Fatty Acid Composition of Some Mycolic Acid-containing Coryneform Bacteria

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(Received 19 January 1982; revised 9 April 1982)

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; Keddie & 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 methanalysis 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, 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-SCP 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 (about 50 mg) were degraded by acid methanalysis 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, 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-SCP 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).

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 (C16:0) and hexadecenoic (C16:1) acids with octadecenoic acid (C18:1) present in only small amounts (Table 2). In contrast, the animal-associated corynebacteria (e.g. C. kutscheri, C. pseudopseudopseudopseudomycetica, C. xerosis), saprophytic glutamic acid-producing strains (e.g. Brevibacterium divaricatum, 'B. flavum', 'B. chang-fu', '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 (C16:0) and octadecenoic (C18:1) 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. C11:1, C12:1, C13:1, C14:1, C15:1, C16:1 and C17:1). 'Corynebacterium mycetoides' C63 also formed a distinct pattern as it contained substantial amounts of C17:0 in addition to C16:0 and C18:1 (Table 2). The presence of high proportions of odd-numbered straight-chain acids is unusual, but the mycolic acids of
### Laboratory no. | Strain and source*
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D6 | \textit{Arthrobacter albidus}; NCIB 10266
D87 | \textit{Arthrobacter hydrocarboglutamicus}; ATCC 15583
C228 | \textit{Arthrobacter paraffinis}; NCIB 10699
C112 | \textit{Arthrobacter roseoparaffinis}; NCIB 10700
D2† | \textit{Arthrobacter variabilis}; NCIB 9455
B53† | \textit{Bacterionema matruchotii}; NCIB 10254
B60 | \textit{Bacterionema matruchotii}; Dr G. H. Bowden, Dept of Dental Bacteriology, The London Hospital Dental Institute, London; J1163
C80 | \textit{Brevibacterium ammoniagenes}; NCIB 8143
C790 | \textit{Brevibacterium butanicum}; ATCC 21196
C794 | \textit{Brevibacterium chang-fua}; ATCC 14017
D3 | \textit{Brevibacterium divaricatum}; NCIB 9379
C81 | \textit{Brevibacterium flavum}; NCIB 9565
C123† | \textit{Brevibacterium immariophilum}; NCIB 9544
C260 | \textit{Brevibacterium ketoglutaricum}; DSM 20165
C124 | \textit{Brevibacterium lactofermentum}; NCIB 9567
C113 | \textit{Brevibacterium parafinolyticum}; NCIB 11160
C82 | \textit{Brevibacterium roseum}; NCIB 9654
C127 | \textit{Brevibacterium saccharolyticum}; NCIB 9543
C188 | \textit{Brevibacterium sterolicum}; NCIB 11161
C357†, C358 | \textit{Caseobacter polymorphus}; NCDO 2097, NCDO 2099
C340 | \textit{Corynebacterium acetatocidophilum}; NCIB 9661
D901 | \textit{Corynebacterium alboflavum}; ATCC 21194
C126, C873 | \textit{Corynebacterium hovis}; NCTC 3224, NCDO 1931
C130† | \textit{Corynebacterium callunae}; NCIB 10338
C13, C66, C67 | \textit{Corynebacterium diphtheriae}; NCTC 3985, NCTC 3529, NCTC 10681
C39, C104 | \textit{Corynebacterium fascians}; ATCC 12974, NCDO 1488
D4 | \textit{Corynebacterium flavescenti}; NCIB 8707
C19 | \textit{Corynebacterium glutamicum}; NCIB 10025
C85† | \textit{Corynebacterium hercula}; NCIB 9694
D8† | \textit{Corynebacterium hydrocarboceastus}; Dr K. Komagata, Ajinomoto Co., Kawasaka, Japan; AJ 1386
C20, C59 | \textit{Corynebacterium kutscheri}; NCTC 949; NCTC 1386
C133 | \textit{Corynebacterium lilium}; NCIB 10337
C136 | \textit{Corynebacterium melassecola}; NCIB 10336
C63 | \textit{Corynebacterium miyoyoides}; NCIB 9864
D24†, D24B | \textit{Corynebacterium minutissimum}; NCTC 10288, NCTC 10285
C265† | \textit{Corynebacterium pautmetabolum}; DSM 20162
C73 | \textit{Corynebacterium pseudodiphtheriticum}; Dr D. Jones, Dept of Microbiology, University of Leicester; C10
C150, C152, C153, C155, C215 | \textit{Corynebacterium pseudotuberculosis}; 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† | \textit{Corynebacterium renale}; NCTC 7448
C190† | \textit{Corynebacterium rubrum}; NCIB 9433
C137 | \textit{Corynebacterium xerosis}; NCIB 9956
C234 | \textit{Mycobacterium flavum} subsp. \textit{melaninum}; NCIB 9738
C237 | \textit{Mycobacterium lactis}; NCIB 9740
C239 | \textit{Mycobacterium rubrum} subsp. \textit{propanicum}; NCIB 9741
N174† | \textit{Rhodococcus copephilus}; NCIB 11211
C7†, C24, C56, C57, C58, D19–D22, R59, R71† | \textit{Rhodococcus equi}; NCTC 1621, NCTC 10673 (\textit{Corynebacterium hooagii})
NCTC 5649, NCTC 5650, NCTC 4219, Dr H. R. Carne: UNP 20343, Jeffcott I, 149, 1499, ATCC 25715, ATCC 25729
N31 | \textit{Rhodococcus rhodochrous}; Dr R. E. Gordon, Institute of Microbiology, Rutgers University, New Jersey, U.S.A.; W21


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

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Rhodococcus have been reclassified in the genus 'R. rubrum' and 'C. fascians'.

Minor components having the chromatographic properties of monounsaturated fatty acid methyl esters were found in A. hexadecenoic acid; 7,9:1 for 10-methyloctadecanoic acid.

Abbreviations for fatty acids are illustrated by the following examples: C16:0 for the straight-chain saturated hexadecanoic acid; C16:1 for the monounsaturated hexadecenoic acid; t,10:1 for 10-methylpentadecanoic acid.

† Minor components having the chromatographic properties of monounsaturated fatty acid methyl esters were found in A. paraffinis (C19:1, 1.5%), C. bovis C12 (C11:0, 0.5%; C12:0, 0.5%; C13:0, 1.0%; C14:1, 1.5%; C15:1, 4.0%), C. bovis C823 (C11:0, 0.5%; C12:0, 0.5%; C13:1, 1.5%; C14:1, 2.0%; C15:1, 5.5%) and M. lacticolum (C9:0, 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., 1982a, 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., 1982a) and the discontinuous distribution of 10-methyl branched acids promises to be of value in the classification and identification of mycolic acid-containing bacteria.

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 paurumetabolum 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 (C_68 to C_76), 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, Nocardiа 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.

We are indebted to colleagues who kindly provided cultures (Table 1). This investigation was supported, in part, by MRC grant G974/522/S and one of us (M. D. C.) gratefully acknowledges receipt of a Luccock Scholarship (Medical Scholarships and Research Committee, Faculty of Medicine, University of Newcastle upon Tyne).
REFERENCES


