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Fatty Acids from Vegetative Cells and Spores of *Bacillus stearothermophilus*[1]

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In spores of *Bacillus stearothermophilus* produced at 45 and 55 C, branched-chain fatty acids predominated in the former and straight-chain acids in the latter.

Heat resistance of bacterial cells and spores has been related to the type and melting point of protoplasmic lipids (5). Gaughran (4) and Ingraham (7) contend that the total lipid and saturation vary little in thermophilic bacteria with changes in growth temperature. Long and Williams (12), however, reported an increase in degree of unsaturation of lipids in spores of *Bacillus stearothermophilus* grown at elevated temperatures. This report describes changes in the distribution of lipids in spores and vegetative cells of *B. stearothermophilus* ATCC 7953 produced at temperatures of 45 and 55 C.

Vegetative cells and spores were produced as described previously (16). At 55 C, vegetative cells were collected after 16 hr and spores were collected after 24 hr. At 45 C, vegetative cells were collected after 36 hr and spores were collected after 48 hr. Cells were washed with distilled water until free from media constituents and vegetative debris.

Lipids were extracted by the method of Long and Williams (12), and free fatty acids were esterified by the method of Bills et al. (2). Lipids not adsorbed in the previous step were collected by the method of Metcalfe et al. (13). The pooled methyl esters of the fatty acids were analyzed by gas chromatography. Helium was the carrier gas with an outlet flow of 65 ml/min and a column inlet pressure of 40 psi. Two copper columns [outer diameter, 0.25 inch (0.63 cm)], 6 ft (1.8 m) in length, were packed with 15% LP-71 (ethylene glycol succinate on 60- to 80-mesh Diatoport ST-111). The effluent was monitored by a hydrogen flame ionization detector in an analytical gas chromatograph (model 810-29, F & M Scientific Corp., Avondale, Pa.). Isothermal determinations were made at 169 and 180 C. Retention times of standard samples (Applied Science Laboratories, State College, Pa.) of saturated even-numbered acids from C_4 to C_{18} as well as $C_{12:1}$, $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, iso-C_{18}, anteiso-C_{19}, iso-C_{20}, and anteiso-C_{21} were compared with retention times of fractions in the extracted samples. Relative amounts of fatty acids were calculated from areas under the recorded peaks. Identification was also based on the linear relationship between the logarithm of the retention volume and the number of carbon atoms in homologous fatty acids (8) and on the criteria of Landowne and Lipsky (11).

The fatty acids recovered in greatest quantities were those containing 15 to 17 carbon atoms (Table 1). The most prevalent saturated, straight-chain acid was C_{16}. The percentages of straight-chain acids were always higher in cells incubated at the higher temperature. Palmitic acid occurs frequently and in large amounts in bacteria (14). Branched-chain fatty acids were also recovered in large quantities from both vegetative cells and spores. When the temperature of incubation was increased from 45 to 55 C, branched-chain acids decreased by slightly over half, accompanied by the accumulation of larger proportions of straight-chain acids, particularly C_{16}. The most prevalent of the branched-chain acids was iso-C_{15}, with lesser quantities of anteiso-C_{15}, iso-C_{17}, and anteiso-C_{17}. Kaneda (9) stated that an abundance of branched-chain fatty acids seems characteristic of the genus *Bacillus*. A monoenoic C_{16} acid was recovered from vegetative cells and spores grown at 55 C but not from those grown at 45 C. Comparison of fatty acids from the vegetative cells may not be too meaningful, since the age of the culture influences the type of lipids recovered from bacterial cells (1).

Gaughran (5) suggested that the melting point

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TABLE 1. *Percentage composition of fatty acids recovered from vegetative cells and spores of Bacillus stearothermophilus grown at 45 and 55 C*

Fatty acid	Vegetative cells		Spores	
	45 C	55 C	45 C	55 C
iso-C_8	<0.1	0.3	<0.1	
iso-C_9	<0.1	0.4	<0.1	0.3
C_9	0.2		<0.1	0.6
iso-C_{10}		0.3		0.5
C_{10}			0.2	0.7
iso-C_{11}	0.2	0.2		
iso-C_{13}	0.2			
iso-C_{14}	0.5	0.3	0.4	1.1
C_{14}	0.3	2.3	0.8	3.7
iso-C_{15}	43.2	19.6	36.2	11.7
anteiso-C_{15}	22.3	13.0	24.7	5.2
iso-C_{16}	2.4	2.1	2.7	3.5
C_{16}	2.1	42.1	14.3	48.0
C_{16}-		10.9		5.5
iso-C_{17}	16.9		11.2	
anteiso-C_{17}	11.7	7.6	9.5	17.6
C_{17}		0.9		1.6

of lipid material may fix the minimal temperature for growth of an organism. A preponderance of branched-chain fatty acids at the lower temperature suggests the presence of lipid complexes with diminished melting points, since a methyl group in a fatty acid will lower the melting point (6). Conversely, the presence of nC_{16} acid in relatively large amounts in cells produced at 55 C indicates lipids with a higher melting point. These fatty acids could also influence the permeability and stability of these cells, since fatty acids in gram-positive bacteria are associated mainly with phospholipids which are localized in the cell membrane (3, 10, 15).

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