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Fatty Acid Composition of *Spirochaeta stenostrepta*

RONALD JOSEPH[1]

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01002

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The fatty acid composition of *Spirochaeta stenostrepta* consists primarily of saturated, branch-chained fatty acids. Iso-C_{15}, anteiso-C_{15}, iso-C_{17}, and anteiso-C_{17} represent 66% of the total fatty acids.

The fatty acid composition of several pathogenic and saprophytic leptospires and treponemes has been determined after growth of these organisms in media containing horse serum (9), lipid-depleted bovine albumin (fraction V) supplemented with saturated and unsaturated even carbon number fatty acids (3, 4), or in rabbit testicles (9). The major fatty acids contained 14, 16, and 18 carbons and were saturated or unsaturated, or both, depending on the fatty acids added to the growth medium (4). Growth of leptospires in the presence of odd carbon number fatty acids resulted in a profile consisting mostly of saturated and unsaturated odd carbon number fatty acids (4). Branch-chained fatty acids were either not detected or were present in trace amounts in leptospires or treponemes (2-4, 9), but represented 27 and 9% of the total fatty acid composition of *Borrelia vincentii* and *Spirochaeta zuelzerae*, respectively (2).

As part of the characterization of the free-living anaerobic spirochetes, the fatty acid composition of *Spirochaeta stenostrepta*, cultivated in a medium which was not supplemented with serum, albumin, or fatty acids, was examined. The fatty acid composition of this organism consisted primarily of saturated branch-chained odd carbon number fatty acids which were dramatically different from that reported for other spirochetes (2-4, 9).

S. stenostrepta strain Z-1 (ATCC 25083) was cultivated on GYPT medium (5). The cells were harvested during the logarithmic phase of growth, washed once with distilled water, and used either immediately or following lyophilization. For isolation of the extractable lipids, 1 g (dry weight) of cells was extracted in sequence for 30-min periods at room temperature under reduced light and a nitrogen atmosphere with 50-ml volumes each of chloroform: methanol, 2:1 and 1:1 (v/v) two times each, and absolute methanol, one time. After the above extractions, the bound lipids were released by acid hydrolysis of the cell residue with 2 N HCl under a nitrogen atmosphere for 2 hr at 121 C, 18 psi. The bound lipids were extracted with three washes of diethyl ether. Nonlipid contaminants were removed by Sephadex chromatography (10).

The fatty acid methyl esters were isolated by thin-layer chromatography on silica gel G plates developed with hexane:diethyl ether: acetic acid, 70:30:2 (v/v), after methylation (7). Rhodamine 6G sprayed on a portion of the plate was used to locate the methylated fatty acids. Catalytic hydrogenation (8) was used to detect the presence of unsaturated fatty acids. *Bacillus cereus*, ATCC 6074, was used as a control for the extraction procedure, and its fatty acid composition has been previously reported (6).

A dual-column Perkin Elmer 880 gas-liquid chromatograph equipped with a temperature programmer, a differential flame ionization detector, and a Leeds and Northrup model S recorder was used to analyze the purified fatty acid methyl esters. The methyl esters were separated on both a 7-ft copper column containing diethyleneglycol-succinate (12%, w/v, on Chrom-sorb P) and on a 7-ft copper column containing Apiezon L (15%, w/v, on Gas-sorb P). Solutions containing known fatty acid methyl esters (Applied Science Laboratories, Inc.) were used as standards. The composition percentages of the fatty acids were calculated by the method of Carrol (1). The results reported are the average values for five separate experiments.

[1] Present address: Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pa. 19140.

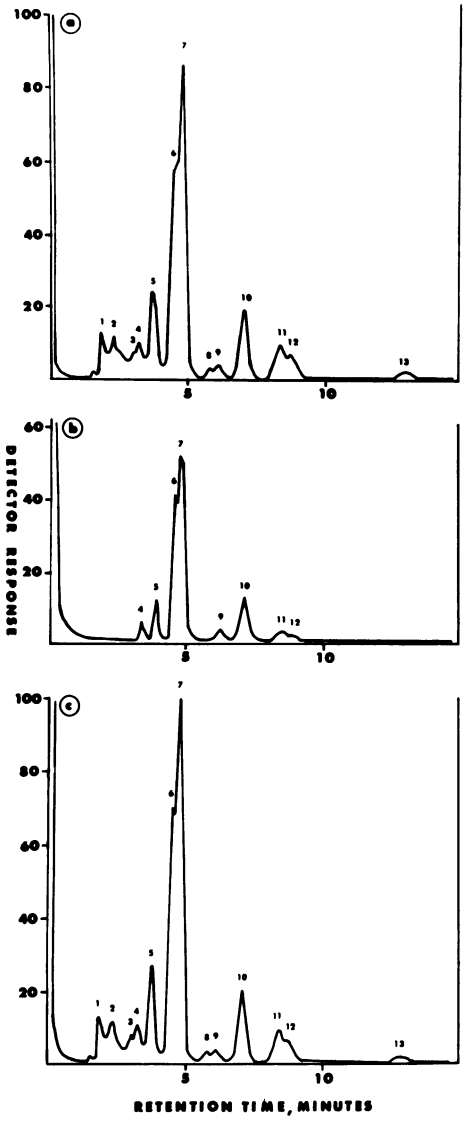

FIG. 1. *Gas-liquid chromatograms of fatty acid methyl esters from the lipids of S. stenostrepta. Fatty acid methyl esters from the extractable lipids (a); fatty acid methyl esters from the bound lipids (b); and fatty acid methyl esters from the total (combined extractable and bound) lipids (c). Separation was on a 7-ft copper column containing diethyleneglycol-succinate (12%, w/v, on Chrom-sorb P) and operated at 190 C. Injector-detector temperature was maintained at 250:220 C. Flow rates: 85 ml of He, 70 ml of H_2 and 260 ml of O_2 per minute.*

The lipids of *S. stenostrepta* extractable into organic solvents before acid hydrolysis comprised 22.3% of the cell dry weight. After acid hydrolysis, an additional 1.5% of the cell dry weight was extractable by diethyl ether. The extractable lipids contained phospholipid (51%), glycolipid (40 to 50%), and a small amount of free fatty acids. Gas-liquid chromatography of the solvent-extractable and total fatty acid methyl ester fractions resolved 13 peaks (Fig. 1a and c). Saturated branch-chain fatty acids represented 73 to 76% of each fraction (Table 1), of which iso-C_{15}, anteiso-C_{15}, iso-C_{17}, and anteiso-C_{17} comprised 63 to 66%.

Gas-liquid chromatography of the bound fatty acid methyl esters revealed the presence of only eight fatty acids in contrast to 13 for the solvent-extractable fraction (Fig. 1b, Table 1). The bound lipid fraction contained a greater percentage of iso-C_{15} and anteiso-C_{15} and a smaller percentage of iso-C_{17} and anteiso-C_{17} than the extractable lipid fraction. C_{12}, iso-C_{13}, C_{16}, and two unidentified fatty acids, (a) and (b) in Table 1, which were present in trace amounts in the extractable lipids were not detected in the bound lipid fraction. The two unidentified acids accounted for less than 4% of the total fatty acids. There were no shifts in either the retention times or peak heights after catalytic hydrogenation of the fatty acids, indicating the absence of unsaturated fatty acids.

The ability of *S. stenostrepta* to grow in the absence of added fatty acids to serum and to synthesize only saturated and primarily branch-chained fatty acids implies that the mechanism for fatty acid biosynthesis and oxidation in this organism must be different from that in either the treponemes or the leptospires. These differences in fatty acid com-

TABLE 1. *Percentage of fatty acids detected in S. stenostrepta*

Peak no.[a]	Fatty acid[b]	Extractable	Bound	Total
1	12:0	2.2	ND[c]	2.0
2	iso 13:0	2.7	ND	2.1
3	(a)	2.2	ND	1.8
4	iso 14:0	3.1	2.6	3.0
5	14:0	8.5	8.1	8.6
6	iso 15:0	12.8	19.3	13.7
7	anteiso 15:0	38.8	44.9	41.9
8	(b)	1.6	ND	1.5
9	iso 16:0	2.4	3.4	2.0
10	16:0	12.3	15.0	11.4
11	iso 17:0	7.3	4.7	6.6
12	anteiso 17:0	4.7	2.0	4.1
13	18:0	1.4	ND	1.3

[a] Peak number refers to peaks in Fig. 1.

[b] Number preceding colon indicates number of carbons; number following colon designates degree of unsaturation.

[c] No peak detected.

position may serve as a useful characteristic in taxonomic studies of spirochetes.

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