PROBLEMS IN THERMOPHILY

III. The Amino Acid Composition of Thermophilic Bacilli

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Summary

Circular paper chromatographic methods were made use of to study the amino acid composition of thermophilic sporeforming bacteria grown at incubation temperatures of 37 and 55° C. No difference in the amino acid composition of the bacteria at the two temperatures of incubation was observed. The following amino acids were identified:

1. Alanine
2. Arginine
3. Aspartic acid
4. Cystine
5. Glutamic acid
6. Glycine
7. Histidine
8. Lysine
9. Methionine
10. Phenylalanine
11. Proline
12. Serine
13. Threonine
14. Tyrosine

It is suggested that an investigation of the free amino acids present in the bacilli grown at the two temperatures of incubation may reveal quantitative if not qualitative differences in the amino acid composition.

Introduction

The ability of thermophilic micro-organisms to prolifer at temperatures at which most proteins coagulate has baffled the scientist ever since their discovery and even to this day no definite factor to which thermophily can be attributed has been pointed out. Various explanations, ranging from the possession of a low grade of protoplasmic organisation[7] and a lower moisture content[14, 23] to the presence of certain elements such as calcium in a higher proportion[2, 6] have been put forth to account for life at such elevated temperatures.

With the advance made in the knowledge of enzymes, bacteriologists sought the answer to this problem of thermophily in the enzymic constitution of thermophilic and mesophilic bacteria. Several workers[5, 8, 17-21] have shown the presence of thermostable hydrolytic and respiratory enzymes in thermophilic bacteria and have suggested that the presence of thermostable enzymes explains growth at high temperatures. It may also be mentioned that the ability to grow

at high temperatures has often been associated with the nature of the protoplasmic fats.[1, 11, 15]

The fact that several proteins begin to coagulate at and over 55° C. leads one to believe that the proteins of thermophilic organisms may differ from the proteins of mesophilic forms, and this difference is further indicated by the presence of thermostable enzymes in the heat resistant forms. It would be no surprise, therefore, if a difference in the amino acid composition of proteins from thermophilic and mesophilic forms was found. The purpose of this communication is to present the results obtained from a study of the amino acid make-up of the two groups of bacteria, *viz.*, thermophilic and the mesophilic forms.

MATERIALS, METHODS AND RESULTS

Selection of cultures.—The organisms we selected for study were strains belonging to the *Bacillus subtilis* species which could grow under both thermophilic and mesophilic conditions. Further, the selection of this particular organism for studying the differences, if any, in the amino acid make-up under thermophilic and mesophilic conditions was prompted by our belief that thermophiles are heat-loving mutants of mesophilic forms.[3]

Preparation of the hydrolysate.—Four strains Aglb., 2, El5A and P_3 of the *B. subtilis* species were selected for study. Each isolate under study was inoculated into two 250 ml. flasks each containing 50 ml. of 1 per cent glucose broth. One of the flasks was incubated at 55° C. and the other at 37° C. At the end of a 48-hour period of growth at the two temperatures of incubation, the cells were harvested from the medium by centrifugation and washed thrice with 10 ml. physiological saline. The bacterial proteins were then hydrolysed with 6 N·HCl at a temperature of 100–110° C. in an oven for about 12 hours at the end of which the hydrolysate was evaporated under vacuum in a desiccator in presence of NaOH pellets. The residue so obtained was washed with glass distilled water and dried. This process of washing and drying was repeated several times and finally stored under a layer of toluene in a refrigerator.

Determination of total nitrogen.—The total nitrogen content was determined by Kjeldahl's volumetric method on a microchemical scale.

Chromatographic technique.—The technique followed was essentially the same as described by Giri *et al.*[12, 13] The hydrolysates were spotted on a circle 4 cm. in diameter drawn in the centre of a Whatman No. 1 filter sheet 16×18 inches. The method suggested by Rao and Wadhwani[22] resulted in a much better separation of the slow running amino acids than with the solvent suggested by Giri *et al.* The solvent employed was *n*-butanol, acetic acid and water in the proportion of 8 : 1 : 4 and the chromatograms were run thrice for better separation.

The method of Giri *et al.*[12, 13] permitted the identification of the following amino acids:—

Phenylalanine	Alanine
Tyrosine	Arginine
Proline	Cystine

The limitation of this method is that several amino acids are not separated in spite of the multiple development of the chromatograms. Bands were present which indicated the presence of either leucine or isoleucine or both. It may be mentioned here that tryptophan is destroyed by acid hydrolysis and consequently it could not be detected on the chromatogram.

The method suggested by Rao and Wadhwani[22] permitted the identification of several more amino acids. The following amino acids were identified:

Methionine	Serine
Proline	Arginine
Glutamic acid	Lysine
Threonine	Histidine
Aspartic acid	Cystine
Glycine	

From the chromatograms it was clear that the amino acids present in the bacterial growths obtained at the incubation temperatures of 37 and 55° C. were identical. Neither qualitative nor quantitative difference was apparent in spite of the spotting being done on an equal nitrogen basis.

Discussion

Chromatographic analysis of the amino acid composition of sporeforming bacteria grown under mesophilic and thermophilic conditions indicate a striking similarity between the proteins obtained from the isolates grown under different temperature conditions. Although previous workers[4, 10, 24] who studied the amino acid composition of several mesophilic bacteria and yeasts grown under different conditions of growth with respect to media and the hydrogen-ion concentration reported a similarity in the amino acid composition of these micro-organisms, a difference in the amino acid composition of micro-organisms grown under different temperature conditions was to be expected in the light of what has been shown before, *viz.*, that thermophiles are heat resistant mutants of the common mesophilic forms.[3] That a mutation is accompanied by a change in the amino acid composition has already been suggested by Knight and Stanley[16] as a result of their studies on the amino acid composition of the cucumber viruses 3 and 4 and of the Holme's ribgrass virus, all of which are considered mutants of the tobacco mosaic virus.

From this, it would appear that neither mutation nor changes in the condition of growth affect the amino acid make-up of bacterial proteins and tends to support the results obtained by Eureinova[9] with respect to amino acids of nucleoproteins of mesophiles and thermophiles. Furthermore, analysis of the proteins of even obligate thermophiles has not shown in our hands a difference in the amino

acid contents as compared to the proteins of obligate mesophiles. However, it is possible that the free amino acids of thermophilic bacilli and the corresponding mesophilic forms may reveal a quantitative if not qualitative difference. This remains to be established.

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