Journal of General Microbiology (1977), **98**, 205–213
Printed in Great Britain

Fatty and Mycolic Acid Composition of *Bacterionema matruchotii* and Related Organisms

By L. ALSHAMAONY AND M. GOODFELLOW
Department of Microbiology, The Medical School, The University, Newcastle upon Tyne NE1 7RU
AND D. E. MINNIKIN
Department of Organic Chemistry, School of Chemistry, The University, Newcastle upon Tyne
AND G. H. BOWDEN AND J. M. HARDIE
Dental Bacteriology Laboratory, The London Hospital Medical College, Turner Street, London E1 2AD

(*Received* 22 *July* 1976)

SUMMARY

Whole-organism methanolysates of bacterionemae contained mycolic acids in addition to other long-chain fatty acids. These mycolic acids were similar in general structure and overall size to those found in strains of *Corynebacterium diphtheriae* and *Corynebacterium xerosis*. The long-chain fatty acids of bacterionemae, mainly straight-chain saturated and unsaturated acids, were similar to those of certain coryneform bacteria including *C. diphtheriae*. On the basis of these lipid data, and results of earlier studies, we recommend that the genus *Bacterionema* be transferred from the family Actinomycetaceae to the Coryneform Group of Bacteria.

INTRODUCTION

The genus *Bacterionema* (Gilmour, Howell & Bibby, 1961) was proposed for a group of bacteria previously classified as *Leptothrix buccalis* (Bulleid, 1925), *Leptotrichia buccalis* (Bibby & Berry, 1939) and *Leptotrichia dentium* (Davis & Baird-Parker, 1959). The taxon contains the single species *B. matruchotii* (Gilmour, 1974), commonly isolated in low numbers from the oral cavity of man and primates. Presently the genus *Bacterionema* is classified in the family Actinomycetaceae (Slack, 1974).

On the basis of wall composition, metabolic and fermentation characteristics, Pine (1970) considered that the families Corynebacteriaceae or Mycobacteriaceae might provide a more suitable niche. In a numerical phenetic study, Holmberg & Hallender (1973) found *B. matruchotii* to be a homogeneous and distinct phenon showing a relationship to the genera *Actinomyces*, *Arachnia*, *Corynebacterium* and *Nocardia* and considered that further work was needed to establish the taxonomic position of the bacterionemae.

There is good evidence that lipid markers are of value in classifying coryneform and nocardioform taxa (Lechevalier, Horan & Lechevalier, 1971; Bowie *et al.*, 1972; Pommier & Michel, 1973; Alshamaony, Goodfellow & Minnikin, 1976*a*; Alshamaony *et al.*, 1976*b*; Goodfellow, Collins & Minnikin, 1976). Mycolic acids, i.e. long-chain 3-hydroxy fatty acids with a long alkyl chain on C-2, have been particularly useful in clarifying the taxonomy of the genera *Nocardia*, *Mycobacterium*, *Corynebacterium* and the '*rhodochrous*' complex.

Table 1. *Designation and sources* of test strains*

B10	*Bacterionema matruchotii*, NCTC10592; M. Magnus, Toronto, Canada. Foot abscess.
B52	*B. matruchotii*, NCTC10206.
B57–B59	*B. matruchotii*, G. H. Bowden, J1247; J1429; A1839. Oral isolates
B62	*B. matruchotii*, ATCC14266; M. N. Gilmour, New York. Oral calculus. NCTC10254. Type strain
C33	*Corynebacterium xerosis*, NCTC9755
PW8	*Corynebacterium diphtheriae*, Park Williams 8 strain, autoclaved cell paste supplied by A. F. B. Standfast, The Lister Institute of Preventive Medicine, Elstree, Hertfordshire

* ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; NCTC, National Collection of Type Cultures, London; B and C, laboratory numbers.

The mycolic acids which occur in true corynebacteria (Jones, 1975; Goodfellow *et al.*, 1976) are of relatively low molecular weight (20 to 30 carbon atoms) (Lederer *et al.*, 1952; Diara & Pudles, 1959; Etémadi, Gasche & Sifferlen, 1965; Welby-Gieusse, Lanéelle & Asselineau, 1970; Yano & Saito, 1972). At the other extreme, mycobacteria usually contain complex mixtures of mycolic acids (Minnikin, Alshamaony & Goodfellow, 1975) of high molecular weight (60 to 90 carbon atoms) (Etémadi, 1967). Nocardiae contain mycolic acids with about 50 carbon atoms, those from gordonae have about 60 carbon atoms, and many rhodochrous strains have acids with about 40 carbon atoms (Ioneda, Lederer & Rozanis, 1970; Maurice, Vacheron & Michel, 1971; Azuma *et al.*, 1974; Alshamaony *et al.*, 1976*a*, *b*).

Bowie *et al.* (1972) have shown that two groups of coryneform organisms can be distinguished by gas chromatographic analyses of their long-chain fatty acids. This study reports the isolation and analysis of long-chain fatty acids and mycolic acids from strains of *B. matruchotii*, *Corynebacterium diphtheriae* and *C. xerosis*.

METHODS

Cultures. The strains are listed in Table 1. Bacterionema strains were maintained on brain–heart infusion agar (Oxoid), and *C. xerosis* on Dorset Egg and Loeffler serum slopes (Cowan, 1974).

The identity of the strains of *Bacterionema* was confirmed by physiological tests (Gilmour, 1974), cell-wall analysis (Bowden, 1969; Rhuland *et al.*, 1955), and analysis of the acid products of glucose fermentation (Carlsson, 1973).

Cultivation. Corynebacterium xerosis was grown in shake flasks of nutrient broth (Oxoid) supplemented with 1 % (v/v) Tween 80 for 2 days at 37 °C, and the bacterionemae were grown in brain–heart infusion broth (Oxoid) in stationary culture for 2 weeks at 37 °C. Cultures were checked for purity at maximum growth, killed with formalin (1 %, v/v), harvested by centrifuging, washed with distilled water and freeze-dried.

Preparation of fatty acid methyl esters. Samples of freeze-dried bacteria (50 to 100 mg) were degraded by acid methanolysis as described by Minnikin *et al.* (1975) and the hexane extracts were examined for long-chain components by thin-layer chromatography using Merck silica gel H (0·5 mm layers) and a developing mixture of petroleum ether (b.p. 60 to 80 °C)/diethyl ether (85:15, v/v). Long-chain components were isolated by preparative thin-layer chromatography on layers (1 mm) of Merck silica gel $PF_{254+366}$ using the same developing mixture, separated bands being detected by illumination with ultraviolet light (366 nm).

Gas chromatography. Long-chain fatty acid methyl esters were analysed using a glass

Table 2. *Fatty-acid composition of strains of Bacterionema and Corynebacterium*

		Fatty acids (% w/w, total fatty acid)							
Equivalent chain length		12·0	14·0	14·7	15·6	16·0	16·4	18·0	18·3
Assignment*		12:0	14:0	14:1	?	16:0	16:1	18:0	18:1
B. matruchotii	B10	.	1	.	.	53	21	10	15
	B52	.	.	.	.	49	.	10	41
	B57	.	1	.	2	48	.	5	44
	B58	.	1	.	.	47	.	6	46
	B59	.	1	7	2	44	3	5	38
	B62	4	2	1	.	49	.	11	33
C. diphtheriae	PW8	4	14	1	.	27	54	.	.
C. xerosis	C33	.	4	.	.	20	9	.	67

* 12:0, 14:0, 16:0 and 18:0 represent do-, tetra-, hexa- and octa-decanoic acids, respectively; 14:1, 16:1 and 18:1 represent tetra-, hexa- and octa-decenoic acids, respectively; ?, unknown.

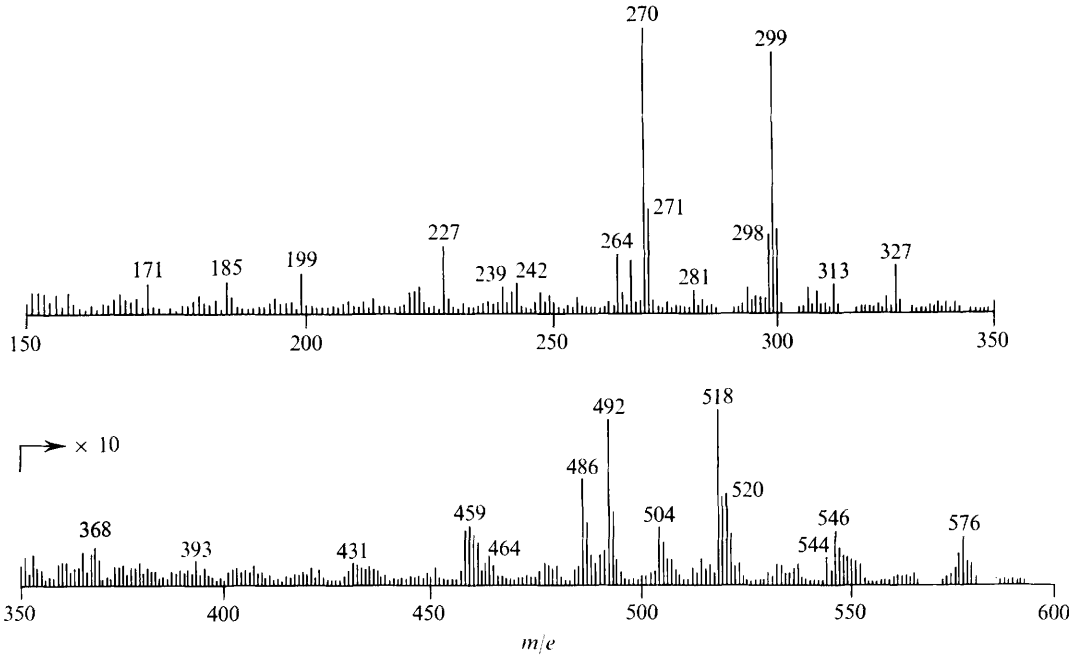


Fig. 1. Partial mass spectrum of the methyl esters of total mycolic acids from *B. matruchotii* (B62); the spectrum was copied directly from the original (not normalized), base peak *m/e* 270.

column (200 × 0·4 cm) packed with 10 % (w/w) polyethyleneglycol adipate on Chromosorb W (100 to 120 mesh) with nitrogen as carrier gas and a temperature of 180 °C. The identity of individual esters was determined by comparison of retention times and equivalent chain lengths (Miwa *et al.*, 1960) with those of a standard mixture of straight-chain and unsaturated methyl esters.

Mass spectrometry. Mass spectra of mycolic esters were taken on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature of 180 to 220 °C. Partial mass spectra of the methyl esters of the total mycolic acids of all the strains listed in Table 1, with the exception of that shown in Fig. 1, have been deposited with the

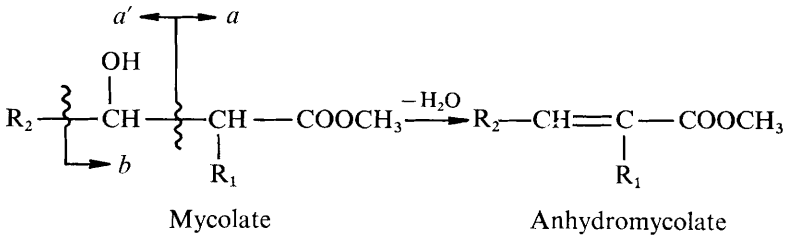
British Lending Library, Boston Spa, Yorkshire LS23 7BQ, as Supplementary Publication No. SUP 28005 (7 pages). Copies may be obtained from the BLL on demand. Where possible, requests should be accompanied by prepaid coupons (held by many university and technical libraries and by the British Council).

RESULTS

Thin-layer chromatographic analysis of the whole-organism methanolysates of the Bacterionema and Corynebacterium strains showed the presence of two components. The most mobile fractions (R_F > 0·6) corresponded to methyl esters of long-chain fatty acids and the least mobile components were similar in their migration to methyl esters of mycolic acids isolated from strains of true corynebacteria (Minnikin *et al.*, 1975; Goodfellow *et al.*, 1976).

Methyl esters of long-chain fatty acids were examined by gas chromatography (Table 2). Each fatty-acid profile consisted mainly of mixtures of straight-chain and unsaturated components, but in extracts of two strains (B57 and B59) small amounts of an unidentified component, possibly a C_{16} branched-chain ester, were detected.

A partial mass spectrum of the methyl ester of the total mycolic acid from the type strain of *B. matruchotii* (B62) is shown in Fig. 1. Mycolic esters from nocardioform bacteria and true corynebacteria fragment on mass spectrometry (Etémadi, 1967; Maurice *et al.*, 1971; Alshamaony *et al.*, 1976*a*) according to the following scheme:



Mycolate Anhydromycolate

Cleavage corresponding to *a* and *a'* involves net transfer of the hydroxyl hydrogen atom to C-2 to produce an aldehyde and straight-chain ester. The fragments due to cleavage *b* complement those at *a* and allow the mass of the side-chain on C-2 (R_1) to be calculated. The highest peaks in the mass spectra of methyl mycolates correspond to anhydromycolates formed by elimination of water from the parent molecule.

Peaks in the mass spectra at *m/e* 214, 242, 270 and 298 correspond to straight-chain esters; the fragments at *m/e* 243, 271, 299 and 327 (cleavage *b*) confirm the presence of side-chains (R_1) having the overall composition $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$. The nature of the side-chains derived from analyses of the mass spectra of the mycolic esters studied are summarized in Table 3.

Peaks in the range *m/e* 408 to 546 in the mass spectra of the mycolic esters correspond to anhydromycolates. Table 4 summarizes the range of molecular size and degree of unsaturation of the anhydromycolates derived from the mycolic esters of the strains investigated.

As noted previously (Alshamaony *et al.*, 1976*a*), peaks corresponding to aldehydes (cleavage *a'*) are unreliable in their appearance. Etémadi *et al.* (1965) found a peak attributable to a mono-unsaturated aldehyde (*m/e* 266) but for a saturated component only an aldehyde dehydration product (*m/e* 250) was detected. Peaks due to saturated or mono-

Table 3. *Peaks in the mass spectra of methyl mycolates of Bacterionema and Corynebacterium strains corresponding to fragments derived from the 2-branched 3-hydroxy ester unit*

The main component of each series is denoted by + + +, any component greater than 50 % of the main peak by + +, and all other significant components by +.

Mass spectra peaks corresponding to cleavage*		*a* m/e	214	242	270	298
		b m/e	243	271	299	327
R_1 branch*			$C_{10}H_{21}$	$C_{12}H_{25}$	$C_{14}H_{29}$	$C_{16}H_{33}$
Straight-chain ester*			C_{12}	C_{14}	C_{16}	C_{18}
B. matruchotii	B10		.	+	+ + +	+
	B52		.	+	+ + +	+
	B57		.	.	+ + +	+
	B58		.	.	+ + +	+
	B59		.	.	+ + +	+
	B62		.	+	+ + +	+
C. diphtheriae	PW8		.	+	+ + +	+
C. xerosis	C33		+	+ + +	+ +	.

* Explanation of assignment of peaks is given in Results.

Table 4. *Peaks in the mass spectra of methyl mycolates of Bacterionema and Corynebacterium strains corresponding to anhydromycolates*

The main component of each series is denoted by + + +, any component greater than 50 % of the main peak by + +, and all other significant components by +.

No. of C in parent mycolic acid	m/e	No. of double bonds	*Bacterionema matruchotii* B10	B52	B57	B58	B59	B62	*C. diphtheriae* PW8	*C. xerosis* C33
C_{26}	408	0	.	.	.	.	.	.	+	.
C_{28}	436	0	.	.	.	.	.	.	+	.
C_{30}	462	1	.	.	.	.	.	.	.	+
	464	0	.	.	.	.	.	+	+ +	.
C_{32}	488	2	.	.	.	.	.	.	.	+ +
	490	1	+ +	.	.	.	.	.	+	+ +
	492	0	+ + +	+ + +	+ +	+ +	+ +	+ +	+ + +	.
C_{34}	516	2	.	.	.	.	.	.	.	+ + +
	518	1	+	+	+ + +	+ + +	+ + +	+ + +	.	+
	520	0	+	+	+	+	+	+ +	+	.
C_{36}	544	2	.	.	+	+	+	+	.	+ +
	546	1	.	.	+	+	+	+	.	.

unsaturated aldehydes or their dehydration products were not clearly discernible but in the mass spectrum of the mycolic ester from *C. xerosis*, which contained di-unsaturated mycolates (Table 4), peaks at *m/e* 236 and 264 possibly correspond to di-unsaturated aldehydes.

DISCUSSION

The presence of mycolic acids in whole-organism methanolysates of bacterionemae distinguishes these bacteria from other taxa (*Actinomyces*, *Arachnia*, *Bifidobacterium*, *Rothia*) of the family Actinomycetaceae (Slack, 1974) which do not contain these characteristic components (Minnikin *et al.*, 1975). The mycolic acids of *B. matruchotii* are similar in their

general structure and overall size to those of the two strains of *Corynebacterium* studied here and to those of other corynebacteria (Lederer *et al.*, 1952; Pudles & Lederer, 1954; Diara & Pudles, 1959; Etémadi *et al.*, 1965; Welby-Gieusse *et al.*, 1970; Yano & Saito, 1972), but are distinct from those of nocardiae, mycobacteria and rhodochrous strains which have higher molecular weights (Etémadi, 1967; Maurice *et al.*, 1971; Azuma *et al.*, 1974; Alshamaony *et al.*, 1976*a*, *b*).

The mycolic acids of *C. xerosis* C33 are interesting in that the main components are di-unsaturated acids that apparently have both double-bonds in the main-chain since only fragments from saturated straight-chain esters (Table 3) are produced on mass spectrometry. Di-unsaturated mycolic acids have also been found in extracts of *Corynebacterium hofmanii* (Welby-Gieusse *et al.*, 1970) and *Brevibacterium genitalis* (Okazaki *et al.*, 1969) but in these instances single double-bonds were present in the main and branched chains. Detailed chemical studies of the mycolic acids of *C. xerosis* and related strains are required to determine the significance of these apparent differences. Minor di-unsaturated components (C_{36}) were also present (Table 4; Fig. 1) in the mycolates of several strains of *B. matruchotii*.

The patterns of long-chain fatty acids, consisting mainly of straight-chain saturated and unsaturated acids (Table 2), are similar to those found in strains of *C. diphtheriae* (Asselineau, 1961; Brennan & Lehane, 1971), and in one of the groups (*Arthrobacter tumescens*, *Brevibacterium ammoniagenes*, *Corynebacterium fascians*) of coryneform bacteria studied by Bowie *et al.* (1972). Representatives of other bacteria probably related to the coryneform group, such as *Corynebacterium acnes* (Voss, 1970), propionibacteria and anaerobic corynebacteria (Moss & Cherry, 1968; Moss *et al.*, 1967, 1969; Lanéelle & Asselineau, 1968; Shaw & Dinglinger, 1969) as well as most arthrobacters (Walker & Fagerson, 1965; Shaw & Stead, 1971; Bowie *et al.*, 1972; Kostiw, Boylen & Tyson, 1972), do not contain substantial amounts of unsaturated acids but contain high proportions of iso and anteiso branched-chain acids in addition to the straight-chain saturated acids. Similar mixtures, rich in branched-chain acids, have been isolated from members of the actinomycete taxa Actinoplanaceae, *Microbispora*, *Micromonospora*, *Thermoactinomyces*, *Thermomonospora* and *Thermopolyspora* (Ballio & Barcellona, 1968), and from *Streptomyces* (Hofheinz & Grisebach, 1965; Ballio & Barcellona, 1968; Lanéelle, Asselineau & Castelnuovo, 1968; Behal, Prochazkova & Vanek, 1968; Pommier & Michel, 1973). The fatty acids of mycobacteria (Asselineau, 1966; Thoen, Karlson & Ellefson, 1971*a*, *b*, 1972) and mycolic acid-containing nocardioform bacteria (Lanéelle, Asselineau & Castelnuovo, 1965; Ballio & Barcellona, 1968; Farshtchi & McClung, 1970; Bowie *et al.*, 1972; Pommier & Michel, 1973) consist mainly of straight-chain saturated and unsaturated acids with the frequent addition of 10-methyloctadecanoic (tuberculostearic) acid. The latter acid was not detected in the present study. Small amounts of iso and anteiso acids have been found, however, in certain nocardiae (Farshtchi & McClung, 1970; Pommier & Michel, 1973) and in *C. diphtheriae* (Brennan & Lehane, 1971); it is possible, therefore, that the uncharacterized minor component found in strains B57 and B59 of *B. matruchotii* (Table 2) is an iso or anteiso branched-chain acid. The relatively shorter chain lengths of the fatty acids of *C. diphtheriae* PW8 (Table 2) may be a result of the different growth conditions of this organism.

Gilmour *et al.* (1961) classified the genus *Bacterionema* in the order Actinomycetales, family Actinomycetaceae because of its mycelial growth and Gram-positive filamentous morphology, though they did point out that it differed extensively from the two existing genera *Actinomyces* and *Nocardia*. However, although the genus has subsequently been found to form a recognizable taxonomic entity on the basis of numerical phenetic and sero-

logical data (Melville, 1965; Snyder *et al.*, 1967; Holmberg & Hallender, 1973), its generic location is unsettled. Pine (1970) suggested the family Corynebacteriaceae might be a more suitable niche for *B. matruchotii* on the basis of its propionic acid fermentation (Howell & Pine, 1961). Bacterionemae and true corynebacteria also have a wall type IV (Pine, 1970; Cross & Goodfellow, 1973) and a DNA base composition in the range 55 to 60% GC (Rogosa *et al.*, 1974; Gilmour, 1974). The discovery that bacterionemae contain short-chain mycolic acids and a similar fatty-acid profile to corynebacteria re-emphasizes the relationship between these taxa. We recommend, therefore, that the genus *Bacterionema* be transferred from the family Actinomycetaceae and classified with the Coryneform Group of Bacteria (Rogosa *et al.*, 1974).

Thanks are due to A. Temple for technical assistance, to P. Kelly for mass spectroscopy, and to Dr A. F. B. Standfast for the gift of *C. diphtheriae* organisms. M. G. and D. E. M. thank the Medical Research Council for support (grant G974/522/S).

REFERENCES

ALSHAMAONY, L., GOODFELLOW, M. & MINNIKIN, D. E. (1976*a*). Free mycolic acids as criteria in the classification of *Nocardia* and the '*rhodochrous*' complex. *Journal of General Microbiology* **92**, 188–199.

ALSHAMAONY, L., GOODFELLOW, M., MINNIKIN, D. E. & MORDARSKA, H. (1976*b*). Free mycolic acids as criteria in the classification of *Gordona* and the '*rhodochrous*' complex. *Journal of General Microbiology* **92**, 183–187.

ASSELINEAU, J. (1961). Sur quelques applications de la chromatographie en phase gazeuse a l'étude d'acides gras bactériens. *Annales de l'Institut Pasteur* **100**, 109–119.

ASSELINEAU, J. (1966). *The Bacterial Lipids*. Paris: Hermann.

AZUMA, I., OHUCHIDA, A., TANIYAMA, T., YAMAMURA, Y., SHOJI, K., HORI, M., TANAKA, Y. & RIBI, E. (1974). The mycolic acids of *Mycobacterium rhodochrous* and *Nocardia corallina*. *Biken Journal* **17**, 1–9.

BALLIO, A. & BARCELLONA, S. (1968). Relations chimiques et immunologiques chez les actinomycétales. I. Les acides gras de 43 souches d'actinomycètes aérobies. *Annales de l'Institut Pasteur* **114**, 121–137.

BEHAL, V., PROCHAZKOVA, V. & VANEK, Z. (1968). Regulation of biosynthesis of secondary metabolites. II. Fatty acids and chlortetracycline in *Streptomyces aureofaciens*. *Folia microbiologica (Praha)* **14**, 112–116.

BIBBY, B. G. & BERRY, G. P. (1939). A cultural study of filamentous bacteria obtained from the human mouth. *Journal of Bacteriology* **38**, 263–274.

BOWDEN, G. H. (1969). The components of the cell walls and extracellular slime of four strains of *Staphylococcus salivarius* isolated from human dental plaque. *Archives of Oral Biology* **14**, 685–697.

BOWIE, I. S., GRIGOR, M. R., DUNCKLEY, G. G., LOUTIT, M. W. & LOUTIT, J. S. (1972). The DNA base composition and fatty acid constitution of some gram-positive pleomorphic soil bacteria. *Soil Biology and Biochemistry* **4**, 397–412.

BRENNAN, P. J. & LEHANE, D. P. (1971). The phospholipids of corynebacteria. *Lipids* **6**, 401–409.

BULLEID, A. (1925). An experimental study of *Leptothrix buccalis*. *British Dental Journal* **46**, 289–300.

CARLSSON, J. (1973). Simplified gas chromatographic procedure for identification of bacterial metabolic products. *Applied Microbiology* **25**, 287–289.

COWAN, S. T. (1974). *Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.

CROSS, T. & GOODFELLOW, M. (1973). Taxonomy and classification of the actinomycetes. In *Actinomycetales: Characteristics and Practical Importance*, pp. 11–112. Edited by G. Sykes and F. A. Skinner. London: Academic Press.

DAVIS, G. H. G. & BAIRD-PARKER, A. C. (1959). *Leptotrichia buccalis*. *British Dental Journal* **106**, 70–73.

DIARA, A. & PUDLES, J. (1959). Sur les lipides de *Corynebacterium ovis*. *Bulletin de la Société de chimie biologique* **41**, 481–486.

ETÉMADI, A. H. (1967). Corrélations structurales et biogénétiques des acides mycoliques en rapport avec la phylogenèse de quelques genres d'actinomycétales. *Bulletin de la Société de chimie biologique* **49**, 695–706.

ETÉMADI, A. H., GASCHE, J. & SIFFERLEN, J. (1965). Identification d'homologues supérieurs des acides corynomycolique et corynomycolénique dans les lipides de *Corynebacterium* 506. *Bulletin de la Société de chimie biologique* **47**, 631–638.

FARSHTCHI, D. & MCCLUNG, N. M. (1970). Effect of substrate on fatty acid production in *Nocardia asteroides*. *Canadian Journal of Microbiology* **16**, 213–217.

GILMOUR, M. N. (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 676–679. Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams and Wilkins.

GILMOUR, M. N., HOWELL, A., JR., & BIBBY, B. G. (1961). The classification of organisms termed *Leptotrichia* (*Leptothrix*) *buccalis*. I. Review of the literature and proposed separation into *Leptotrichia buccalis* Trevisan, 1879 and *Bacterionema matruchotii* (Mendel, 1919) comb. nov. *Bacteriological Reviews* **25**, 131–141.

GOODFELLOW, M., COLLINS, M. D. & MINNIKIN, D. E. (1976). Thin-layer chromatographic analysis of mycolic acid and other long-chain components in whole-organism methanolysates of coryneform and related taxa. *Journal of General Microbiology* **96**, 351–358.

HOFHEINZ, W. & GRISEBACH, H. (1965). Die Fettsäuren von *Streptomyces erythreus* und *Streptomyces halsledii*. *Zeitschrift für Naturforschung* **20**, 43–53.

HOLMBERG, K. & HALLENDER, H. O. (1973). Numerical taxonomy and laboratory identification of *Bacterionema matruchotii*, *Rothia dentocariosa*, *Actinomyces naeslundii*, *Actinomyces viscosus* and some related bacteria. *Journal of General Microbiology* **76**, 43–63.

HOWELL, A. & PINE, L. (1961). The classification of organisms termed *Leptotrichia* (*Leptothrix*) *buccalis*. IV. Physiological and biochemical characteristics of *Bacterionema matruchotii*. *Bacteriological Reviews* **25**, 162–171.

IONEDA, T., LEDERER, E. & ROZANIS, J. (1970). Sur la structure des diesters de trehalose ('cord factors') produits par *Nocardia asteroides* et *Nocardia rhodochrous*. *Chemistry and Physics of Lipids* **4**, 379–392.

JONES, D. (1975). A numerical taxonomic study of coryneform and related bacteria. *Journal of General Microbiology* **87**, 52–96.

KOSTIW, L. L., BOYLEN, C. W. & TYSON, B. J. (1972). Lipid composition of growing and starving cells of *Arthrobacter crystallopoietes*. *Journal of Bacteriology* **111**, 103–111.

LANÉELLE, M.-A. & ASSELINEAU, J. (1968). Sur les lipides de *Propionibacterium freudenreichii*. *Compte rendu hebdomadaire des séances de l'Académie des sciences* **266D**, 1901–1903.

LANÉELLE, M.-A., ASSELINEAU, J. & CASTELNUOVO, G. (1965). Études sur les mycobactéries et les nocardiae. IV. Composition des lipides de *Mycobacterium rhodochrous*, *M. pellegrino* sp., et de quelques souches de nocardiae. *Annales de l'Institut Pasteur* **108**, 69–82.

LANÉELLE, M.-A., ASSELINEAU, J. & CASTELNUOVO, G. (1968). Relations chimiques et immunologiques chez les Actinomycétales. IV. Composition chimique des lipides de quatre souches de *Streptomyces* et d'une souche *N.* (*Str.*) *gardneri*. *Annales de l'Institut Pasteur* **114**, 305–312.

LECHEVALIER, M. P., HORAN, A. C. & LECHEVALIER, H. A. (1971). Lipid composition in the classification of nocardiae and mycobacteria. *Journal of Bacteriology* **105**, 313–318.

LEDERER, E., PUDLES, J., BARBEZAT, S. & TRILLAT, J. J. (1952). Sur la constitution chimique de l'acide coryno-mycolique de bacille diphthérique. *Bulletin de la Société chimique de France*, 93–95.

MAURICE, M. T., VACHERON, M. T. & MICHEL, G. (1971). Isolement d'acides nocardiques de plusieurs espèces de *Nocardia*. *Chemistry and Physics of Lipids* **7**, 9–18.

MELVILLE, T. H. (1965). A study of the overall similarity of certain actinomycetes mainly of oral origin. *Journal of General Microbiology* **40**, 309–315.

MINNIKIN, D. E., ALSHAMAONY, L. & GOODFELLOW, M. (1975). Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *Journal of General Microbiology* **88**, 200–204.

MIWA, T. K., MIKOLAJCZAK, K. L., EARLE, F. R. & WOLFF, I. A. (1960). Gas chromatographic characterization of fatty acids. *Analytical Chemistry* **32**, 1739–1742.

MOSS, C. W. & CHERRY, W. B. (1968). Characterization of the C_{15} branched-chain fatty acids of *Corynebacterium acnes* by gas chromatography. *Journal of Bacteriology* **95**, 241–242.

MOSS, C. W., DOWELL, V. R., LEWIS, V. J. & SCHEKTER, M. A. (1967). Cultural characteristics and fatty acid composition of *Corynebacterium acnes*. *Journal of Bacteriology* **94**, 1300–1305.

MOSS, C. W., DOWELL, V. R., JR, FARSHTCHI, D., RAINES, L. J. & CHERRY, W. B. (1969). Cultural characteristics and fatty acid composition of propionibacteria. *Journal of Bacteriology* **97**, 561–570.

OKAZAKI, H., SUGINO, H., KANZAKI, T. & FUKUDA, H. (1969). L-Glutamic acid fermentation. VI. Structure of a sugar lipid produced by *Brevibacterium thiogenitalis*. *Agriculture and Biological Chemistry* **33**, 764–770.

PINE, L. (1970). Classification and phylogenetic relationship of microaerophilic actinomycetes. *International Journal of Systematic Bacteriology* **20**, 445–474.

POMMIER, M.-T. & MICHEL, G. (1973). Phospholipid and acid composition of *Nocardia* and nocardoid bacteria as criteria of classification. *Biochemical Systematics* **1**, 3–12.

PUDLES, J. & LEDERER, E. (1954). Sur l'isolement et la constitution chimique de l'acide coryno-mycolénique et de deux cétones des lipides du bacille diphthérique. *Bulletin de la Société de chimie biologique* **36**, 759–777.

RHULAND, L. E., WORK, E., DENHAM, R. F. & HOARE, D. S. (1955). The behavior of isomers of α,ε-diaminopimelic acid on paper chromatograms. *Journal of the American Chemical Society* **17**, 4844–4846.

ROGOSA, M., CUMMINS, C. S., LELLIOTT, R. A. & KEDDIE, R. M. (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 599–633. Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams and Wilkins.

SHAW, N. & DINGLINGER, F. (1969). The structure of an acylated inositol mannoside in the lipids of propionic acid bacteria. *Biochemical Journal* **112**, 769–775.

SHAW, N. & STEAD, D. (1971). Lipid composition of some species of *Arthrobacter*. *Journal of Bacteriology* **107**, 130–133.

SLACK, J. M. (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 659–660. Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams and Wilkins.

SNYDER, M. L., SLAWSON, M. S., BULLOCK, W. & PARKER, R. B. (1967). Studies on oral filamentous bacteria. II. Serological relationships within the genera *Actinomyces*, *Nocardia*, *Bacterionema* and *Leptotrichia*. *Journal of Infectious Diseases* **117**, 341–345.

THOEN, C. O., KARLSON, A. G. & ELLEFSON, R. D. (1971 *a*). Fatty acids of *Mycobacterium kansasii*. *Applied Microbiology* **21**, 628–632.

THOEN, C. O., KARLSON, A. G. & ELLEFSON, R. D. (1971 *b*). Comparison by gas–liquid chromatography of the fatty acids of *Mycobacterium avium* and some other non-photochromogenic mycobacteria. *Applied Microbiology* **22**, 560–563.

THOEN, C. O., KARLSON, A. G. & ELLEFSON, R. D. (1972). Differentiation between *Mycobacterium kansasii* and *Mycobacterium marinium* by gas–liquid chromatographic analysis of cellular fatty acids. *Applied Microbiology* **24**, 1009–1010.

VOSS, J. G. (1970). Differentiation of two groups of *Corynebacterium acnes*. *Journal of Bacteriology* **101**, 392–397.

WALKER, R. W. & FAGERSON, I. S. (1965). Studies of the lipids of *Arthrobacter globiformis*. I. Fatty acid composition. *Canadian Journal of Microbiology* **11**, 229–233.

WELBY-GIEUSSE, M., LANÉELLE, M.-A. & ASSELINEAU, J. (1970). Structure des acides corynomycoliques de *Corynebacterium hofmanii* et leur implication biogénétique. *European Journal of Biochemistry* **13**, 164–167.

YANO, I. & SAITO, K. (1972). Gas chromatographic and mass spectrometric analysis of molecular species of corynomycolic acids from *Corynebacterium ulcerans*. *FEBS Letters* **23**, 352–356.