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Cellular fatty acids of treponemes

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EVIDENCE is rapidly accumulating which indicates that gas liquid chromatography (GLC) is a useful aid in the identification and classification of micro-organisms (Abel, de Schmertzing, and Peterson, 1963; Reiner, 1965; Henis, Gould, and Alexander, 1966). Recently, several workers have analysed micro-organisms for fatty acids by GLC and, by comparing their profiles, have established some useful taxonomic relationships (Moss and Lewis, 1967; Yamakawa and Ueta, 1964). The present study describes the fatty acid composition of fifteen different cultivable treponemes.

Material and methods

The following cultures were obtained, both as lyophilized and as freshly cultivated treponemes, from the Venereal Disease Research Laboratory of the National Communicable Disease Center: Reiter, Kazan, Kazan 2, Kazan 5, Kazan 8, Nichols, Noguchi, *Treponema skoliodonta, Treponema phagedenis, Treponema calligyra, Treponema ambigua, Treponema microdentium, Treponema refringens, Treponema zuelzerae,* and the N-9 strain of *Borrelia vincentii*. The source, growth medium (which was the same for all cultures), and the techniques employed in cultivating and harvesting these cultures have been described by Cannefax (1965). No attempt was made to study the effects of culture age, media composition, and temperature of incubation on cellular fatty acids (Moss and Lewis, 1967). Both lyophilized and freshly cultivated treponemes were tested in duplicate. The procedures used for saponification of the treponemal cells and for extraction and methylation of acidic components have already been described (Moss and Lewis, 1967). The methylated fatty acid samples were analysed immediately or were stored at −20°C.

The methyl esters were analysed with a Barber-Colman Model 5000 gas chromatograph (Barber-Colman Company, Rockford, Illinois) equipped with a hydrogen-flame ionization detector and a disc integrator recorder (Series 8000). Samples were analysed on an 8 ft (2·4 m.) U-tube glass column containing 15 per cent. ethylene glycol succinate (EGS), coated on 80/100 mesh Chromosorb W (Applied Science Laboratories, State College, Pennsylvania), and also on a 6 ft (1·83 m.) column of 2 per cent. SE-30 methyl silicone rubber gum, coated on 80/100 mesh Chromosorb P (Applied Science Laboratories).

The following operating parameters were used for the SE-30 column: injection temperature, 230°C; detector temperature, 250°C.; column temperature, 110° to 230°C. at 3°C./min.; carrier gas, nitrogen.

The EGS column was operated under the same conditions except that the column bath temperature was maintained at 160°C.

For routine analysis, 3 μl. of the methylated bacterial fatty acid samples were analysed for 45 min. after injection on to the SE-30 column under the conditions stated. This time interval was sufficient for detecting normal saturated fatty acid methyl esters of chain length from 8 to 23 carbons. The fatty acid methyl ester peaks were tentatively identified by comparing retention times on both columns (EGS and SE-30) with retention times of highly purified methyl ester standards (Applied Science Laboratories and National Institutes of Health). Peak areas were determined by disc integrator, and the percentage of each acid was calculated from the ratio of the area of its peak to the total area of all peaks. In this study, data are presented on those acids that were present at concentrations greater than 1 per cent.

Results and comment

The Table shows the percentages of the principal fatty acids obtained from the fifteen strains of cultivable treponemes. In all the strains tested, the major fatty acids were palmitic, stearic, and C_{18} monoenoic and C_{18} dienoic straight chain acids. With the exception of *T. zuelzerae, T. refringens, B. vincentii,* and *T. skoliodonta,* the predominant acids were palmitic and stearic. Although C_{18} dienoic acid has been observed in other micro-organisms, the relative amounts of this acid generally are much smaller than those observed in most of the treponeme cultures. Essentially identical results were obtained with duplicate analyses of both lyophilized and freshly cultivated treponemes.

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Trade names are used for identification only and do not represent an endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

TABLE *Percentages of principal cellular fatty acids of treponemes*

Organisms	*Fatty acids*																		
	10 : 0	*Un*	12 : 0	*Un*	14 : 0	15 : 0*Br*	15 : 0	16 : 1	16 : 0	*Un*	17 : 0*Br*	17 : 0	18 : 1	18 : 2	18 : 0	*Un*	19 : 0	20 : 0
*T. pallidum**																		
Kazan 2	3	1	1	1	1	nt	nt	6	22	3	3	3	15	15	21	nt	3	2
Kazan 5	2	nt	1	nt	2	nt	nt	6	21	4	4	4	14	14	25	nt	3	tr
Kazan 8	nt	nt	1	nt	2	nt	2	4	27	4	3	4	16	16	21	nt	nt	tr
Kazan	2	nt	2	tr	2	nt	1	5	25	3	5	2	18	14	18	nt	2	1
Nichols	2	1	1	nt	2	nt	1	6	22	3	3	4	14	13	28	nt	tr	tr
Reiter	1	tr	1	nt	3	nt	1	4	19	1	1	3	12	19	28	nt	7	tr
Noguchi	nt	nt	1	nt	4	2	3	10	20	13	tr	4	5	19	19	nt	tr	tr
T. refringens	1	nt	1	nt	2	nt	4	7	30	4	2	4	8	20	17	nt	tr	nt
T. phagedenis	1	tr	1	nt	2	nt	1	4	23	5	2	nt	12	18	27	nt	4	nt
T. skoliodonta	1	nt	1	nt	2	1	1	3	29	nt	4	1	14	22	20	nt	nt	1
T. calligyra	2	nt	4	nt	3	nt	nt	3	32	1	5	1	12	15	21	nt	1	tr
T. ambigua	3	nt	6	nt	3	1	1	5	23	tr	7	2	14	14	20	nt	1	tr
T. microdentium	1	nt	2	2	12	8	3	7	22	1	tr	2	12	11	15	nt	1	1
B. vincentii	tr	nt	tr	nt	6	21	2	tr	31	2	6	tr	12	5	13	nt	1	1
T. zuelzerae	tr	nt	1	nt	6	6	1	2	30	1	3	1	33	tr	8	8	tr	tr

In the column headings, the number to the left of the colon refers to the number of carbon atoms; that to the right refers to the number of double bonds; Br denotes branched-chain acids; Un denotes unidentified
*Nonpathogenic strains
In the body of the Table, the numbers refer to percentages of total acids: nt indicates not detected; tr indicates less than 1 per cent.

Of the treponemes studied, only *B. vincentii, T. zuelzerae,* and *T. microdentium* had patterns that differed from those of the others. The principal distinguishing feature of *B. vincentii* was the presence of relatively large amounts (21 per cent.) of a C_{15} saturated branched chain acid. Only two other treponemes, *T. zuelzerae* and *T. microdentium,* had appreciable amounts of this acid. Also, unlike the other treponemes, *B. vincentii* had relatively small (trace) amounts of C_{16} monoenoic and C_{18} dienoic acid (5 per cent.).

T. zuelzerae was easily ditinguished by the presence of C_{16} saturated and C_{18} monoenoic acids, an unidentified component which eluted between the C_{18} and C_{19} saturated straight chain acids, and only trace amounts of C_{18} dienoic acid (Table). In addition, this strain contained relatively small amounts (8 per cent.) of stearic acid.

T. microdentium differed from the other treponemes by the presence of relatively large amounts of C_{14} saturated straight chain acid (12 per cent.) and C_{15} saturated branch chain acid (8 per cent.).

The observation that *B. vincentii, T. zuelzerae,* and *T. microdentium* can be distinguished from each other on the basis of cellular fatty acids is in agreement with recent immunological studies which indicate serological differences between these three treponemes (Cannefax, 1965; Meyer and Hunter, 1967). The similarities in the fatty acid profiles of the other treponemes indicate, however, that there is no apparent relationship between cellular fatty acids and serological grouping, since these species constitute diverse sero-groups.

The similarities in fatty acid composition of the treponemes studied presumably reflect common enzyme systems frequently encountered in nature. On the other hand, the observations on *B. vincentii, T. microdentium,* and *T. zuelzerae* must reflect some basic difference in fatty acid synthesis in these cultures. The relationship, if any, between the antigenic behaviour of the treponemes and their cellular fatty acids composition is not clear. Serological specificity appears to be more closely related to the polysaccharide antigen than to the lipid component (Christiansen, 1962). Studies now in progress on the carbohydrate composition may provide additional information to aid in a more complete taxonomic classification of the cultivable treponemes.

Summary

The cellular fatty acid composition of fifteen different cultivable treponemes was examined by gas liquid chromatography. The principal fatty acids present were palmitic, stearic, and C_{18} monoenoic and C_{18} dienoic straight chain acids.

Of the treponemes studied, only *Borrelia vincentii, Treponema zuelzerae,* and *Treponema microdentium* had fatty acid patterns that differed from those of others.

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Les acides gras cellulaires des tréponèmes

SOMMAIRE

La composition en acides gras cellulaires de quinze différents tréponèmes cultivables fut examinée par chromatographie en gaz-liquide. Les principaux acides gras présents furent les acides palmitique, stéarique et C_{18} monoénoique et C_{18} diénoique en chaîne droite.

Parmi les tréponèmes étudiés, seuls *Borrelia vincentii*, *Treponema zuelzerae* et *Treponema microdentium* présentent un ensemble d'acide gras différent de celui des autres.