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# Fatty Acid Distribution in Mesophilic and Thermophilic Strains of the Genus *Bacillus*

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The fatty acid distribution of three mesophilic and three thermophilic strains of the genus *Bacillus* was determined by gas chromatography of the fatty acid methyl esters. Fatty acid i-15:0 was the most abundant in both the mesophiles (51%) and the thermophiles (41%). The second most abundant fatty acid was a-15:0 in the mesophiles (22%), and i-17:0 in the thermophiles (27%). The fatty acid pair i-15:0, i-17:0 was the most predominant pair in both the mesophiles (61%) and the thermophiles (66%). The fatty acid pair a-15:0, a-17:0 was the second most predominant pair and was much higher in the mesophiles (30%) than in the thermophiles (15%). The average fatty acid chain length was 15.5 for the mesophiles and 16.0 for the thermophiles. The significance of these results for the lipid theory of thermophily is discussed.

Three major theories have been advanced to explain the phenomenon of thermophily. The first of these theories considers thermophily to be due to the protective action of lipids and attempts to correlate heat stability of the organism with the melting point of cell lipid material (3). The second theory views thermophily as a special type of metabolic state characterized by high rates of breakdown and resynthesis (1). The third theory ascribes thermophily to the structure and function of macromolecules and emphasizes physical-chemical differences of macromolecules from mesophiles and thermophiles (4). The latter theory has received most support so far. Previous papers from this laboratory have demonstrated macromolecular differences of ribosomes (13), ribosomal ribonucleic acid (RNA; reference 10), deoxyribonucleic acid (DNA; reference 11), and cell-free amino acid-incorporating systems (12) for preparations from mesophilic and thermophilic strains of the genus *Bacillus*. In view of these findings, it was of interest to assess the relative importance of the lipid theory of thermophily in these organisms by a comparison of their overall fatty acid composition. This paper presents the results of this study.

## MATERIALS AND METHODS

**Organisms and growth conditions.** Three mesophilic and three thermophilic strains of the genus *Bacillus* were used for this study. The mesophiles included *B. pumilus* (NRS 236), *B. licheniformis* (NRS 243), and an unclassified *Bacillus* species (X1). The thermophiles were strains of *B. stearothermophilus* (FJW, 10, 2184). The cells were grown and collected as described previously (12) except that they were harvested in the late logarithmic phase (absorbance of 1.8 at 540 nm). The medium consisted of 1% Trypticase (BBL) and 0.2% yeast extract (Difco) and also contained 1 ml of antifoam (Union Carbide Corp., SAG-471) per 25 liters. The mesophiles were grown at 37 C and the thermophiles were grown at 55 C.

**Lipid preparation and gas-liquid chromatography.** Total lipids were extracted by the method of Card (5) with two modifications. (i) The ratio of solvent to cell paste was about 80:1, and (ii) the combined extracts were washed directly with 0.29% NaCl. The yield of lipid was approximately 65 mg per 5 g of cells (wet weight). Acid methanolysis was performed on approximately 10 mg of the total lipid, and the resulting methyl esters were analyzed by gas-liquid chromatography (7). In addition to the diethylene glycol succinate polyester columns, a column [4 ft (1.2 m) by 3 mm] packed with 3.8% SE-30 gum rubber on 80-100 mesh Diatoport S, and a column [6 ft (1.8 m) by 3 mm] packed with 5% ethylene glycol adipate on 80-100 mesh Diatoport S were used. The fatty acid methyl esters were separated by programming the gas chromatograph from 150 to 225 C at a rate of 3 C per min and using a nitrogen flow rate of 2 to 4 ml per min. Standard methyl esters of the fatty acids were obtained from Supelco Inc. (mixture GLC-100) and Applied Science Laboratories (mixture L-203).

**Other methods.** Hydrogenation of methyl ester samples was done by bubbling hydrogen into an ethanol solution of the esters with platinum oxide present as a catalyst. Mass determinations were made by using a gas chromatograph-mass spectrometer (LKB model 9000).

## RESULTS AND DISCUSSION

**Identification of fatty acids.** Best results were obtained with the ethylene glycol adipate column.

Analyses using the other two columns were in excellent agreement with those employing the ethylene glycol adipate columns except that they failed to resolve the iso- and anteiso- isomers of the 15-, 16-, and 17-carbon fatty acids.

Thirteen peaks were obtained by gas-liquid chromatography for the samples from the mesophiles (Table 1). The samples from the thermophiles yielded the same peaks with the exception that fatty acids a-16:0 and n-16:1 were missing. Fatty acid methyl esters were identified by comparing their retention time with those of standards and by simultaneous injection of standards with samples. Unsaturated fatty acids n-16:1 and i-17:1 were identified by chromatography of a hydrogenated sample as well as by their order of appearance from the various chromatographic columns. The identity of fatty acid i-17:1 was further confirmed by mass determination using the gas chromatograph-mass spectrometer.

**Fatty acid distribution patterns.** The fatty acid distribution patterns obtained with ethylene glycol adipate columns are shown in Table 1. In all cases, two separate samples were isolated and duplicate analyses were performed on each sample. The relative deviation of duplicate analyses was 2.5% or less for components amounting to 10% or more of the fatty acids. The relative deviation for the same components analyzed as two separate samples was 8.3% or less.

With the exception of *B. licheniformis*, the fatty acid distribution patterns for the mesophiles were similar. The predominant fatty acid was i-15:0 and the second most abundant fatty acid was a-15:0. The fatty acid distribution patterns for the thermophiles were also similar, and fatty acid-i-15:0 was still the most predominant fatty acid; however, it constituted only 41% of the total fatty acids as compared to 51% for the mesophiles. Furthermore, the second most abundant fatty acid was not a-15:0 but i-17:0. Fatty acid a-15:0 was present to a much smaller extent and longer chain fatty acids (i-16:0, a-17:0) were more predominant in the thermophiles as compared to the mesophiles. In most cases, the fatty acid distribution pattern for *B. licheniformis*

TABLE 1. *Fatty acid distribution patterns in the genus Bacillus*[a]

| Fatty acid | Mesophiles | | | | Thermophiles | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | Avg | D | E | F | Avg |
| i-14:0 | T | 1.6 | T | T | T | T | T | T |
| n-14:0 | T | T | T | T | T | T | T | T |
| i-15:0 | 56.1 | 57.4 | 38.1 | 50.8 | 34.0 | 48.7 | 37.2 | 40.5 |
| a-15:0 | 18.4 | 22.7 | 24.8 | 22.1 | 3.8 | 2.8 | 3.6 | 3.4 |
| n-15:0 | T | T | T | T | T | T | 2.0 | T |
| i-16:0 | 2.8 | 3.5 | 5.5 | 3.9 | 9.3 | 4.0 | 10.3 | 8.0 |
| a-16:0 | T | T | T | T | | | | |
| n-16:0 | 1.9 | 1.2 | 3.1 | 2.1 | 6.8 | 1.8 | 1.2 | 3.3 |
| n-16:1 | T | T | T | T | | | | |
| i-17:0 | 8.6 | 6.7 | 15.6 | 10.4 | 31.1 | 28.4 | 21.5 | 27.3 |
| a-17:0 | 6.9 | 4.8 | 13.0 | 8.2 | 15.0 | 8.8 | 10.9 | 11.7 |
| i-17:1 | 5.4 | 2.1 | T | 2.5 | T | 5.4 | 11.5 | 5.7 |
| n-17:0 | T | T | T | T | T | T | 1.7 | T |

[a] A, *B. pumilus;* B, *Bacillus* sp. (X1); C, *B. licheniformis;* D, *B. stearothermophilus* FJW; E, *B. stearothermophilus* 10; F, *B. stearothermophilus* 2184. Values are in per cent and represent averages of four determinations (two samples and duplicate analyses of each). T denotes trace (less than 1%).

TABLE 2. *General characteristics of the fatty acid distribution*[a]

| Fatty acid | Mesophiles | | | | Thermophiles | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | Avg | D | E | F | Avg |
| i-15:0 + i-17:0 | 65.2 | 53.7 | 64.1 | 61.0 | 64.7 | 75.1 | 58.6 | 66.1 |
| a-15:0 + a-17:0 | 25.2 | 37.8 | 27.5 | 30.2 | 18.7 | 11.2 | 14.4 | 14.8 |
| i-14:0 + i-16:0 | 2.8 | 5.5 | 3.5 | 3.9 | 9.3 | 3.9 | 10.3 | 7.8 |
| n-14:0 + n-16:0 | 1.9 | 3.1 | 1.2 | 2.1 | 6.8 | 1.8 | 1.2 | 3.3 |
| Avg chain length | 16.1 | 16.1 | 15.9 | 16.0 | 15.5 | 15.3 | 15.7 | 15.5 |

[a] See footnote *a*, Table 1.

came closest to that for the thermophiles. This is not unexpected since this organism has the highest maximal growth temperature of the three mesophiles studied (10).

The fatty acid distribution pattern in *Bacillus* has been determined by Kaneda (8) for a large number of mesophiles and by Daron (6) for one thermophile. These authors have reported the predominance of branched-chain fatty acids in these organisms. Our findings are in agreement with these reports. Kaneda (8) has suggested that the relative abundance of branched-chain fatty acids reflects the availability of precursors and may, therefore, be affected by the composition of the growth medium. In our experiments, the same medium (which was rich in amino acids) was used throughout, and the availability of amino acids was constant and, presumably, sufficient. Any differences observed must, accordingly, be due to other factors. On the assumption that fatty acids of similar structure but differing by two carbons arise from the same precursors, Kaneda (8) has grouped the major fatty acids as shown in Table 2. Our results corroborate Kaneda's finding that the pairs i-15:0, i-17:0 and a-15:0, a-17:0 are the most abundant in the mesophiles and show that this holds for the thermophiles as well. The data in Table 2 indicate that a major difference between the fatty acids of the mesophiles and those of the thermophiles lies in the synthesis of a-15:0 and a-17:0 fatty acids.

The differences in fatty acid distribution patterns for the two types of organisms result in a significant longer average chain length for the fatty acids from the thermophiles as compared to that from the mesophiles (Table 2). There is, however, no simple correlation between the chain length of a fatty acid and its melting point. For example, the five major fatty acids a-15:0, i-15:0, i-16:0, a-17:0, and i-17:0 have melting points of 25.8, 52.2, 62.4, 38.0, and 60.5 C, respectively (9). It can be seen from Table 1 that the thermophiles were definitely richer in the iso-fatty acids with higher melting points (i-16:0, i-17:0), whereas the mesophiles contained more of the fatty acid having a lower melting point (i-15:0). For the anteiso- fatty acids, the differences were especially pronounced for fatty acid a-15:0 which has a very low melting point; the mesophiles contained about seven times as much of this fatty acid as the thermophiles. Furthermore, considering the fatty acid pairs (Table 2), the thermophiles contained less of the lower melting point pair (a-15:0, a-17:0) and more of the higher melting point pair (i-15:0, i-17:0) than was the case for the mesophiles.

The various findings discussed above provide support for the theory that thermophiles contain lipids of higher melting points than mesophiles. It is plausible that the physical state or viscosity of these lipids is important in protecting and maintaining the structural integrity of subcellular components. It should be noted, however, that the fatty acids exist primarily as phosphatides and glycolipids within the cell (2) and, therefore, a comparison of melting points of those compounds would be more meaningful. Unfortunately, no such data are currently available. Furthermore, polar or very long-chain fatty acids were not determined by our method. These may also be of importance in assessing mesophiles and thermophiles.

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