JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1988, p. 484–492
0095-1137/88/030484-09$02.00/0

Vol. 26, No. 3

Cultural and Chemical Characterization of CDC Groups EO-2, M-5, and M-6, *Moraxella* (*Moraxella*) Species, *Oligella urethralis*, *Acinetobacter* Species, and *Psychrobacter immobilis*

C. WAYNE MOSS,[1]* P. LYNN WALLACE,[1] DANNIE G. HOLLIS,[2] AND ROBERT E. WEAVER[2]

Analytical Chemistry[1] and Special Bacterial Reference[2] Laboratories, Centers for Disease Control, Atlanta, Georgia 30333

Received 14 September 1987/Accepted 16 November 1987

We determined phenotypic characteristics, cellular fatty acid composition, and isoprenoid quinone content of representative strains of CDC groups EO-2, M-5, and M-6, *Moraxella* (*Moraxella*) species, *Oligella urethralis*, *Acinetobacter* species, and *Psychrobacter immobilis*. All organisms contained ubiquinone with eight isoprene units as the major isoprenolog, but distinct differences were observed in fatty acid composition. Twenty-eight of the original collection of CDC group EO-2 strains were further identified as *P. immobilis*, EO-2, or EO-3 by distinctive cellular fatty acid profiles, cellular morphology, and pigment production. The cellular fatty acid compositions of M-5 and M-6 were similar but were clearly different from those of other organisms. The genus *Acinetobacter* was differentiated from other organisms in the study by small amounts of 2-hydroxydodecanoic acid (2-OH-12:0), and *P. immobilis* was differentiated by small amounts of decanoic acid (10:0) and a branched-chain 17-carbon acid (i-17:0). All *Moraxella* species were distinguished by small amounts of decanoic acid (10:0) and the absence of i-17:0. *M. bovis*, *M. nonliquefaciens*, and some strains of *M. lacunata* formed a single fatty acid group, while *M. osloensis*, *M. phenylpyruvica*, *M. atlantae*, and other strains of *M. lacunata* (*M. lacunata* II) had species-specific fatty acid profiles. *O. urethralis* differed from *Moraxella* species by the presence of large amounts (49%) of *cis*-vaccenic acid (18:1 ω7c), small amounts (1%) of 3-hydroxyhexadecanoate (3-OH-16:0), and the absence of 10:0 and 3-hydroxydodecanoate (3-OH-12:0). The combined use of chemical data and a small number of conventional tests permitted rapid identification and differentiation of these organisms from each other and from related organisms.

In recent years, our laboratories have used gas-liquid chromatography (GLC), mass spectrometry, and associated analytical techniques to study the chemical composition and metabolic activity of microorganisms as a basis for their identification and classification. Chemical data such as short-chain acid and amine metabolites, cellular fatty acids, isoprenoid quinones, and sphingolipids have provided valuable information for the recognition of genus and species of various bacteria (6, 7, 15). These chemical data have been particularly useful in recent studies with several unclassified groups of gram-negative aerobic and facultative anaerobic bacteria from clinical specimens (6, 13, 16).

In this report, we extended our studies to Centers for Disease Control (CDC) group EO-2 (EO, eugonic oxidizer), a gram-negative, oxidase-positive, nonmotile coccobacillus which has been isolated from a variety of sources (5). The cellular fatty acids and quinone contents of EO-2 are compared with those of cultural and biochemically related organisms including *Moraxella* (*Moraxella*) species, *Oligella urethralis* (formerly *Moraxella urethralis*; 16), *Acinetobacter* species, *Psychrobacter immobilis*, CDC group M-5, and CDC group M-6.

MATERIALS AND METHODS

Cultures. Cultures used in this study were isolated from a variety of sources (Table 1). The cultures were identified by the Special Bacterial Reference Laboratory, CDC, using conventional cultural and biochemical tests described previously (5).

Transformation assay. The transformation assay was done by the procedure of Juni and Heym (11, 12). In this assay, crude DNA from each test strain was prepared and tested for the ability to transform a single competent auxotrophic strain of *P. immobilis* (ATCC 43117) to prototrophy (12). Results of the transformation analysis were kindly provided by Jane Hudson (8).

Culture conditions. Cells for fatty acid and isoprenoid quinone analysis were obtained by inoculating strains onto heart infusion agar plates supplemented with 5% rabbit blood (HIAB). The plates were incubated at 35°C for 24 h for cellular fatty acids and 48 h for quinone analysis.

Fatty acid analysis. Approximately 0.5 ml of sterile distilled water was added to the surface of one HIAB plate, and the growth was removed by gently scraping. The turbid cell suspension was placed in a screw-cap tube (13 by 100 mm) fitted with a Teflon-lined cap and saponified by heating at 100°C for 30 min after adding 1 ml of 15% NaOH in 50% aqueous methanol. The sample was cooled to ambient temperature, 1.5 ml of 25% hydrochloric acid–methanol reagent was added, and the mixture was heated at 100°C for 15 min. After cooling to room temperature, 1.5 ml of ether-hexane (1:1, vol/vol) was added, and the contents were mixed by shaking. The phases were allowed to separate by standing 1 to 2 min, and the aqueous (lower) layer was carefully removed with a Pasteur pipette and discarded. Then, 1.0 ml of phosphate buffer (pH 11.0) was added, the contents were mixed by shaking, and the phases were allowed to separate by standing 2 to 3 min. About two-thirds of the top (organic) layer containing the fatty acid methyl esters was removed to a septum-capped sample vial for subsequent analysis by automated GLC.

GLC of cellular fatty acids. Fatty acid methyl esters were

* Corresponding author.

TABLE 1. Strains used in this study

Organism and strain[a]	Other strain designation(s)[b]	Source	Sender[b]
EO-2			
F4829		Cerebral spinal fluid	P.R.
D5834		Throat	Md.
E9789		Eye	Mo.
F784		Vagina	Maine
F7648		Sinus	Wash.
E6062		Abdomen	Tex.
E6235		Dog bite	Pa.
E9721		Ear exudate	Mo.
F974		Wound	Mo.
F934		Urine	Iowa
E9070		Foot wound	Va.
E7487(1)		Eye	Tenn.
E9355		Wound, shoulder	Hawaii
E6463		Blood	Ill.
E5629		Pacemaker site	Ariz.
E7655	Now group EO-3	Vagina	Mo.
F4914	Now group EO-3	Eye	N.C.
F6203	Now group EO-3	Stitch abscess	Canada
F1303	Now group EO-3	Forearm	Kans.
F6397	Now group EO-3	Blood	Pa.
Psychrobacter immobilis			
KC1837	ATCC 43116, type strain		ATCC
KC1838	ATCC 43117, auxotroph		ATCC
KC1839	ATCC 15174, *Micrococcus cryophilus*		ATCC
KC1840	ATCC 17955, *Moraxella phenylpyruvica*		ATCC
F9256		Catheter site	Tex.
F8880		Vagina	Colo.
A3584		Eye	Calif.
F7790		Blood	Ga.
A7344		Poultry	Canada
E5252		Finger wound	Ark.
F6202		Cerebral spinal fluid	Canada
A4508		Urethra	Ala.
E5433		Blood	Iowa
A3014(2)		Brain tissue	N.Y.
Moraxella lacunata (profile I)			
KC784	ATCC 17967, neotype strain	Eye	ATCC
KC1376	NCTC 10358, ATCC 17956	Eye, guinea pig	NCTC
F9292		Blood	Wash.
KC749	ATCC 17952, NCTC 7911, biotype liquefaciens		ATCC
KC757	ATCC 17950, NCTC 7985		ATCC
KC758	ATCC 17951, NCTC 7986		ATCC
Moraxella lacunata (profile II)			
KC756	ATCC 11748	Eye	ATCC
E9650		Eye	Colo.
F4037		Eye	N. Mex.
F3963		Eye	N. Mex.
F6509		Eye	Oreg.
F8531		Eye	N. Mex.
F9222		Eye	N. Mex.
F9223		Eye	N. Mex.
F9224		Eye	N. Mex.
F9225		Eye	N. Mex.
F9242		Eye	N. Mex.
F9243		Eye	N. Mex.
F9244		Eye	N. Mex.
Moraxella bovis			
KC746	ATCC 17947, NCTC 8561, ATCC 17973	Cattle	ATCC
KC745	ATCC 10900, neotype	Cattle, pink eye	ATCC
KC4161(1)		Nasal, cow	Wisc.
5562		Eye, cow	Ga.
5563		Eye, cow	Ga.
Moraxella nonliquefaciens			
KC798	4663/82, ATCC 19975, neotype	Nose swab	Henriksen
KC799	7784		Henriksen
KC801	A1526		Henriksen

Continued on following page

TABLE 1—*Continued*

Organism and strain[a]	Other strain designation(s)[b]	Source	Sender[b]
KC802	A1603		Henriksen
F8529		Vocal cord	Pa.
F7670		Exudate, corneal ulcer	Tex.
KC800	A1195		Henriksen
F9466		Nasal swab	N. Mex.
Moraxella osloensis			
A1920	Type strain, ATCC 19976	Cerebral spinal fluid	CDC
E7317		Wound, leg	Pa.
E398		Pilonidal cyst	Md.
KC1279	ARG 13-9, ATCC 19961		Juni
KC1375	NCTC 10749		NCTC
E9997		Blood	N.H.
Moraxella phenylpyruvica			
A1019		Scalp lesion	Calif.
KC1327	CDC 2863, type strain, ATCC 23333	Blood	Ill.
A2163(1)		Cerebral spinal fluid	N.Y.
A1232(1)		Vulva	Pa.
D8326		Blood	Tex.
E3405		Blood	Finland
B7925		Ear	Mich.
B5856		Urine	Canada
D6988		Blood	Pa.
D7423		Urine	Wash.
D7723		Blood	Ariz.
Moraxella atlantae			
8330	ATCC 29528	Spleen	Ga.
A279	ATCC 29524	Blood	Conn.
A1922	ATCC 29526	Blood	Fla.
KC1353	CDC 5118, ATCC 29525, type strain	Blood	Ohio
Oligella urethralis			
E8240		Urine	Col.
KC744	ATCC 17960, CDC 7603, type strain	Ear	Wash.
E8229		Urine	Pa.
KC1290	Lautrop WM 20		Mitchell
E8717		Urine	Wash.
M-5			
B9108		Tongue, dog	Ga.
E7900		Wound, dog bite	Maine
E9924		Wound, hand	N. Mex.
E7557		Wound	Ala.
E8000		Wound, dog bite	Va.
F8888		Wound, dog bite	Calif.
D6680		Dog bite	Fla.
M-6			
E6808		Bronchial wash	Kans.
E7434		Blood	Calif.
E7792		Blood	Hawaii
E6825		Blood	New Zealand
F6169		Blood	Calif.
E8494		Blood	Ohio
F9253		Blood	R.I.
F7615		Blood	Canada
Acinetobacter calcoaceticus[c] KC1127	ATCC 23055, type strain	Soil	ATCC
Acinetobacter baumannii			
KC722	ATCC 17904, NCTC 10303	Urine	ATCC
KC731	ATCC 15151		ATCC
KC738	ATCC 9955	Cerebral spinal fluid	ATCC
KC741	ATCC 17961, CDC 7788	Blood	ATCC
Acinetobacter genospecies 3			
KC726	ATCC 17922, NCIB 9017		ATCC
KC739	ATCC 19004	Cerebral spinal fluid	ATCC
Acinetobacter haemolytica			
KC735	ATCC 19002	Ocular pus	ATCC
KC737	ATCC 19194	Nose	ATCC
Acinetobacter junii KC716	ATCC 17908, type strain	Urine	ATCC

Continued on following page

TABLE 1—*Continued*

Organism and strain[a]	Other strain designation(s)[b]	Source	Sender[b]
Acinetobacter genospecies 6, KC754	ATCC 17979	Throat	ATCC
Acinetobacter johnsonii KC723	ATCC 17923, NCIB 9018		ATCC
Acinetobacter lwoffii			
KC123	NCTC 5866, ATCC 15309, type strain		NCTC
KC725	ATCC 17925, NCIB 9020		ATCC
Acinetobacter genospecies 9, KC743	ATCC 9957	Gangrenous lesion	ATCC
Acinetobacter genospecies 10, KC724	ATCC 17924	Throat	ATCC
Acinetobacter genospecies 11, KC1843	PB 73, CIP 63.46		Bouvet
Acinetobacter genospecies 12, KC1844	PB 76, SEIP 12.81	Urine	Bouvet
Acinetobacter, ungrouped strain close to genospecies 1 and 3, KC728	ATCC 17903, NCTC 8102		ATCC
Acinetobacter, ungrouped, KC727	ATCC 17988	Urine	ATCC

[a] Strain designations are those of the Special Bacterial Reference Laboratory, CDC, Atlanta.

[b] ATCC, American Type Culture Collection, Rockville, Md.; Bouvet, P. Bouvet, Institut Pasteur, Paris, France; CIP, Collection de l'Institut Pasteur, Paris, France; Juni, E. Juni, University of Michigan, Ann Arbor; Henriksen, S. D. Henriksen, University of Oslo, Oslo, Norway; Lautrop, H. Lautrop, Statens Seruminstitut, Copenhagen, Denmark; Mitchell, J. Mitchell, Brook Air Force Base, San Antonio, Tex.; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; SEIP, Service des Enterobacteries de l'Institut Pasteur, Paris, France; other abbreviations, states or territory from which cultures were referred for identification to the Special Bacterial Reference Laboratory, CDC, Atlanta; cultures from Canada, Finland, and New Zealand were received for identification.

[c] The strains of *Acinetobacter* include representative strains of each of the 12 DNA hybridization groups (genospecies) recently described by Bouvet and Grimont (1).

analyzed by GLC with the 5898A-GLC Microbiol Identification System (Hewlett-Packard Inc., Avondale, Pa.). This system includes a gas chromatograph with a flame ionization detector and automatic sample injector with controller, an electronic integrator, and a minicomputer. The gas chromatograph was equipped with a fused silica capillary column (25 m by 0.2 mm [inner diameter]) with cross-linked methylphenyl silicone (SE 54) as the stationary phase. The operating parameters of the instrument that were automatically controlled by the computer software were as follows: injector temperature, 250°C; detector temperature, 300°C; column temperature, programmed from 170 to 300°C at 5°C/min and maintained at 300°C for 1 min before recycle back to 170°C. The fatty acid methyl esters were identified by comparing retention times with those of reference standards (Hewlett-Packard; Supelco, Inc., Bellefonte, Pa.), and this identity was confirmed by trifluoroacetylation, hydrogenation, and mass spectrometry (13). The chromatograms with retention times and peak areas were recorded with the electronic integrator and transferred to the computer for calculation, storage, and the final reports.

Determination of isoprenoid quinones. Cells from five HIAB plates were hydrolyzed by adding 0.2 ml of 50% aqueous KOH and 3 ml of 1% pyrogallol in methanol and heating at 100°C for 10 min. After the mixture cooled to room temperature, 1 ml of saturated NaCl solution and 5 ml of acetone-hexane (1:4, vol/vol) were added and the mixture was vigorously shaken for 5 min on a wrist-action shaker (Burrell Corp., Pittsburgh, Pa.). The phases were allowed to separate by standing or brief centrifugation, and the upper organic layer was removed and placed in a small beaker. The aqueous layer was extracted three additional times, and the combined organic layers were evaporated to dryness under a gentle stream of nitrogen. The extracted quinones were dissolved in 0.5 ml of methanol and examined by reverse-phase high-performance liquid chromatography as described previously (6, 16). Tentative identification was established by retention time comparison with that of authentic standards supplied by Hoffmann-La Roche Co., Basel, Switzerland. Identification was confirmed by collecting fractions from reverse-phase high-performance liquid chromatography followed by analysis by both electron impact and chemical ionization mass spectrometry (6, 16).

RESULTS AND DISCUSSION

Over the past 21 years, the Special Bacterial Reference Laboratory has collected more than 100 strains of an unidentified group of gram-negative bacteria designated EO-2 (5). These organisms were isolated from a variety of human and some nonhuman sources at diverse geographic locations throughout the United States, Puerto Rico, Canada, New Zealand, and Australia (Table 1). This group of eugonic oxidizers (EO) includes aerobic, gram-negative, coccoid to short, thick or slightly thick rods, often appearing vacuolated or peripherally stained (O-shaped), in pairs and short chains or packets, which are strongly oxidase positive, nonmotile, and indole negative, and utilize glucose, xylose, and lactose. In general, these organisms are phenotypically similar to *Acinetobacter calcoaceticus* (formerly "*A. anitratus*," "*Herella vaginicola*") except for oxidase and to *Moraxella* (*Moraxella*) species and CDC groups M-5 and M-6 except for saccharolytic activity (Table 2). In addition, the EO-2 strains have similar characteristics to the type strain of *P. immobilis*, a newly described species of chiefly psychrotropic gram-negative coccobacilli (12). In an attempt to provide additional information for identification of these closely related organisms, representative strains of each species and group were examined for cellular fatty acids and isoprenoid quinones.

The 20 EO-2 strains listed in Table 1 were placed into two distinct groups by cellular fatty acid composition. Fifteen strains were placed into a homogeneous group that retained the designation EO-2; the other five strains formed another group, which we designated EO-3. The quantitative fatty acid data of groups EO-2 and EO-3 as well as *P. immobilis*, *Moraxella* (*Moraxella*) species, *O. urethralis*, and CDC groups M-5 and M-6 are presented in Table 3.

Each of the 15 strains forming group EO-2 (E6062, E6235, E9721, F974, F4829, D5834, E9789, F784, F7648, F934, E9070, E7487(1), E9355, E6463, E5629) was readily characterized by its high content of *cis*-vaccenic acid (18:1 ω7c). The relative amounts of 18:1 ω7c among the 15 strains examined ranged from 65 to 73% with an average of 67%. Each of the 15 strains also contained small amounts (3%) of 3-hydroxydecanoate (3-OH-10:0) and small amounts (3%) of a monounsaturated 12-carbon acid. The presence of these

TABLE 2. Characteristics of EO-2, EO-3, *P. immobilis*, *Acinetobacter* species, *Moraxella* (*Moraxella*) species, *O. urethralis*, M-5, and M-6[a]

Characteristic	*P. immobilis*[b]	EO-2[c]	EO-3[d]	*A. calcoaceticus*[e]	*A. calcoaceticus*[f]	*M. lacunata*	*M. nonliquefaciens*	*M. bovis*	*M. osloensis*	*M. phenylpyruvica*	*M. atlantae*	*Oligella urethralis*	M-5	M-6
No. of strains	13	10	5	501	253	25	243	7	163	50	23	22	59	40
O-shaped cells	−(0)	+(100)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Beta-like hemolysis	−(0)	−(0)	−(0)	−(9)	−(6)	−(0)	−(0)	+(100)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Oxidase	+(100)	+(100)	+(100)	−(0)	−(0)	+(100)	+(100)	+(100)	+(100)	+(100)	+(100)	+(100)	+(100)	+(100)
Growth on MacConkey agar	+(92)	+(+) (60;30)	+(+) (60;40)	+(99;1)	+(90;7)	−(4)	−(8;2)	−(0)	v(70)	v(80;6)	+(+) (87;13)	+(96)	v(42;20)	v(20;28)
Catalase	+(100)	+(100)	+(100)	+(100)	+(100)	+(100)	+(95)	v(14)	+(95)	+(90)	+(91)	+(100)	+(100)	−(8)
Acid from:														
Glucose	v(85)	+(100)	+(100)	+(100)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Xylose	v(85)	+(100)	+(100)	+(99)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Lactose	v(85)	+(90;10)	+(+) (80;20)	+(97;2)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Mannitol	−(0)	−(10)	w+(100)	−(2)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Yellow pigment	−(0)	−(0)	+(100)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Nitrate reduction	v(77)	+(90)	−(0)	−(1)	−(3)	+(100)	+(95)	v(14)	v(24)	v(68)	−(5)	−(0)[g]	−(0)	+(100)[h]
Urea hydrolysis	v(15)	+(+) (40;50)	+(+) (20;80)	v(28;18)	−(5;4)	−(0)	−(0)	−(0)	−(0)	+(100)	−(0)	−(0)	−(0)	−(0)
Gelatin hydrolysis[i]	−(0)	−(0)	−(0)	−(9)	−(4)	v(74)[j]	−(0)	+(100)	−(0)	−(0)	−(0)	−(0)	−(0)	−(0)
Sodium acetate as carbon source	v(75)	NT	NT	NT	NT	−(7)	−(0)	NT	+(100)	v(43)	v(21;21)	v(60)	v(25)	v(83)
Growth at:														
25°C	+(100)	v(89)	+(100)	+(97)	+(100)	v(47)	+(93)	+(100)	+(96)	v(85)	v(68)	v(50)	+(95)	v(78)
35°C	v(23)	+(100)	+(100)	+(97)	+(100)	v(87)	v(88)	+(100)	+(98)	+(100)	+(100)	+(100)	+(100)	+(100)
42°C	−(0)	−(10)	v(20)	v(87)	v(63)	−(0)	v(15)	−(0)	v(51)	v(29)	v(50)	v(59)	v(63)	v(47)

[a] Except for *P. immobilis*, EO-2, and EO-3, data from the Special Bacterial Reference Laboratory, CDC, were previously published (5). Not all strains were tested in every test. Signs and symbols: −, less than 10% positive at 7 days; +, 90% or more positive at 48 h; v, 11 to 89% positive at 48 h; +(+), positives and later positives together total 90% or more; w, weak; NT, not tested; number in parentheses, percent positive; (;), number before semicolon is percent positive at 48 h, and number after semicolon is percent positive at 3 to 7 days.

[b] Phenylethyl alcohol-like odor often detected; grow in NB (0% NaCl) and usually grow in NB with 6% NaCl.

[c] Usually do not grow in NB (0% NaCl) or in NB with 6% NaCl.

[d] Usually grow in NB (0% NaCl) and usually do not grow in NB with 6% NaCl.

[e] Saccharolytic strains.

[f] Nonsaccharolytic strains.

[g] Nitrite reduction with gas formation, 100%.

[h] Nitrite reduction, no gas formation, 100%.

[i] 7 to 14 days of incubation.

[j] Loeffler slant, digestion +100%.

TABLE 3. Cellular fatty acid composition of CDC groups EO-2, M-5, M-6, *Moraxella* (*Moraxella*) species, *O. urethralis*, *Acinetobacter* species, and *P. immobilis*

Organism[a]	Fatty acid[b]									
	10:0	3-OH-10:0	i-11:0	11:0	12:1	12:0	12:1(2)	2-OH-12:0	3-OH-12:0	14:0
EO-2 (15)	–	3	–	–	3	–	–	–	–	–
EO-3 (5)[d]	–	–	–	–	–	–	–	–	–	–
M-5 (7)	–	T	–	–	–	5	–	–	3	5
M-6 (8)	–	–	–	–	–	6	–	–	4	4
O. urethralis (5)	–	–	–	–	–	–	–	–	–	6
P. immobilis (14)	3	–	T	T	–	2	T	–	4	–
M. bovis (5)	2	–	–	–	–	5	1	–	5	–
M. nonliquefaciens (8)	3	–	–	T	–	5	1	–	7	T
M. lacunata I (6)	3	–	–	–	–	5	1	–	5	T
M. lacunata II (13)	2	–	–	–	–	1	1	–	5	1
M. osloensis (6)	9	–	–	–	–	–	1	–	4	T
M. phenylpyruvica (11)	5	–	3	4	–	4	1	–	7	1
M. atlantae (4)	4	–	–	–	–	3	1	–	6	1
Acinetobacter (20)	T	–	–	T	–	6	1	2	4	1

two acids and large amounts of 18:1 ω7c and the absence of a 16:1 acid differentiate these organisms from others listed in Table 3 and many other organisms examined previously in this laboratory (6, 7, 13, 15). In addition to differences in cellular fatty acid composition, none of the 15 group EO-2 strains reacted in the genetic transformation assay for *P. immobilis* (8; Jane Hudson, personal communication). Thus, it appears that these 15 group EO-2 organisms are genetically unrelated to *P. immobilis* even though they share many cultural and biochemical characteristics.

The five strains of group EO-3 (E7655, E4914, F1303, F6203, F6397) were previously found to be unreactive in the *P. immobilis* transformation assay (8). Thus, these five strains are genetically unrelated to *P. immobilis* even though they are similar in morphological and cultural characteristics. The fatty acid compositions of these five strains were clearly different from both *P. immobilis* and EO-2 as well as from the other organisms listed in Table 3. Strains of EO-3, like EO-2, contained large amounts (80%) of 18:1 ω7c, but each strain lacked the 3-OH-10:0 and 12:1 acids that were characteristic of EO-2 organisms. In addition, each EO-3 strain contained small amounts of a 19-carbon cyclopropane acid (19:0 cyc), a 20-carbon monounsaturated acid (20:1), and a 2-hydroxy 18-carbon monounsaturated acid (2-OH-18:1). These three acids were absent or were not detected in more than trace amounts (0.8%) in EO-2, *P. immobilis*, and all other organisms listed in Table 3.

The fatty acid compositions of the type strain of *P. immobilis* (ATCC 43116), as well as the auxotroph of this strain (ATCC 43117), were essentially identical and were characterized by large amounts (58%) of oleic acid (18:1 ω9c), moderate amounts (9%) of a monounsaturated 17-carbon acid (17:1), and small amounts (2%) of an iso-branched-chain 17-carbon acid (i-17:0). This same fatty acid profile was observed in 12 additional *P. immobilis* strains tested: 10 clinical isolates (A3584, F7790, A7344, E5252, F6202, A4508, E5433, A3014, F8880, F9256), ATCC strain 15175 listed as "*Micrococcus cryophilus*," and ATCC strain 17955 listed as *Moraxella phenylpyruvica*. The last two strains were established as psychrobacters in earlier transformation studies (11); DNA from each of the other 10 strains was observed to transform the auxotroph strain of *P. immobilis* to prototrophy (8; Jane Hudson, personal communication). Thus, on the basis of this genetic interaction and their chemical similarity in cellular fatty acid content, these 14 strains were grouped together as *P. immobilis* in Table 3.

The combined data from fatty acid analysis and the transformation assay clearly indicate that the 20 original EO-2 organisms form two distinct groups. Closer examination of cultural and biochemical data revealed two additional characteristics (cellular morphology, yellow nondiffusable pigment) which correlated with grouping by fatty acid data. Each of the 15 strains designated EO-2 by fatty acid data have a distinctive O-shaped cellular morphology (vacuolated or peripherally stained) (Fig. 1). This O-shaped morphology was not observed with the 14 strains of *P. immobilis* or with any of the five EO-3 organisms. The five strains of group EO-3 organisms have a definite yellow nondiffusable pigment which is not observed with either *P. immobilis* or with EO-2 organisms. These key characteristics for distinguishing *P. immobilis*, EO-2, and EO-3 are summarized in Table 4. It should be noted that two asaccharolytic *P. immobilis* reference strains (ATCC 15174 and ATCC 17955) were phenotypically more similar to *Moraxella (Moraxella)* species than to EO-2 or EO-3. However, these two strains were readily identified as *P. immobilis* by cellular fatty acid composition and by the transformation assay. Additional studies are planned with the remaining strains in the CDC collection to determine whether they fit into *P. immobilis*, EO-2, or EO-3 according to the characteristics listed in Table 4.

Like EO-2 and EO-3 organisms, *O. urethralis* was characterized by large amounts of 18:1 ω7c with an average value of 49% and a range of 47 to 51% for the five strains tested (Table 3). However, this acid was not detected in *Moraxella (Moraxella)* species, all of which contained oleic acid (18:1 ω9c) as the major 18-carbon monounsaturated component. *O. urethralis* also contained large amounts (31%) of palmitic acid (16:0) with small amounts of myristic (14:0), 3-hydroxymyristic (3-OH-14:0), palmitoleic (16:1), and stearic (18:0) acids. It was the only organism tested that contained 3-hydroxyhexadecanoate (3-OH-16:0), which was consistently present in each strain at concentrations of 1 to 2%

TABLE 3—*Continued*

Fatty acid[b]													
3-OH-14:0	16:0 alc	16:1 ω7c[c]	16:0	i-17:0	17:1	17:0	3-OH-16:0	18:0 alc	18:2	18:1 ω9c	18:1 ω7c	18:0	20:4
1	–	–	16	–	–	1	–	–	1	2	67	6	–
–	–	T	8	–	T	1	–	–	–	T	80	3	–
T	–	25	25	–	–	T	–	–	4	2	26	2	T
1	–	21	39	–	–	–	–	–	6	4	10	3	T
5	–	3	31	–	–	1	1	–	T	T	49	2	–
T	–	12	2	2	9	T	–	–	3	58	–	2	–
T	2	18	9	–	7	–	–	2	8	37	–	2	T
–	2	19	9	–	5	–	–	6	6	31	–	2	1
T	1	25	8	–	2	–	–	3	7	37	–	2	1
1	6	1	27	–	–	1	–	8	14	10	–	20	2
2	–	6	9	–	T	T	–	T	5	52	–	10	T
–	–	11	12	–	T	T	–	–	22	21	–	5	2
1	–	T	22	–	–	1	–	1	23	21	–	11	2
1	–	19	18	T	4	3	–	–	2	34	2	1	–

[a] Number in parenthesis is numbers of strains examined.

[b] Number before the colon is the number of carbon atoms and number after the colon is the number of double bonds; 2-OH and 3-OH indicate a hydroxyl group at the 2- and 3-carbon, respectively; i indicates a branched-chain acid with the branched methyl group at the iso position; alc indicates a primary alcohol group. Values are percentages of total fatty acids and are arithmetic means; T, trace (less than 0.7%); –, not detected.

[c] Represents the total of 16:1 ω7c and small amounts (0 to 2%) of other 16:1 isomers.

[d] EO-3 organisms contained about 4% lactobacillic acid (19:0 cyc) and 1% each of 2-OH-18:1 and 20:1.

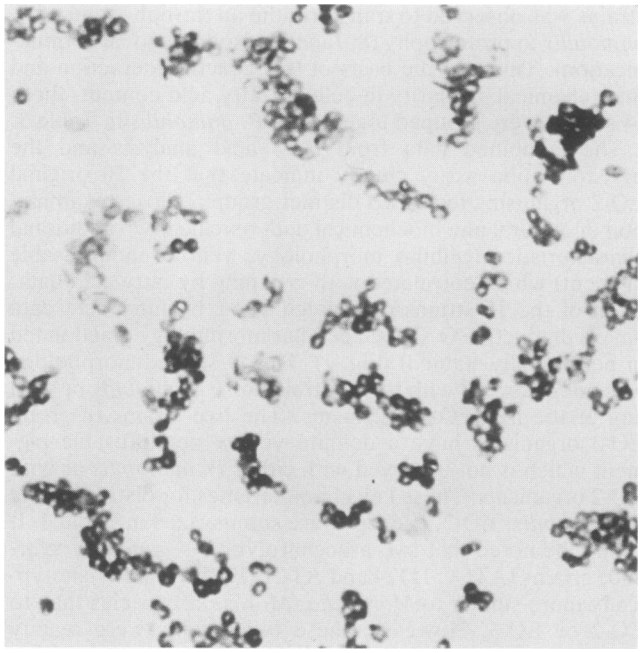

FIG. 1. Gram stain preparation of group EO-2 (strain F4829) after 24 h of incubation at 35°C on heart infusion agar with 5% rabbit blood. Note the peripherally stained O-shaped cellular morphology. Magnification, ×1,700.

(Table 3). The overall fatty acid profile of *O. urethralis* is essentially identical to that of *Oligella ureolytica* (formerly CDC group IVe; 7) the second species of this newly proposed genus (18). Thus, both species of *Oligella* are readily differentiated from *Moraxella* (*Moraxella*) species by the presence of 18:1 ω7c, the absence of decanoic acid (10:0), and larger amounts of myristic acid (14:0) (Table 3) (7).

CDC groups M-5 and M-6 also contained 18:1 ω7c, but the relative amounts of this acid were only approximately 20 to 30% of that found in *O. urethralis*, EO-2, and EO-3 (Table 3). Overall, groups M-5 and M-6 contained the same fatty acids, but they differed in the relative amounts of 16:1, 16:0, and 18:1 ω7c acids. M-5 organisms contained approximately equal amounts (25%) of 16:1, 16:0, and 18:1 ω7c, whereas group M-6 contained about twice as much 16:0 as 16:1 acids (39 versus 21%, respectively) and only 10% of 18:1 ω7c. Although these quantitative differences were consistent for the strains examined, groups M-5 and M-6 should be considered as having the same fatty acid profile until additional strains of each group are tested to confirm these initial findings. Thus, even though groups M-5 and M-6 are readily distinguished from other organisms in Table 3 by cellular fatty acids, clear differentiation between the two groups requires conventional biochemical tests such as catalase and reduction of nitrate (5).

TABLE 4. Characteristics for distinguishing *P. immobilis*, EO-2, and EO-3

Test	*P. immobilis*	EO-2	EO-3
O-shaped cells	−	+	−
Yellow pigment	−	−	+
P. immobilis transformation	+	−	−
Characteristic fatty acids	18:1 ω9c, 17:1, i-17:0	18:1 ω7c, 12:1, 3-OH-10:0	18:1 ω7c, 19:0 cyc

The characteristic features of all *Moraxella* species were the presence of small amounts of 10:0, moderate to large amounts of 18:1 ω9c, and the absence of 18:1 ω7c (Table 3). Two different fatty acid profiles were observed for strains of *M. lacunata* and these were designated *M. lacunata* I and *M. lacunata* II. *M. lacunata* I (KC784 [type strain], KC1376, F9292, KC749, KC757, KC758), *M. bovis*, and *M. nonliquefaciens* were grouped together on the basis of essentially identical fatty acid composition. The presence of small amounts of 17:1 distinguished this group from all other organisms except *P. immobilis* and *Acinetobacter* species. This group also contained small amounts of a diunsaturated 18-carbon acid (18:2), a 12-carbon monounsaturated acid [12:1(2)] and small amounts of *n*-hexadecanol (16:0 alc) and *n*-octadecanol (18:0 alc). These two primary alcohols were identified by their identical retention time match both as free alcohols and as trifluoroacetyl derivatives when compared with authentic alcohol standards. The two alcohols were not detected in *P. immobilis*, which also differed from this group by the presence of a branched-chain 17-carbon acid (i-17:0) and significantly more 18:1 ω9c (58 versus 37%; Table 3). Although the fatty acid composition is useful for rapid grouping of *M. lacunata* I, *M. bovis*, and *M. nonliquefaciens*, their further differentiation requires conventional microbiological tests such as hemolysis and digestion of Loeffler blood serum medium (5).

The 13 strains designated *M. lacunata* II (KC756, E9650, F4037, F3963, F6509, F8531, F9222, F9223, F9224, F9225, F9242, F9243, F9244) differed from *M. lacunata* I by the absence of 17:1, lower amounts of 16:1 and 18:1 ω9c, higher amounts of 18:0, and higher amounts of the two primary alcohols (16:0 alc, 18:0 alc; Table 3). *M. lacunata* II was most similar to *M. atlantae* but could be distinguished from this organism by the presence of approximately equal amounts of 16:0 and 18:0, smaller amounts of 18:1 ω9c, and larger amounts of 18:0 alc.

The distinguishing features of the fatty acid profile of *M. osloensis* were large amounts (52%) of 18:1 ω9c (range, 46 to 61% for six strains), about equal amounts (9%) of 10:0, 16:0, and 18:0, and the absence of 12:0 (Table 3). Large amounts of 18:1 ω9c were also observed in *P. immobilis*, but this organism contained three acids (12:0, 17:1, i-17:0) that were not detected in *M. osloensis*.

The presence of 18:2 as a major fatty acid in *M. phenylpyruvica* and *M. atlantae* distinguished these two species from other organisms in Table 3. The relative amounts of 18:2 among the four strains of *M. atlantae* ranged from 20 to 25% with an average of 22%, and for the 11 strains of *M. phenylpyruvica*, the range was 18 to 28% with an average of 22%. In addition to the 18:2 acid, *M. phenylpyruvica* was further distinguished from other organisms in Table 3 by the presence of small amounts of two 11-carbon acids (i-11:0, 11:0). *M. phenylpyruvica* contained equal amounts of 16:1 and 16:0, whereas only trace amounts of 16:1 were present in *M. atlantae*.

The *Acinetobacter* strains examined for cellular fatty acids were selected to include representative strains of each of the 12 DNA hybridization groups (genospecies) of this genus as described recently by Bouvet and Grimont (1). The 20 cultures listed in Table 1 include one or more strains of *Acinetobacter calcoaceticus*, *Acinetobacter lwoffii*, and the four proposed new species, *Acinetobacter baumannii*, *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, and *Acinetobacter junii* (1). No differences in fatty acid composition were observed among these *Acinetobacter* species or DNA groups as the 20 strains had essentially identical fatty acid

profiles. The major fatty acids in each strain were 18:1 ω9c, 16:1 ω7c, and 16:0 followed by small amounts of 12:0, 12:1(2), 3-OH-12:0, 17:1, 17:0, 18:2, 18:1 ω7c, and 18:0 (Table 3). Each strain, except *A. lwoffii* KC725 (type strain), also contained a small amount (2%) of 2-hydroxydodecanoic acid (2-OH-12:0) which distinguished *Acinetobacter* from all other organisms listed in Table 3. Although 2-OH-12:0 was present as a minor component, it was consistently present in repeat analysis of the *Acinetobacter* strains processed under both acid and base hydrolysis and generally absent or present in only trace amounts in other organisms listed in Table 3.

The presence of small amounts of decanoic acid (10:0) in all *Moraxella* (*Moraxella*) species distinguished this genus from *Acinetobacter*, which generally contained only trace amounts of this acid. Strains of *Acinetobacter* also contained 17:1 and 17:0 acids as well as 18:1 ω9c and 18:1 ω7c, whereas 18:1 ω7c was absent in all *Moraxella* (*Moraxella*) species and 17:0 was not present in the three *Moraxella* (*Moraxella*) species that contained 17:1 (Table 3). Although *A. lwoffii* KC725 did not contain 2-OH-12:0, the other characteristic acids of *Acinetobacter*, including 12:1(2), 17:1, 17:0, 18:1 ω9c, and 18:1 ω7c, were present in this strain. The value of 2-OH-12:0 as a marker fatty acid to distinguish *Acinetobacter* species from *Moraxella* (*Moraxella*) species and *Neisseria* species has been noted previously (10).

Overall, our fatty acid results with *Moraxella* (*Moraxella*) and *Acinetobacter* species are in general agreement with the earlier studies of Jantzen et al. (9, 10) and Nishimura et al. (17), and Bøvre et al. (2). A major difference is our finding of decanoate (10:0) in *Moraxella* (*Moraxella*) species which was not reported in these earlier studies. In addition, our use of a fused silica capillary column permitted complete resolution and accurate quantitation of the two monounsaturated 18-carbon isomers (18:1 ω9c, 18:1 ω7c) which were not resolved with the packed column used by the earlier workers. The ability to resolve these isomers provides a clear means of differentiating unnamed CDC groups EO-2, EO-3, M-5, and M-6 from *P. immobilis*, *Acinetobacter* species, *Moraxella* (*Moraxella*) species, and *O. urethralis*. Moreover, the resolution and identification of 18:1 ω7c as the major acid of *O. urethralis* provides an important differential marker for this organism, since *Moraxella* (*Moraxella*) species lack this acid. Thus, *O. urethralis* differs from *Moraxella* (*Moraxella*) species not only by the presence of 3-OH-16:0 and the absence of 3-hydroxydodecanoic acid (3-OH-12:0) as reported for a single strain in the earlier study (9) and confirmed with five strains in the present report, but also by the absence of 10:0 and large amounts of 18:1 ω7c (Table 3). These fatty acid data and those from earlier studies (7, 18) support genetic studies which show little or no genetic affinity between *Oligella* species and the *Moraxella* (*Moraxella*) species (3, 18).

The potential importance of *n*-octadecanol (18:0 alc) as a marker to distinguish *M. atlantae* from *M. phenylpyruvica* was noted earlier by Bøvre et al. (2), who reported trace amounts (0.5 to 0.7%) of 18:0 alc in each of five strains of *M. atlantae* but not in 11 strains of *M. phenylpyruvica*. We confirmed the presence of 18:0 alc in *M. atlantae*; however, our data indicate that i-11:0 and 11:0 in *M. phenylpyruvica* at about 3% concentrations are more reliable markers for distinguishing these two species than are the trace amounts of 18:0 alc detected in the present and earlier study (2). Our finding of i-11:0, 11:0, and 10:0, which were not reported in earlier studies (2, 9, 10), is most likely due to our use of a capillary column operated under optimum chromatographic conditions to resolve these early eluting acids. The capillary column was also useful for clear resolution of *n*-hexadecanol (16:0 alc), which we also identified in some *Moraxella* (*Moraxella*) species. The general occurrence of both 16:0 alc and 18:0 alc together in the same strain is not unexpected, since they are chemical homologs which are thought to originate from wax esters (4).

The isoprenoid quinone contents of *P. immobilis*, *Moraxella* (*Moraxella*) species, *O. urethralis*, *Acinetobacter* species, and CDC groups EO-2, EO-3, M-5, and M-6 were essentially identical. Representative strains of each species or group contained ubiquinones (Q) with eight isoprene units (Q-8) as their major isoprenologs. Some strains also contained small to trace amounts of Q-6 and Q-7 as well as hydrogenated Q-7 and Q-8. The identity of these components was firmly established by mass spectrometry which showed a base peak at *m/e* 235, derived from the benzoquinone nucleus, with intense peaks corresponding to the molecular ions. The molecular ions were verified by chemical ionization spectra which gave intense m + 1 ions at the expected mass values (i.e., M + 1 = 727 for Q-8).

In summary, our data indicate that cellular fatty acids provide valuable information for rapid differentiation of *Moraxella* (*Moraxella*) species from *Acinetobacter species and Moraxella* (*Moraxella*) species from *P. immobilis*, *O. urethralis*, and CDC groups EO-2, EO-3, M-5, and M-6. *M. bovis*, M. nonliquefaciens, and some strains of *M. lacunata* form a single homogeneous fatty acid group, while *M. osloensis*, *M. phenylpyruvica*, *M. atlantae*, and strains of *M. lacunata* (*M. lacunata* II) have species-specific fatty acid profiles. The strains originally classified as CDC group EO-2 were further identified as *P. immobilis*, EO-2, and EO-3 by distinctive cellular fatty acid profiles, cellular morphology, and pigment production. The GLC procedure for cellular fatty acids is simple, accurate, and reproducible. Use of the Hewlett-Packard 5898A-GLC Microbiol Identification System decreases the experience required for instrument operation and interpretation of data. Cellular fatty acid results, in combination with selected conventional microbiological tests, provide a rapid and reliable means for identifying the organisms described in this study as well as many others encountered in the clinical laboratory (6, 13, 14, 16).

ACKNOWLEDGMENTS

We thank Jane Hudson for transformation data, Sally Dees for preliminary studies, and Ellen Lamb for secretarial assistance.

LITERATURE CITED

1. **Bouvet, P. J. M., and P. A. D. Grimont.** 1986. Taxonomy of the genus *Acinetobacter* with the recognization of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov., and emended description of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. Int. J. Syst. Bacteriol. **36**:228–240.
2. **Bøvre, K., J. E. Fuglesang, N. Hagen, E. Jantzen, and L. O. Froholm.** 1976. *Moraxella atlantae* sp. nov. and its distinction from *Moraxella phenylpyruvica*. Int. J. Syst. Bacteriol. **26**:511–521.
3. **Bøvre, K., and N. Hagen.** 1981. The family *Neisseriaceae*; rod-shaped species of the genera *Moraxella*, *Acinetobacter*, *Kingella*, and *Neisseria*, and the *Branhamella* groups of cocci, p. 1506–1529 *In* M. P. Starr, H. Stolp, H. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes: a handbook on habitats, isolation, and identification of bacteria. Springer-Verlag, KG, Berlin.
4. **Bryan, K., and E. Jantzen.** 1977. Occurrence and patterns of waxes in Neisseriaceae. J. Gen. Microbiol. **102**:33–43.

5. **Clark, W. A., D. G. Hollis, R. E. Weaver, and P. Riley.** 1984. Identification of unusual pathogenic gram negative aerobic and facultative anaerobic bacteria. Centers for Disease Control, Atlanta.
6. **Dees, S. B., C. W. Moss, D. G. Hollis, and R. E. Weaver.** 1986. Chemical characterization of *Flavobacterium odoratum*, *Flavobacterium breve*, and *Flavobacterium*-like groups IIe, IIh, and IIf. J. Clin. Microbiol. **23**:267–273.
7. **Dees, S., S. Thanabalasundrum, C. W. Moss, D. G. Hollis, and R. E. Weaver.** 1980. Cellular fatty acid composition of group IVe, a nonsaccharolytic organism from clinical sources. J. Clin. Microbiol. **11**:664–668.
8. **Hudson, M. J., D. G. Hollis, R. E. Weaver, and C. G. Galvis.** 1987. Relationship of CDC group EO-2 and *Psychrobacter immobilis*. J. Clin. Microbiol. **25**:1907–1910.
9. **Jantzen, E., K. Bryn, T. Bergan, and K. Bøvre.** 1974. Gas chromatography of bacterial whole cell methanolysates. V. Fatty acid composition of neisseriae and moraxellae. Acta. Pathol. Microbiol. Scand. Sect. B **82**:767–779.
10. **Jantzen, E., K. Bryn, T. Bergan, and K. Bøvre.** 1975. Gas chromatography of bacterial whole cell methanolysates. VII. Fatty acid composition of *Acinetobacter* in relation to the taxonomy of neisseriaceae. Acta. Pathol. Microbiol. Scand. Sect. B **83**:569–580.
11. **Juni, E., and G. A. Heym.** 1980. Transformation assay for identification of psychrotropic achromobacters. Appl. Environ. Microbiol. **40**:1106–1114.
12. **Juni, E., and G. A. Heym.** 1986. *Psychrobacter immobilis* gen. nov., sp. nov.: genospecies composed of gram-negative, aerobic, oxidase-positive coccobacilli. Int. J. Syst. Bacteriol. **36**: 388–391.
13. **Lambert, M. A., C. M. Patton, T. J. Barrett, and C. W. Moss.** 1987. Differentiation of *Campylobacter* and *Campylobacter*-like organisms by cellular fatty acid composition. J. Clin. Microbiol. **25**:706–713.
14. **Moss, C. W.** 1981. Gas-liquid chromatography as an analytical tool in microbiology. J. Chromatogr. **203**:337–347.
15. **Moss, C. W., and S. B. Dees.** 1976. Cellular fatty acids and metabolic products of *Pseudomonas* species obtained from clinical specimens. J. Clin. Microbiol. **4**:492–502.
16. **Moss, C. W., A. Kai, M. A. Lambert, and C. M. Patton.** 1984. Isoprenoid quinone content and cellular fatty acid composition of *Campylobacter* species. J. Clin. Microbiol. **19**:772–776.
17. **Nishimura, Y., H. Yamamoto, and H. Iizuka.** 1979. Taxonomic studies of *Acinetobacter* species—cellular fatty acid composition. Z. Allg. Mikrobiol. **19**:307–308.
18. **Rossau, R., K. Kersters, E. Falsen, E. Jantzen, P. Segers, A. Union, L. Nehls, and J. De Ley.** 1987. *Oligella*, a new genus including *Oligella urethralis* comb. nov. (formerly *Moraxella urethralis*) and *Oligella ureolytica* sp. nov. (formerly CDC group IVe): relationship to *Taylorella equigenitalis* and related taxa. Int. J. Syst. Bacteriol. **37**:198–210.