The Amino Acid Composition of *Sarcina lutea* Grown on Different Media

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SUMMARY: The free amino acid composition of the 75 % ethanol extracts of a strain of *Sarcina lutea* varied considerably depending on the composition of the growth medium. Differences in hydroxyproline content were particularly significant. The amino acid composition of the ethanol-extracted cell residues remained constant.

In the course of some enzyme studies on acetone-dried *Sarcina lutea*, paper chromatography was used to identify amino acid reaction products, and it was observed that free amino acids diffused out of the organisms into the suspending medium. After spraying the chromatograms with ninhydrin, a bright orange spot due to hydroxyproline was particularly prominent. A qualitative examination of the free amino acids inside the organisms was undertaken, and comparisons were made between organisms grown on various media.

EXPERIMENTAL

Organism. *Sarcina lutea* was obtained from the Medical Research Council's Unit for Chemical Microbiology, Cambridge.

Culture media. A casein digest was prepared according to the method of Gladstone & Fildes (1940) for 'casein hydrolysate A'. The casein hydrolysate was stored in a dark bottle at +2°. Casein digest agar contained: yeast extract (Marmite Food Extract Co. Ltd., London, E.C. 3), 2 g.; Oxoid Lab-Lemco (Oxo Ltd., London, E.C. 4), 1 g.; NaCl, 5 g.; casein digest, 85 ml.; agar, 20 g.; distilled water to 1 l. Nutrient agar contained: proteose peptone (Difco Laboratories Inc., Detroit 1, Michigan), 5 g.; yeast extract (Marmite), 2 g.; Oxoid Lab-Lemco, 1 g.; NaCl, 5 g.; agar, 20 g.; distilled water to 1 l. 'Hydrolysed peptone agar' was made in the same way as nutrient agar except that the proteose peptone was replaced by an equivalent amount of acid hydrolysed peptone. 'Nutrient broth', used only for initial inocula, was made as for nutrient agar, but with omission of the agar. Glucose (10 g./l. when added) was autoclaved separately.

Cultivation and harvesting. The organism, *Sarcina lutea*, was maintained on nutrient agar slopes; it was inoculated, when required, into 2 ml. volumes of nutrient broth in bijoux bottles and incubated for 18-20 hr. (overnight) at 25°. The contents of one bijoux bottle were used to surface-inoculate 200 ml. solid medium contained in a Roux bottle. The organisms were harvested after 3 days of growth at 25°, physiological saline being used to wash the organisms

from the surface of the agar. The organisms were centrifuged for 20 min. at 5000 g, were resuspended (once) in saline and again centrifuged down. The packed organisms were then coagulated in acetone, filtered off, dried *in vacuo* and weighed.

Preparation of extracts of dried organisms. Aqueous ethanolic extracts were prepared and treated with chloroform as described by Lindan & Work (1951). Acetone-dried cells (200–250 mg.) were extracted with three successive 10 ml. batches of 75 % (v/v) ethanol in water with continuous shaking at room temperature (*c.* 18°) for 24 hr. The extracted residues of the organisms were hydrolysed by acid according to the procedure of Work & Dewey (1953), and the solutions were electro dialysed using the apparatus described by Work (1950).

Nitrogen estimations were carried out by the micro-Kjeldahl method.

Paper chromatography. Chromatograms were made using Whatman no. 4 paper ($22\frac{1}{2} \times 18\frac{1}{4}$ in.) with phenol (ammonia atmosphere) as first solvent, and butanol + acetic acid (Campbell, Work & Mellanby, 1951) as second solvent. Ninhydrin-reacting spots were identified by reference to marker squares with known amino-acid mixtures; the strengths of spots were arbitrarily estimated by eye after drying and spraying, scoring with numbers ranging from 1 to 10.

RESULTS

The yields of *Sarcina lutea* grown on the three different solid media with and without glucose are shown in Table 1. In all cases the presence of glucose stimulated growth; growth was best on casein digest + glucose. Under these conditions *S. lutea* formed thick and raised colonies firmly attached to the surface of the agar. Growth on the nutrient agar and hydrolysed peptone agar media was much thinner and the organisms were easily washed from the agar surface.

Table 1. *Yields of Sarcina lutea grown on various solid media*

Culture medium	Yield of organism (mg. dry wt./ Roux bottle)	Increase in yield of organism on addition of 1 % (w/v) glucose (%)
Nutrient agar	220	—
Nutrient agar + glucose	420	91
Hydrolysed peptone agar	250	—
Hydrolysed peptone agar + glucose	420	68
Casein digest agar	1000	—
Casein digest agar + glucose	1660	66

The ethanolic extracts of organisms from each medium after chloroform treatment comprised 14–18 % of the corresponding total dry matter (Table 2).

Qualitative amino acid composition of the ethanol-extracted residues

Chromatograms of acid hydrolysates of the ethanol-extracted residues of organisms grown on the six different media were remarkably similar (Table 3). The hydrolysates were all subsequently electro dialysed and the neutral fractions on chromatography were again very similar to one another. No unusual amino acids were present.

*Qualitative amino acid composition of the ethanolic extracts
of organisms*

Samples of the 'extract' (100 μ l. equivalent to 3–5 μ g. N) were first examined on chromatograms to indicate the general amino acid distribution. Each extract was then electrodialysed and the contents of the three fractions: anode fraction, neutral fraction (centre compartment) and cathode fraction, were concentrated and retained for separate examination. The neutral fractions were chromatographed in excess (equiv. 10–15 μ g. N) to reveal neutral amino acids present in amounts too low to be detected in the whole (untreated) extract (before electrodialysis). The approximate relative amounts of the known amino acids are given in Table 3.

Table 2. *Distribution of dry matter between soluble and insoluble fractions of Sarcina lutea grown on various media*

Culture medium	Wt. of organisms extracted (mg.)	Wt. of insoluble residue (mg.)	Amount of residue as % total dry wt. (%)	Wt. of ethanolic extract (mg.)	Amount of extract as % total dry wt. (%)	Amount of lipid as % total dry wt. (by difference) (%)
Nutrient agar	220	167	76	37	16	8
Nutrient agar + glucose	220	160	73	34	15	12
Hydrolysed peptone agar	250	188	75	36	14	11
Hydrolysed peptone agar + glucose	220	137	62	36	16	22
Casein digest agar	220	175	79	40	18	3
Casein digest agar + glucose	200	105	52	34	17	31

Glutamic acid and alanine were the most abundant amino acids under all growth conditions; aspartic acid was equally abundant except when nutrient agar was used. With nutrient agar or hydrolysed peptone agar as growth media, a very pronounced orange spot due to hydroxyproline was present; this was absent from extracts of organisms grown on the casein digest medium. Chromatographic examination of the growth media confirmed the absence of hydroxyproline from the casein hydrolysate, whereas a peptone hydrolysate contained hydroxyproline as the most abundant amino acid; unhydrolysed peptone did not appear to contain any free hydroxyproline. *Sarcina lutea* appeared to contain more free amino acids when grown on the casein digest medium; this was the most favourable medium for growth (see Table 1). Under these growth conditions appreciable amounts of proline were present in the ethanolic extracts, in contrast to trace amounts under the other growth conditions. Also of interest is the low amount of the basic amino acids in the extracts except when casein digest was the growth medium.

Compounds other than the common amino acids were found in the ethanolic extracts of *Sarcina lutea*. Organisms grown on nutrient agar and hydrolysed

Table 3. *Amino acids in Sarcina lutea grown on various media*

Culture medium	Fraction examined	Aspartic acid	Glutamic acid	Glycine + serine	Threonine	Alanine	Tyrosine	Valine	Phenylalanine	Leucines	Proline	Hydroxyproline	Basic amino acids	Asparagine	Glutamine
Nutrient agar	Whole extract	3	10	1	0	6	0	0	0	0	0	10	1	0	2
	Neutral fraction	—	—	8	2	10	0	1	0	1	0	10	—	2	4
Nutrient agar + glucose	Whole extract	3	10	2	0	6	0	2	0	0	0	10	1	0	2
	Neutral fraction	—	—	7	0	10	0	6	0	1	1	10	—	4	10
Hydrolysed peptone agar	Whole extract	10	10	2	0	2	0	0	0	0	0	8	1	0	2
	Neutral fraction	—	—	10	0	10	0	1	0	1	2	10	—	0	10
Hydrolysed peptone agar + glucose	Whole extract	9	10	1	0	8	0	1	0	0	0	5	1	0	1
	Neutral fraction	—	—	2	0	10	0	2	0	0	0	10	—	0	1
Casein digest agar	Whole fraction	8	10	2	0	9	0	2	0	6	3	0	3	0	0
	Neutral fraction	—	—	2	0	10	3	7	3	9	10	0	—	0	4
Casein digest agar + glucose	Whole extract	8	10	2	0	8	0	3	0	6	3	0	4	0	0
	Neutral fraction	—	—	5	0	10	1	7	1	9	10	0	—	0	0
Any of the above six media	Acid hydrolysate of extracted cell residue	7	10	6	5	10	5	10	6	10	7	0	6	—	—

Arbitrary figures ranging from 1 to 10 indicate the approximate relative strengths of the amino-acid spots on the chromatograms.

peptone agar media contained glutathione, glutamine and asparagine. Acid labile substances, probably peptides, were also present, the most conspicuous spot, which disappeared on hydrolysis, was found 'under alanine'. Another spot, which ran very fast in phenol but only slowly in butanol + acetic acid, was also probably a peptide. Other weak spots, which disappeared on hydrolysis, were also probably due to peptides. The presence of β -alanine in the extracts was suspected from its known chromatographic behaviour and characteristic bright blue colour with ninhydrin, but was not thoroughly confirmed. The bacterial amino acid $\alpha\alpha'$ diaminopimelic acid, not found in acid-hydrolysates of Gram-positive cocci (Work & Dewey, 1953), was not found in any of the extracts or extracted residues of *S. lutea*.

Examination of the contents of the anode and cathode fractions with and without hydrolysis failed to reveal any further useful information.

DISCUSSION

The above qualitative examination of the amino acids in *Sarcina lutea* shows that the composition of the free amino acid pool depends on the composition of the growth medium. A similar dependence was observed by Gale (1947) in the case of *Streptococcus faecalis*, and by Fernell & King (1953) for *Escherichia coli* and *Bacillus subtilis*. The latter workers disintegrated the bacteria before examining any soluble cell fractions. The free amino acids of a number of bacteria were also investigated by Taylor (1947); specific decarboxylase preparations were used to estimate the concentrations of glutamic acid, arginine, ornithine, lysine, histidine and tyrosine.

Attempts were made to estimate hydroxyproline in extracts of *Sarcina lutea* by the method of Troll (1953), but it was not found to yield reproducible results since other components of the extracts interfered. The high hydroxyproline content of extracts of *S. lutea* grown on media other than the casein hydrolysate contrasts sharply with its complete absence from the acid-hydrolysed residues under all growth conditions. It would seem that *S. lutea* can absorb hydroxyproline in large amounts from a suitable growth medium, and may hydrolyse peptone to liberate free hydroxyproline, but does not incorporate it into cellular protein.

In contrast to the variations in the ethanolic extracts of *Sarcina lutea*, the amino acid composition of the ethanol-extracted residues is constant irrespective of the growth conditions. Stokes & Gunness (1946) have stated that the amino acid composition of an organism is a stable and characteristic property of the cell under fixed conditions of growth. Freeland & Gale (1947) studied the amino acid composition of certain bacteria and yeast and found that the amino acid composition of the protein of *Escherichia coli* and *Aerobacter aerogenes* was unaffected by widely varying growth conditions. Work (1949) also observed that the amino acids of the insoluble cell residues of *Corynebacterium diphtheriae* grown on a casein digest or a synthetic amino acid medium were the same.

Sarcina lutea is capable of absorbing a wide variety of free amino acids in

varying proportions depending on the medium on which it is growing; it appears, however, to have a specifically selective mechanism for incorporating amino acids, from those already absorbed, into its cellular protein.

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