Fatty Acid Composition of Some Mycolic Acid-containing Coryneform Bacteria

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The fatty acid profiles of 74 strains of mycolic acid-containing coryneform bacteria were examined by gas-liquid chromatography. All of the strains contained major amounts of straight-chain and monounsaturated fatty acids although some also possessed substantial amounts of 10-methyloctadecanoic acid. *Iso*- and *anteiso*-branched acids were not present. Five distinct fatty acid patterns were evident: (i) *Corynebacterium diphtheriae*, *C. pseudotuberculosis* and '*C. ulcerans*' strains contained major amounts of hexadecanoic and hexadecenoic acids; (ii) *C. glutamicum*, *C. xerosis* and related saprophytic and animal-associated strains, predominantly hexadecanoic and octadecenoic acids; (iii) *C. bovis*, major amounts of octadecenoic and 10-methyloctadecanoic acids; (iv) '*C. mycetoides*', significant amounts of heptadecanoic acid as well as hexadecanoic and octadecenoic acids; and (v) strains related to *Rhodococcus* possessed significant quantities of 10-methyloctadecanoic acid in addition to straight-chain and monounsaturated acids.

INTRODUCTION

Lipid markers are being increasingly used in the classification and identification of coryneform and related taxa (Minnikin *et al.*, 1978). The most productive studies to date have involved analyses of mycolic acids (Goodfellow *et al.*, 1976; Keddle & Cure, 1977; Collins *et al.*, 1982) and isoprenoid quinones (Yamada *et al.*, 1976; Collins *et al.*, 1977, 1979). The use of simple long-chain fatty acids as chemotaxonomic markers in coryneform taxonomy has received less attention, although preliminary data are encouraging (Minnikin *et al.*, 1978, 1979; Collins & Jones, 1980; Collins *et al.*, 1980).

The simple non-hydroxylated fatty acids of coryneform bacteria are conventional in type and generally fall into two broad patterns, those containing high proportions of straight-chain and monounsaturated acids and those with high proportions of *iso*- and *anteiso*-methyl branched-chain acids (Minnikin *et al.*, 1978). Preliminary data indicate that mycolic acid-containing coryneform bacteria fall into the first category, whereas those taxa lacking mycolic acids contain substantial amounts of branched-chain acids. The single report (Brennan & Lehane, 1971) of high proportions of *iso*- and *anteiso*-acids in mycolic acid-containing strains labelled *Corynebacterium diphtheriae* and *C. pseudotuberculosis* has not been substantiated. Mycolic acid-containing strains assigned to the genera *Mycobacterium*, *Nocardia* and *Rhodococcus*, however, contain 10-methyloctadecanoic acid (tuberculostearic acid) in addition to the simple straight-chain and monounsaturated acid types (Kroppenstedt & Kutzner, 1978; Minnikin *et al.*, 1978).

In the present study the fatty acid composition of 74 strains of mycolic acid-containing coryneform bacteria was determined.

METHODS

Cultures and cultivation. Details of the test strains and their sources are given in Table 1. Most of the strains were grown in shake flasks of nutrient broth no. 2 (Oxoid) for 3 to 5 d at 30 °C. In the case of the *Corynebacterium bovis* strains the medium was supplemented with Tween 80 (0.2%, v/v). Cultures were checked for purity at maximum growth, killed with formaldehyde (1%, v/v), harvested by centrifugation (10000 g), washed with distilled water and freeze-dried.

Extraction and analysis of fatty acids. Dried organisms (about 50 mg) were degraded by acid methanolysis after Minnikin *et al.* (1975). Fatty acid methyl esters were isolated by preparative thin-layer chromatography on layers (1 mm) of Merck silica gel PF₂₅₄₊₃₆₆ using petroleum ether (b.p. 60–80 °C)/diethyl ether (85:15, v/v) as developing solvent. A Perkin-Elmer F11 flame ionization gas chromatograph fitted with stainless steel columns (4 mm o.d.) was used, with nitrogen as carrier gas, for the analysis of the purified fatty acid methyl esters. Analyses were performed using both non-polar (2 m, 2.5% OV-1 silicone on 80–100 mesh Chromosorb G.AW-DMCS, 180 °C; Phase Separations) and polar (2 m, 3% Silar-5CP on 80–100 mesh Gas-chrom A, 160 °C; Applied Science Laboratories) columns. The identity of individual esters was established by comparison of the retention times with those of standard straight-chain and monounsaturated esters. Identification of fatty acid esters was also assisted by calculation of equivalent chain lengths (Miwa *et al.*, 1960) for each stationary phase employed. The relative proportions of the fatty acid esters were estimated by calculating the product of the retention time and peak height for each component in a sample and taking percentages of the sum of the products for all the components (Kates, 1972).

RESULTS AND DISCUSSION

Results of the gas-liquid chromatographic analysis of the non-hydroxylated long-chain fatty acid methyl esters of the test strains are shown in Table 2. All of the strains contained major amounts of straight-chain and monounsaturated fatty acids. *Iso*- and *anteiso*-branched acids were not detected, although in some strains 10-methyl-branched acids (e.g. tuberculostearic acid) were present in substantial amounts (see Table 2).

The test strains were divided into five groups on the basis of their fatty acid profiles. *Corynebacterium diphtheriae*, *C. pseudotuberculosis* and '*C. ulcerans*' contained major amounts of hexadecanoic (C_{16:0}) and hexadecenoic (C_{16:1}) acids with octadecenoic acid (C_{18:1}) present in only small amounts (Table 2). In contrast, the animal-associated corynebacteria (e.g. *C. kutscheri*, *C. pseudodiphtheriticum*, *C. xerosis*), saprophytic glutamic acid-producing strains (e.g. *Brevibacterium divaricatum*, '*B. flavum*', '*B. chang-fua*', '*B. immariophilum*', '*B. lactofermentum*', '*B. roseum*', '*B. saccharolyticum*', '*C. acetoacidophilum*', *C. callunae*, *C. glutamicum*, '*C. herculis*', *C. lilium*, '*C. melassecola*') and *Bacterionema matruchotii* contained major amounts of hexadecanoic (C_{16:0}) and octadecenoic (C_{18:1}) acids (Table 2). The close relationship found between *C. diphtheriae*, *C. pseudotuberculosis* and '*C. ulcerans*' is of interest as strains assigned to these species are the only known corynebacteria able to produce diphtheria toxin upon lysogenization with the beta phage of *C. diphtheriae* (Maximescu *et al.*, 1974). Additional representatives of the *C. diphtheriae* and *C. glutamicum* groups need to be examined to determine whether the differences in fatty acid profiles can be weighted for diagnostic purposes. The fatty acid data on the *Bacterionema matruchotii* strains are similar to those obtained in an earlier and more extensive study (Alshamaony *et al.*, 1977). These data do not contradict the suggestion (Collins, 1982; Goodfellow *et al.*, 1982b) that *B. matruchotii* should be reclassified in the genus *Corynebacterium sensu stricto*.

The *C. bovis* strains were distinguished from all of the other true corynebacteria as they contained major amounts of octadecenoic and 10-methyloctadecanoic acids. Lechevalier *et al.* (1977) previously detected 10-methyloctadecanoic acid in a strain of this organism. *Corynebacterium bovis* also has significantly shorter mycolic acids (about C₂₂ to C₃₂) than those of other corynebacteria (about C₂₈ to C₃₆; Collins *et al.*, 1982) and is sharply separated from other species of *Corynebacterium sensu stricto* in numerical phenetic surveys (Jones, 1975; Goodfellow *et al.*, 1982b). The *C. bovis* strains are also unusual as they contain small amounts of fatty acids with chromatographic properties of monounsaturated acids (i.e. C_{11:1}, C_{12:1}, C_{13:1}, C_{14:1}, C_{15:1}, C_{16:1} and C_{17:1}). '*Corynebacterium mycetoides*' C63 also formed a distinct pattern as it contained substantial amounts of C_{17:0} in addition to C_{16:0} and C_{18:1} (Table 2). The presence of high proportions of odd-numbered straight-chain acids is unusual, but the mycolic acids of

Table 1. Designation and sources of test strains

Laboratory no.	Strain and source*
D6	<i>Arthrobacter albidus</i> ; NCIB 10266
D87	<i>Arthrobacter hydrocarboglutamicus</i> ; ATCC 15583
C228	<i>Arthrobacter paraffineus</i> ; NCIB 10699
C112	<i>Arthrobacter roseoparaffinus</i> ; NCIB 10700
D2†	<i>Arthrobacter variabilis</i> ; NCIB 9455
B53†	<i>Bacterionema matruchotii</i> ; NCIB 10254
B60	<i>Bacterionema matruchotii</i> ; Dr G. H. Bowden, Dept of Dental Bacteriology, The London Hospital Dental Institute, London: J1163
C80	<i>Brevibacterium ammoniagenes</i> ; NCIB 8143
C790	<i>Brevibacterium butanicum</i> ; ATCC 21196
C794	<i>Brevibacterium chang-fua</i> ; ATCC 14017
D3	<i>Brevibacterium divaricatum</i> ; NCIB 9379
C81	<i>Brevibacterium flavum</i> ; NCIB 9565
C123†	<i>Brevibacterium immariophilum</i> ; NCIB 9544
C260	<i>Brevibacterium ketoglutamicum</i> ; DSM 20165
C124	<i>Brevibacterium lactofermentum</i> ; NCIB 9567
C113	<i>Brevibacterium paraffinolyticum</i> ; NCIB 11160
C82	<i>Brevibacterium roseum</i> ; NCIB 9654
C127	<i>Brevibacterium saccharolyticum</i> ; NCIB 9543
C188	<i>Brevibacterium sterolicum</i> ; NCIB 11161
C357†, C358	<i>Caseobacter polymorphus</i> ; NCDO 2097, NCDO 2099
C129	<i>Corynebacterium acetoacidophilum</i> ; NCIB 9661
D901	<i>Corynebacterium alkanum</i> ; ATCC 21194
C12†, C873	<i>Corynebacterium bovis</i> ; NCTC 3224, NCDO 1931
C130†	<i>Corynebacterium callunae</i> ; NCIB 10338
C13, C66, C67	<i>Corynebacterium diphtheriae</i> ; NCTC 3985, NCTC 3529, NCTC 10681
C39, C104	<i>Corynebacterium fascians</i> ; ATCC 12974, NCPPB 1488
D4	<i>Corynebacterium flavescens</i> ; NCIB 8707
D7	<i>Corynebacterium glutamicum</i> ; NCIB 10025
C85†	<i>Corynebacterium herculis</i> ; NCIB 9694
D8†	<i>Corynebacterium hydrocarboclastus</i> ; Dr K. Komagata, Ajinomoto Co., Kawasaki, Japan: AJ 1386
C20, C59	<i>Corynebacterium kutscheri</i> ; NCTC 949; NCTC 1386
C133	<i>Corynebacterium lilium</i> ; NCIB 10337
C136	<i>Corynebacterium melassecola</i> ; NCIB 10336
C63	<i>Corynebacterium mycetoides</i> ; NCTC 9864
D24†, D24B	<i>Corynebacterium minutissimum</i> ; NCTC 10288, NCTC 10285
C265†	<i>Corynebacterium paurometabolum</i> ; DSM 20162
C73	<i>Corynebacterium pseudodiphtheriticum</i> ; Dr D. Jones, Dept of Microbiology, University of Leicester: C10
C150, C152, C153, C155, C215	<i>Corynebacterium pseudotuberculosis</i> ; Dr H. R. Carne, Dept of Pathology, University of Cambridge: Ov 1134 (ovine strain), Cap 16 (caprine strain), Cap 16-F302-4 (caprine strain), E107 (equine strain), NCTC 4681
C17†	<i>Corynebacterium renale</i> ; NCTC 7448
C190†	<i>Corynebacterium rubrum</i> ; NCIB 9433
C170, C172, C174, C176, C178, C179, C180	<i>Corynebacterium ulcerans</i> ; Dr H. R. Carne: JW2, JW7, JW33, JW40 (bovine strains), PM39C, PM298G, PM319C (equine strains)
C137	<i>Corynebacterium xerosis</i> ; NCIB 9956
C234	<i>Mycobacterium flavum</i> subsp. <i>methanicum</i> ; NCIB 9738
C237	<i>Mycobacterium lacticolum</i> ; NCIB 9740
C239	<i>Mycobacterium rubrum</i> subsp. <i>propanicum</i> ; NCIB 9741
N744†	<i>Rhodococcus coprophilus</i> ; NCIB 11211
C7†, C24, C56, C57, C58, D19–D22, R59, R71†	<i>Rhodococcus equi</i> ; NCTC 1621, NCTC 10673 (<i>Corynebacterium hoagii</i>), NCTC 5649, NCTC 5650, NCTC 4219, Dr H. R. Carne: UNP 20343, Jeffcott I, 149, 1499, ATCC 25715, ATCC 25729
N31	<i>Rhodococcus rhodochrous</i> ; Dr R. E. Gordon, Institute of Microbiology, Rutgers University, New Jersey, U.S.A.: W21

* ATCC, American Type Culture Collection, Rockville, Md, U.S.A.; DSM, Deutsche Sammlung von Microorganismen, Teilsammlung Darmstadt, 61, Darmstadt, F.R.G. NCDO, National Collection of Dairy Organisms, Shinfield, U.K.; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, U.K.; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, Herts., U.K.; NCTC, National Collection of Type Cultures, London, U.K.

Species not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) are inserted between quotation marks.

† Type strains.

Table 2. Percentage fatty acid composition of the test strains

Equivalent chain lengths	Assignment* { Silar 5CP column OV-1 column		C _{14:0}	C _{15:0}	C _{16:0}	C _{16:1}	t _{17:0}	C _{17:0}	C _{17:1}	t _{18:0}	C _{18:0}	C _{18:1}	t _{19:0}
I	<i>Corynebacterium diphtheriae</i> C13 C66 C67		14.0	15.0	16.0	16.4	16.4	17.0	17.35	17.35	18.0	18.3	18.3
			14.0	15.0	16.0	15.75	16.45	17.0	16.7	17.45	18.0	17.7	18.4
			2.5	0.5	27.5	53.0	—	—	—	—	2.0	14.5	—
	<i>C. pseudotuberculosis</i> C150 C152 C153 C155 C215		5.0	0.5	32.5	55.0	—	—	—	—	2.0	5.0	—
			5.0	—	35.0	54.0	—	—	—	—	1.5	4.5	—
			1.5	—	56.0	35.5	—	—	—	—	2.5	4.5	—
			2.0	—	61.5	32.0	—	—	—	—	2.0	2.5	—
			1.5	—	56.0	35.5	—	—	—	—	2.0	5.0	—
	<i>C. ulcerans</i> C170 C172 C174 C176 C178 C179 C180		1.5	—	56.0	36.0	—	—	—	—	2.0	4.5	—
			2.5	—	57.0	34.0	—	—	—	—	2.5	4.0	—
			4.5	0.5	54.0	38.0	—	—	—	—	0.5	2.5	—
			3.5	0.5	49.0	45.0	—	—	—	—	—	2.0	—
			4.0	—	52.5	40.5	—	—	—	—	1.0	2.0	—
			4.5	1.0	50.0	39.0	—	—	—	—	1.5	4.0	—
			6.5	1.5	39.5	47.0	—	—	—	—	0.5	3.5	—
II	<i>Arthrobacter albidus</i> D6 <i>Bacterionema matruchotii</i> B53 B60		8.5	1.5	43.0	43.0	—	—	—	—	0.5	3.5	—
			7.5	1.5	43.0	46.0	—	—	—	—	—	2.0	—
			0.5	1.0	41.0	1.0	—	—	—	—	1.5	55.0	—
	<i>Brevibacterium chang-fua</i> C794 <i>B. divaricatum</i> D3 <i>B. flavum</i> C81 <i>B. immariophilum</i> C123 <i>B. lactofermentum</i> C124		1.5	—	46.5	—	—	—	—	—	6.5	46.0	—
			0.5	—	42.0	0.5	—	—	—	—	6.5	43.0	—
			1.0	0.5	43.0	1.0	—	—	—	—	0.5	51.0	—
			0.5	1.0	42.0	1.0	—	—	—	—	1.0	56.0	—
			0.5	1.0	39.0	1.0	—	—	—	—	0.5	54.0	—
	<i>B. roseum</i> C82 <i>B. saccharolyticum</i> C127 <i>Corynebacterium acetoacidophilum</i> C129 <i>C. callunae</i> C130 <i>C. flavescens</i> D4 <i>C. glutamicum</i> D7 <i>C. herculis</i> C85 <i>C. kutscheri</i> C20 C59		1.0	1.0	43.0	1.0	—	—	—	—	1.5	54.0	—
			0.5	0.5	42.0	0.5	—	—	—	—	4.5	54.0	—
			0.5	1.0	44.0	1.0	—	—	—	—	1.5	52.5	—
			0.5	1.0	44.0	1.0	—	—	—	—	1.0	55.5	—
			1.0	—	38.0	2.0	—	—	—	—	1.0	52.5	—
			1.0	1.0	35.5	1.0	—	0.5	—	—	2.0	57.0	—
			0.5	1.0	41.5	1.0	—	—	—	—	1.0	60.0	—
	<i>C. melassecola</i> C136 <i>C. pseudodiphtheriticum</i> C73 <i>C. renale</i> C17 C. xerosis C137		0.5	2.0	40.0	0.5	—	0.5	—	—	1.5	55.0	—
			1.5	0.5	48.5	1.0	—	—	—	—	5.0	43.5	—
			1.5	0.5	48.0	1.5	—	—	—	—	2.0	46.5	—
			0.5	1.5	38.5	0.5	—	0.5	—	—	0.5	58.0	—
			0.5	1.0	43.0	1.5	—	—	—	—	—	54.0	—
III	<i>C. botis</i> C12† C825†		1.0	—	33.0	2.0	—	—	—	—	10.5	53.5	—
			—	—	40.5	—	—	—	—	—	—	55.0	—
			1.5	1.0	48.5	1.0	—	—	—	—	1.5	46.5	—
			2.0	1.0	13.0	0.5	—	—	0.5	—	0.5	31.0	35.0
			2.0	1.0	10.5	9.5	—	—	0.5	—	0.5	38.5	27.5

IV	[' <i>C. mycetoides</i> ' C63										
	{ ' <i>A. hydrocarboglutamicus</i> ' D87 ' <i>A. paraffineus</i> ' C228† ' <i>A. roseoparaffinus</i> ' C112 <i>A. variabilis</i> D2 <i>Brevibacterium ammoniagenes</i> C80 ' <i>B. butanicum</i> ' C790 ' <i>B. ketoglutamicum</i> ' C260 ' <i>B. paraffinolyticum</i> ' C113 ' <i>B. stericum</i> ' C188 <i>Caseobacter polymorphus</i> C357 C358 ' <i>Corynebacterium alkanum</i> ' D801 <i>C. fascians</i> C39† C104 <i>C. hoagii</i> C204 ' <i>C. hydrocarboclastus</i> ' D8 ' <i>C. minutissimum</i> ' D24 D24B <i>C. paurometabolum</i> C265 ' <i>C. rubrum</i> ' C190† ' <i>Mycobacterium flavum</i> ' C234 ' <i>M. lacticolum</i> ' C237† ' <i>M. rubrum</i> ' C239 <i>Rhodococcus coprophilus</i> N744 <i>R. equi</i> C7 C24 C56 C57 C58 D19 D20 D21 D22 R59 R71 <i>R. rhodochrous</i> N31 }										
	1.0	1.5	23.5	0.5	—	15.5	2.0	—	7.5	48.5	—
	0.5	0.5	40.0	1.0	—	—	—	0.5	2.5	40.0	15.0
	7.0	1.5	38.0	8.5	3.0	1.0	1.0	—	1.5	5.0	32.0
	1.0	0.5	30.0	11.5	—	1.5	4.0	—	1.0	48.0	2.5
	2.0	—	35.0	1.0	—	—	0.5	—	14.0	33.0	14.5
	—	—	19.0	0.5	—	—	—	—	10.0	42.5	28.0
	0.5	0.5	38.5	5.5	—	—	—	0.5	2.5	40.5	11.5
	2.5	2.0	35.5	14.5	0.5	4.0	—	0.5	5.0	28.0	7.5
	5.5	1.0	43.0	7.5	3.0	2.0	—	1.0	1.0	11.0	25.0
	3.0	1.0	38.0	11.0	4.5	1.5	—	—	2.0	10.0	29.0
	1.0	0.5	38.0	0.5	—	—	—	0.5	0.5	48.0	11.0
	1.5	1.0	38.5	0.5	—	—	—	0.5	1.0	40.5	16.5
	3.0	2.0	35.0	10.0	4.0	1.0	—	1.0	2.5	12.0	29.5
	6.0	2.5	34.0	9.0	—	0.5	1.0	—	1.0	22.0	24.0
	6.0	4.0	35.0	7.0	—	0.5	0.5	0.5	0.5	21.0	25.0
	2.5	1.5	30.5	10.5	4.5	3.0	0.5	1.5	1.0	3.5	41.0
	1.5	1.5	35.0	8.0	2.0	2.0	0.5	1.0	1.0	6.5	41.0
	0.5	0.5	40.0	2.0	—	—	—	0.5	2.5	35.5	18.5
	0.5	0.5	41.5	1.5	—	—	—	—	3.5	29.5	23.0
	1.5	—	30.0	10.0	—	0.5	0.5	0.5	4.5	31.5	21.0
	3.0	4.5	39.0	8.0	1.5	1.0	2.5	2.0	—	17.0	21.5
	3.5	0.5	27.0	14.0	0.5	0.5	0.5	—	2.0	28.5	23.0
	2.0	4.0	26.0	11.5	0.5	5.5	12.0	4.0	2.0	21.0	11.0
	2.0	4.0	26.0	22.0	2.5	1.5	2.0	2.5	—	10.0	27.5
	2.5	2.0	23.0	27.5	15.0	—	3.0	2.0	0.5	6.5	18.0
	1.5	1.5	34.0	10.0	3.0	2.0	0.5	1.0	1.5	4.0	41.0
	2.5	1.5	30.5	10.5	4.5	2.5	0.5	2.0	1.0	3.5	41.0
	1.5	1.5	48.5	5.0	3.0	3.0	0.5	0.5	8.0	15.0	13.5
	1.5	1.5	34.0	12.0	2.0	2.0	0.5	1.5	2.0	3.5	39.0
	2.0	2.0	35.0	11.0	4.0	1.5	0.5	1.0	1.5	3.0	38.5
	1.5	1.0	35.0	13.5	5.0	1.0	0.5	1.0	1.5	2.5	37.5
	1.5	1.0	34.5	12.0	5.5	1.5	0.5	1.5	1.0	2.5	38.5
	2.5	2.0	33.0	10.5	3.5	4.5	1.0	2.0	2.0	4.0	35.0
	2.0	2.0	35.0	10.0	4.0	2.0	0.5	1.0	1.5	3.0	39.0
	3.0	1.0	35.5	13.5	8.5	1.5	—	1.5	0.5	4.0	31.0
	1.5	1.0	37.0	10.5	2.0	2.5	—	—	0.5	3.5	41.5
	2.0	1.0	35.0	12.5	2.5	0.5	0.5	1.5	1.0	4.5	39.0

* Abbreviations for fatty acids are illustrated by the following examples: C_{16:0} for the straight-chain saturated hexadecanoic acid; C_{16:1} for the monounsaturated hexadecenoic acid; t_{19:1} for 10-methyloctadecanoic acid.

† Minor components having the chromatographic properties of monounsaturated fatty acid methyl esters were found in *A. paraffineus* (C_{19:1}, 1.5%), *C. bovis* C12 (C_{11:1}, 0.5%; C_{12:1}, 0.5%; C_{13:1}, 1.0%; C_{14:1}, 1.5%; C_{15:1}, 5.5%), *C. bovis* C823 (C_{11:1}, 0.5%; C_{12:1}, 0.5%; C_{13:1}, 1.5%; C_{14:1}, 2.0%; C_{15:1}, 5.5%) and *M. lacticolum* (C_{19:1}, 0.5%).

‡ *C. fascians* and '*C. rubrum*' have been reclassified in the genus *Rhodococcus* (Goodfellow & Alderson, 1977).

'*C. mycetoides*' are also distinctive as they contain side-chains (α -alkyl chains) with odd numbers of carbon atoms (Collins *et al.*, 1982)

In addition to straight-chain and monounsaturated fatty acids, representatives of the genus *Rhodococcus* also possess 10-methyl branched acids (Minnikin *et al.*, 1978). The recovery of major amounts of 10-methyloctadecanoic acid, and smaller homologues, from strains previously labelled *C. equi*, *C. fascians*, *C. hoagii* and '*C. rubrum*' does not contradict the reclassification of these taxa in the genus *Rhodococcus* (Goodfellow & Alderson, 1977; Goodfellow *et al.*, 1982*a, b*). Representatives of the first three species mentioned above form a single DNA homology group (Suzuki *et al.*, 1981) and it has been proposed, on the basis of chemical, numerical phenetic and genetical data, that *C. hoagii* be reduced to a synonym of *R. equi* (Goodfellow *et al.*, 1982*a*). Strains labelled '*Arthrobacter hydrocarboglutamicus*', '*A. paraffineus*', '*Brevibacterium butanicum*', '*B. ketoglutamicum*', '*B. paraffinolyticum*', '*B. sterolicum*', '*Corynebacterium alkanum*', '*C. hydrocarboclastus*', '*Mycobacterium lacticum*' and '*M. rubrum*' also contain substantial amounts of 10-methyloctadecanoic acid (Table 2), have mycolic acids with similar chain lengths to those of most rhodococci (Collins *et al.*, 1982) and should be reclassified in the genus *Rhodococcus*.

'*Arthrobacter roseoparaffinus*' C112 and '*A. variabilis*' D2 also contain major amounts of 10-methyloctadecanoic acid (Table 2), but are in need of further study as they have mycolic acids intermediate in size between those of true corynebacteria and most rhodococci (Collins *et al.*, 1982). Indeed, it must be stressed that a clear distinction cannot be made between *Corynebacterium sensu stricto* and *Rhodococcus* strains solely on the presence or absence of 10-methyl branched acids, as strains of *Brevibacterium ammoniagenes* and '*Corynebacterium minutissimum*', whose mycolic acids appear to be similar to those of corynebacteria (Collins *et al.*, 1982), are similar to *C. bovis* in so far as they contain major amounts of 10-methyloctadecanoic acid (Table 2).

The genus *Caseobacter* was introduced (Crombach, 1978) to accommodate 'grey-white' coryneform bacteria from cheese; the isolates contained *meso*-diaminopimelic acid, arabinose, galactose and mycolic acids in their walls. In the present study the *Caseobacter polymorphus* strains contained major amounts of straight-chain, monounsaturated and 10-methyloctadecanoic acids and could not be distinguished from rhodococci and related strains on this basis. The detailed taxonomic affinities of *Caseobacter*, therefore, remain unresolved, for while caseobacters contain short-chain mycolic acids (about C₃₀ to C₃₆; Collins *et al.*, 1982) similar to those of most true corynebacteria, they have a DNA base composition similar to rhodococci (Crombach, 1978; Suzuki *et al.*, 1981). Further comparative chemotaxonomic and molecular genetical studies are needed to clarify the affinities of *Caseobacter* and other mycolic acid-containing taxa. *Caseobacter polymorphus*, however, has little DNA in common with either *Corynebacterium glutamicum* or *Rhodococcus equi* (Suzuki *et al.*, 1981).

Corynebacterium paurometabolum C265 also contains significant amounts of 10-methyloctadecanoic acid in addition to straight-chain saturated and monounsaturated fatty acids (Table 2). This organism, however, can be clearly distinguished from both true corynebacteria and rhodococci in possessing some long (C₆₈ to C₇₆), highly unsaturated (2 to 6 double bonds) mycolic acids (Collins & Jones, 1982).

It is clear from this and earlier studies (Minnikin *et al.*, 1978, 1979; Collins *et al.*, 1980; Collins & Jones, 1980) that fatty acid analyses provide data of value for both the classification and identification of coryneform and related bacteria. In particular, the presence of predominantly straight-chain and monounsaturated fatty acids in strains of *Corynebacterium sensu stricto* underlines their relationship with *Mycobacterium*, *Nocardia* and *Rhodococcus* and distinguishes them from *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Curtobacterium*, *Microbacterium* and *Oerskovia*, all of which contain major amounts of *iso*- and *anteiso*-methyl branched-chain acids. Further, the discontinuous distribution of 10-methyl branched acids promises to be of value in the classification and identification of mycolic acid-containing bacteria.

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