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Fatty Acid Composition of Spores of the "Thermophilic Anaerobes"

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The lipid content of bacterial spores has occasionally been determined, but the fatty acid components have been less frequently characterized. S. K. Long and O. B. Williams (J. Bacteriol. **79**:629, 1960) found that with different lots of spores of *Bacillus stearothermophilus* 6.90 to 13.11% of the spore dry weight was extractable by a diethyl ether extraction and considered to be surface or adsorbed lipid, but, when such extracted and viable spores were re-extracted after grinding and acid hydrolysis, only an additional 1.34 to 1.88% lipid was obtained. They considered their attempts to characterize the fatty acid components of the extracted lipid unsuccessful but felt that their data (from paper chromatography) suggested that the fatty acids present were 18 carbon or greater in chain length.

During our studies on optimal conditions for sporulation and on the spore characteristics of the resulting spores, it was decided to determine their lipid content and to characterize the component fatty acids. This is a report of our results.

The spores assayed were those of *Clostridium thermosaccharolyticum* National Canners Association strain 3814 and a "thermophilic anaerobe" isolated from molasses (H. Xerones, J. L. Segmiller, and I. J. Hutchings, Food Technol. **19**:111, 1965) referred to as strain TA-37. The spores were produced by an "active culture" technique in a medium containing L-arabinose (0.5%), peptone (0.5%), yeast extract (0.5%), and balanced minerals (C. G. Pheil and Z. J. Ordal, *in preparation*). The spore harvests were cleaned by mild sonic treatment and repeated differential centrifugation. The lipids were extracted after acid hydrolysis by the procedure of M. R. J. Salton (Biochim. Biophys. Acta **10**:512, 1953). The total lipid content on a dry weight basis was 13.5 and 16.3% for the spores of TA-37 and *C. thermosaccharolyticum* 3814, respectively. These lipids were firmly bound since a mild ether extraction (without acid hydrolysis) resulted in a lipid content of less than 1% for both strains. The lipid content of the vegetative cells of TA-37 and *C. thermosaccharolyticum* was 2.4 and 5.3%, respectively.

To prepare the samples for gas-liquid chromatography analysis, the fatty acids and other saponifiable material were saponified with 0.5 N methanolic NaOH by refluxing for 30 min. After diethyl ether extraction and drying, the fatty acids were methylated with an ethereal solution of diazomethane for 1 hr. The ether and residual diazomethane were evaporated by a slow stream of nitrogen.

TABLE 1. *Fatty acid composition of spore lipids of Clostridium thermosaccharolyticum 3814 and TA37 as determined by gas-liquid chromatography*[a]

Fatty acid[c]	Percentage of total area of spore suspension[b]	
	TA-37	3814
12:0	2.5	1.8
14:0	9.5	19.6
14:1	10.9	16.7
16:0	19.3	15.7
16:1	10.1	16.6
18:0	7.4	6.7
18:1	11.7	6.2
18:2	Trace	Trace
18:3	Trace	Trace
OH-18:0	19.6	10.9

[a] The methylated fatty acids were separated on a 6 ft (1.83 m) by 0.25 inch (0.64 cm) (internal diameter) stainless-steel column packed with 15% ethylene glycol succinate polymer on Chromasorb W. A flame ionization detector was used with an injector temperature of 190 C and column temperature of 170 C.

[b] The peak areas were calculated by multiplying peak heights by the widths at half peak heights. The per cent area was determined by dividing the area of the individual peak by the sum of all peak areas (× 100).

[c] Ratio between number of carbon atoms and number of double bonds.

The major fatty acids in the total lipid extracts were 14:0 (ratio between number of carbon atoms and number of double bonds), 14:1, 16:0,

16:1, 18:0, 18:1, and a hydroxy-18:0 (Table 1). The relative amounts did vary between the two strains. The exact position of the double bond in the unsaturated fatty acids was not determined, although the major portion of the monoenoic fatty acids in bacteria are Δ11 (vaccenic) (K. Hoffmann and F. Tausig, J. Biol. Chem. **213**:415, 1955). The procedure used did not satisfactorily separate the shorter chain fatty acids, but it did indicate that such compounds were only present in trace amounts. No further attempts were made to characterize them.

The presence of the hydroxystearic acid was verified by preparing the trimethylsilyl derivative (R. D. Wood, P. Roju, and R. Reese, J. Am. Oil Chem. Soc. **42**:161, 1965) and comparing the retention time on an Apiezon L column with a 12-hydroxystearic acid standard. The exact position of the hydroxyl group in the hydroxystearic acid of the spore lipids was not determined.

The presence of significant quantities of lipid material in the spores of these "thermophilic anaerobes" has been demonstrated. The drastic treatment required to release the lipids suggests that the lipid content was not due to adsorption of the lipids onto the spores from the medium or from the lytic products of the vegetative cells, but rather that they are a structural component of the spore per se. The role of the high lipid content of these spores is yet to be determined.

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