0,69	0,81	2,34
Aspartic acid	0,41	0,86	0,79	0,77	1,01	1,63	1,20	1,04	0,84	0,95	1,39
Serine	0,56	1,06	1,01	0,79	0,94	1,08	1,00	1,08	0,91	1,02	1,21
Glycocol	0,99	1,13	1,04	0,66	1,07	1,02	1,22	1,01	0,92	0,94	1,05
Glutamic acid	3,39	1,66	5,00	5,11	4,50	3,63	2,40	1,38	1,44	1,70	2,69
α-Alanine	3,96	1,36	3,20	1,79	6,40	2,60	2,17	1,95	1,35	1,52	4,37
Tyrosine	0,64	0,57	0,87	0,70	0,76	0,88	1,23	0,72	1,13	0,99	0,83
Tryptophan	0,81	0,94	0,76	0,90	0,75	0,90	1,03	1,15	0,94	0,77	1,28
Methionine	1,06	0,96	0,98	1,04	1,23	1,13	1,20	0,86	0,89	0,80	1,38
Valine	1,62	1,80	1,40	2,10	1,49	3,40	1,85	2,09	1,45	1,43	2,10
Phenyl-alanine	0,76	0,75	0,41	0,55	0,87	1,18	1,00	0,72	0,75	0,91	1,20
Leucine (isoleucine)	1,76	1,70	1,57	1,07	2,13	3,41	2,50	2,86	1,94	2,35	1,90
Total	17,33	15,36	19,65	18,47	27,19	25,63	21,38	18,02	15,14	16,27	25,04

EXPERIMENTAL RESULTS

The results given in Table 1 show that 17 amino acids were identified in the investigated strains and that 16 were determined quantitatively. The qualitative amino-acid composition of the proteins of S. typhi and S. paratyphi A and B was basically the same and independent of the type of cultivation and the strain of bacteria.

The general characteristic feature of the amino-acid composition of the typhi-parathyphoid bacteria is their high content of alanine, glutamic acid, and leucine (isoleucine).

No essential quantitative differences were found in the amino-acid composition, although there were some differences in the content of individual amino acids in the different strains of bacteria.

Investigation of the amino-acid composition of typhoid-paratyphoid strains cultivated on nutrient agar, i.e., under stationary conditions, showed no appreciable differences in the range of amino acids depending on the strains and species of bacteria (Table 1). In all three species of bacteria the amino acids present in the largest amounts were alanine, glutamic acid, and leucine (isoleucine).

Consequently, no significant qualitative differences in the amino-acid composition were detected which depended on the mode of cultivation of the bacteria: on synthetic medium with aeration or on Hottinger's nutrient agar. It is apparent that the fact that all investigated strains were cultivated for the same time and on the same medium played an important role in determining the constancy of the amino-acid composition.

Cultures of S. typhi and S. paratyphi A and B are indistinguishable from other pathogenic microorganisms in their amino-acid composition.

Analysis of the free intracellular amino acids in cultures of the typhoid-paratyphoid group demonstrated their considerable qualitative variation (Table 2). Fourteen amino acids were identified and determined in the fraction of free amino acids.

The few differences in composition of fractions of free amino acids were dependent not so much on the medium used for cultivation as on activity of certain enzyme systems.

Previous investigations [7] showed that cultures of S. typhi and S. paratyphi A and B contain specific enzymes of transamination and reductive amination and that glutamic acid and alanine play an active role in these processes. The presence of alanine, glutamic acid, valine, and leucine in large quantities in the fractions of both bound and free amino acids is directly linked with these processes.

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