Cellular Fatty Acid Composition Comparisons of *Haemophilus equigenitalis* and *Moraxella* Species

C. SUGIMOTO,* E. MIYAGAWA, M. NAKAZAWA, K. MITANI, AND Y. ISAYAMA

First Research Division, National Institute of Animal Health, Yatabe, Tsukuba, Ibaraki 305, Japan

The cellular fatty acid compositions of *Haemophilus equigenitalis* and some other species were compared. The cellular fatty acid composition of *H. equigenitalis* was very similar to the cellular fatty acid compositions of *Moraxella* species. When the double bond positions in monounsaturated fatty acids were determined by a gas chromatography-mass spectrometry analysis of ditrimethylsilyloxy derivatives, *Moraxella* species were divided into two groups, the oleic acid group and the *cis*-vaccenic acid group. The former included eight species (*Moraxella atlantae*, *Moraxella bovis*, *Moraxella caviae*, *Moraxella equi*, *Moraxella lacunata*, *Moraxella nonliquefaciens*, *Moraxella osloensis*, and *Moraxella phenylpyruvica*), and the latter included *Moraxella catarrhalis*, *Moraxella ovis*, and *Moraxella urethralis*. *H. equigenitalis* was closely related to the *cis*-vaccenic acid group.

The taxonomic position of *Haemophilus equigenitalis*, the causative agent of contagious equine metritis, is still unclear because this organism does not require X- or V-factors (7, 29, 31). Recently, we studied the cellular fatty acid composition of *H. equigenitalis* and found that it is characterized by large amounts of 18:1 and 16:0 acids (30). The cellular fatty acid composition of this species is grossly similar to the cellular fatty acid compositions of *Moraxella* species (5, 9, 14, 15, 18, 27). In addition, *H. equigenitalis* is catalase and oxidase positive, asaccharolytic, and very unreactive in the conventional biochemical tests (31) and thus is phenotypically similar to species of the genus *Moraxella* (12). However, guanine-plus-cytosine content of deoxyribonucleic acid (DNA) from this organism (36.1 mol% [31]) is distinctly lower than the range of values found in the genus *Moraxella* (4). In this study, the cellular fatty acid composition of *H. equigenitalis* was compared with the cellular fatty acid compositions of *Moraxella* species and species of other related genera to clarify possible relationships among these species.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The strains used in this study are listed in Table 1. The bacterial cells were cultivated on Eugon agar supplemented with 5% Fildes pepsin-digested sheep blood for 72 h under microaerophilic conditions. The cells were freeze-dried after they were washed three times with saline.

Extraction and methylation of fatty acids. The dried cells were treated with 5% HCl in methanol at 105°C for 3 h, and fatty acid methyl esters were extracted

with hexane as previously described (21).

Determination of double bond positions in monounsaturated fatty acids. The monounsaturated fatty acid methyl esters in each mixture were converted to the corresponding dihydroxy fatty acids by oxidation with OsO₄, as described by Capella and Zorzut (6). These dihydroxy fatty acids were subsequently converted to the corresponding ditrimethylsilyloxy ethers by reaction with hexamethyldisilazane and trimethylchlorosilane in pyridine (TMS-HT kit; Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). The ditrimethylsilyloxy ethers of fatty acid methyl esters were extracted with chloroform and analyzed by gas chromatography-mass spectrometry (GC-MS). Double bond positions were determined by using *H. equigenitalis* NCTC 11184 and three *Moraxella* species (*Moraxella bovis*, *Moraxella osloensis*, and *Moraxella urethralis*).

GLC and GC-MS. Fatty acid methyl esters and the ditrimethylsilyloxy ethers of monounsaturated fatty acid methyl esters were analyzed by gas-liquid chromatography (GLC) and GC-MS as previously described (22). GLC was performed with a Hewlett-Packard model 5710A gas chromatograph equipped with a flexible fused-silica capillary column (0.28 mm by 50 m) coated with OV-101. GC-MS was performed by using a gas chromatograph-mass spectrometer-computer system (model 5992B; Hewlett-Packard) equipped with a glass capillary column (0.28 mm by 30 m) coated with OV-101. Fatty acid methyl esters were analyzed at an oven temperature of 180°C, and the ditrimethylsilyloxy ethers were analyzed at 200°C.

Identification of fatty acids. Fatty acids were identified primarily by equivalent chain length (ECL) calculations, using a standard mixture of fatty acid methyl esters, as previously described (22). The methyl esters of oleic acid (Δ^9 18:1 acid) and *cis*-vaccenic acid (Δ^{11} 18:1 acid) were also used as monounsaturated fatty acid standards. All structural determinations of fatty acids were verified by GC-MS.

Numerical analysis. The similarities among the strains tested based on cellular fatty acid composition

TABLE 1. Strains used in this study

Serial no.	Received as:	Source ^a
1	<i>M. atlantae</i> ATCC 29525 ^{Tb}	1
2	<i>M. bovis</i> ATCC 10900 ^T	1
3	<i>M. catarrhalis</i> ATCC 25238 ^T	1
4	<i>M. caviae</i> ATCC 14659 ^T	1
5	<i>M. equi</i> ATCC 25576 ^T	1
6	<i>M. lacunata</i> ATCC 17967 ^T	1
7	<i>M. nonliquefaciens</i> ATCC 19975 ^T	1
8	<i>M. osloensis</i> ATCC 19976 ^T	1
9	<i>M. ovis</i> ATCC 33078 ^T	1
10	<i>M. phenylpyruvica</i> ATCC 23333 ^T	1
11	<i>M. urethralis</i> ATCC 17960 ^T	1
12	<i>M. anatipestifer</i> ATCC 11845 ^T	1
13	<i>M. anatipestifer</i> NIH 80032	2
14	<i>L. pneumophila</i> ATCC 33154	1
15	<i>L. pneumophila</i> ATCC 33156	1
16	<i>K. kingae</i> ATCC 23330 ^T	1
17	<i>H. equigenitalis</i> NCTC 11184	3
18	<i>H. equigenitalis</i> Kentucky-188	4
19	<i>H. equigenitalis</i> EQ 51	5
20	<i>H. equigenitalis</i> EQ 56	5
21	<i>H. equigenitalis</i> EQ 59	5
22	<i>H. equigenitalis</i> EQ 64	5
23	<i>H. equigenitalis</i> EQ 70	5
24	<i>H. equigenitalis</i> EQ CHIBA	5

^a Sources: 1, American Type Culture Collection, Rockville, Md.; 2, R. Sakazaki, National Institute of Health, Tokyo, Japan; 3, National Collection of Type Cultures, London, England; 4, Y. Akiyama, Tochigi Branch Laboratory, Equine Health Laboratory, Tochigi, Japan (originally supplied by J. T. Bryans, College of Agriculture, University of Kentucky, Lexington); 5, our collection.

^b Type strain.

were calculated by using the formula of Bergan (2), as previously described (21).

RESULTS

The cellular fatty acid compositions of *Moraxella*, *Kingella*, and *Legionella* species and *H. equigenitalis* NCTC 11184 are shown in Table 2. Species belonging to the genus *Moraxella* generally contained large amounts of 16:0 and 18:1 acids, and the fatty acid compositions of these species were grossly similar to the fatty acid composition of *H. equigenitalis* (30). *Kingella kingae* contained 14:0, 16:1, and 16:0 acids as the major fatty acids and produced a fatty acid pattern that was different from the patterns of *Moraxella* species and *H. equigenitalis*. *Moraxella anatipestifer* and *Legionella pneumophila* contained large amounts of branched-chain fatty acids, especially *iso*-15:0 acid, and produced fatty acid patterns that were distinctly different from those of the other species tested.

The ECL values of the 18:1 acid indicated that positional isomers might exist. Because the methyl esters of positional isomers produce very

similar mass spectra, the positions of double bonds could not be determined directly from the spectra. To determine the double bond positions, monounsaturated fatty acid methyl esters were converted to the corresponding ditrimethylsilyloxy derivatives and then analyzed by GC-MS. GLC patterns of fatty acid methyl esters of *H. equigenitalis* before and after derivation are shown in Fig. 1 as examples. After derivation, the peak of the 16:1 acid completely disappeared, whereas a reduced amount of the 18:1 acid was still detected. Two new peaks, which appeared at retention times of about 17.5 and 33 min, were thought to correspond to the ditrimethylsilyloxy derivatives of 16:1 and 18:1 acids, respectively. The mass spectra of the ditrimethylsilyloxy derivatives of monounsaturated fatty acid methyl esters detected in *H. equigenitalis* and *M. bovis*, are shown in Fig. 2. These spectra show a characteristic cleavage between two trimethylsilyloxy groups, resulting in very simple mass spectra that are dominated by the two cleavage fragments, as shown in Fig. 3 (6). The peak at *m/e* 73 was due to the trimethylsilyl ion. *H. equigenitalis* and *M. urethralis* contained Δ 11 18:1 acid and palmitoleic acid (Δ 9 16:1 acid), and *H. equigenitalis* also contained a trace of Δ 9 18:1 acid. *M. bovis* and *M. osloensis* contained Δ 9 18:1 and Δ 9 16:1 acids, and Δ 9 17:1 acid was detected in *M. bovis*. The double bond positions of 18:1 acids detected in the other species, which were not included in our determination of double bond positions by trimethylsilyloxy derivation, were easily deduced from their ECL values. The ECL value of Δ 11 18:1 acid was 17.77 ± 0.01 , and that of Δ 9 18:1 acid was 17.71 ± 0.01 .

After we calculated the correlation coefficient for all of the strains, we constructed a dendrogram based on cellular fatty acid composition (Fig. 4). *Moraxella* species formed two clusters. The first cluster included *Moraxella catarrhalis*, *Moraxella ovis*, and *M. urethralis*, which contained *cis*-vaccenic acid as a major fatty acid. The strains of *H. equigenitalis* were very similar to each other and were closely related to the *cis*-vaccenic acid group of *Moraxella* species. The other *Moraxella* species (*Moraxella atlantae*, *M. bovis*, *Moraxella caviae*, *Moraxella equi*, *Moraxella lacunata*, *Moraxella nonliquefaciens*, *M. osloensis*, and *Moraxella phenylpyruvica*) formed the oleic acid group. *M. anatipestifer* and *L. pneumophila* were separated from the two clusters of *Moraxella* species. *K. kingae* could be distinguished from the *Moraxella* species by cellular fatty acid composition.

DISCUSSION

We previously studied the cellular fatty acid composition of *H. equigenitalis* (30) and found

TABLE 2. Cellular fatty acid compositions of the strains tested

ECL	Fatty acid ^a	% of total acids in strain:																
		1 ^b	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
10.01	10:0																	
11.00	11:0							2.0	4.5		1.1							
11.98	12:0	1.8	2.7	4.4	7.8	2.6	2.2	3.8			1.1							
12.64	i-13:0										1.5							
13.03	13:0	2.0	3.9	5.0	5.8	3.3	2.7	2.0	2.0	11.2	4.2		11.9	0.6			3.7	
13.44	3OH 12:0	2.0											1.1	10.0				
13.64	i-14:0							0.6							1.9	1.3	1.4	1.7
13.76	14:1		0.7		1.1	1.1								1.6	0.6	0.6	1.4	
13.98	14:0	2.1			1.5		0.9	0.5		0.8	0.7	4.2	55.3	41.8	41.6	37.6	28.7	
14.65	i-15:0													0.9	1.6	0.8		
15.00	15:0														0.5			
15.19	?	2.9											1.1		8.5	6.3		
15.36	i-16:1																	
15.44	3OH 14:0	5.5	0.7		2.6	0.5	0.7		0.5	2.7		2.9					0.7	3.6
15.61	16:2		4.9			0.8	0.8	1.7			0.8				14.1	20.0		
15.66	i-16:0														5.7	5.3		
15.72	?															0.6	14.5	1.7
15.76	16:1	3.1	27.7	10.4	3.1	29.5	5.1	37.0	7.7	8.2	8.6	2.6	0.9	3.1		0.6	28.0	1.7
16.00	16:0	22.9	11.7	4.2	13.5	5.2	14.0	8.3	6.4	8.0	5.7	25.3	2.8	6.3	3.2	4.2		29.4
16.13	?	2.1											6.4	5.2				
16.19	?						1.7											
16.33	i-17:1														1.5	1.4		
16.41	at-17:1														1.2	1.0		
16.64	i-17:0														8.9	11.1		
16.71	17:1	0.8		4.8		0.6	1.2	1.1			1.9	0.7		0.5	5.2	3.5		
17.42	3OH 16:0										0.5							
17.58	18:2	5.1	6.1			1.7	5.4	4.2	0.7		20.8		2.2	2.5	0.7	0.7	1.3	0.8
17.71	Δ ⁹ 18:1	38.8	32.3			48.6	41.9	30.1	61.9		39.7		3.9	16.8	1.3	1.9	2.5	
17.77	Δ ¹¹ 18:1							1.2		59.0		59.3	0.6				9.9	55.3
17.99	18:0	7.2	2.8	2.1	22.4	2.0	11.0	2.2	13.0	6.1	2.8	1.6	0.6	1.3			4.4	4.0
18.13	?									0.6			9.1	4.3				
18.51	?			0.5								0.5			0.7	0.5		
18.65	?			0.8														
18.93	?																	
19.14	3OH 18:1		0.6			1.6		0.6			4.2	0.5	0.7	1.0			0.5	1.6
19.57	?						6.6											
19.62	?						1.8											
19.69	?						0.5											
19.73	20:1										0.8						0.5	
19.96	20:0								0.5		0.5							

^a The number to the left of the colon indicates the number of carbon atoms; the number to the right of the colon indicates the number of double bonds; ai and i indicate a methyl branch at the anteiso and iso carbon atoms, respectively; 3OH indicates the position of the hydroxy group; Δ indicates the position of a double bond. Fatty acids that accounted for less than 0.5% of the total are not shown.

^b Serial number (see Table 1).

FIG. 1. Gas chromatograms of fatty acid methyl esters of *H. equigenitalis* NCTC 11184 before (A) and after (B) ditrimethylsilyloxy derivation. The analysis was done on an OV-101 column at an oven temperature of 200°C; 16-OTMS and 18-OTMS indicate the ditrimethylsilyloxy derivatives of 16:1 and 18:1 acids, respectively.

that it is clearly distinct from the cellular fatty acid compositions of *Haemophilus* species (13) and grossly similar to the cellular fatty acid compositions of *Moraxella* species (5, 9, 14, 15, 27). *Moraxella* species have been reported to contain large amounts of 16:0 and 18:1 acids, which we confirmed in this study. The cellular fatty acid composition of *M. anatipestifer*, whose biochemical characteristics are markedly different from those of other *Moraxella* species (1), was peculiar in its abundance of branched-chain fatty acids and was similar to the cellular fatty acid compositions of *Legionella* species (23, 24, 26).

Bøvre and colleagues studied the cellular fatty acid compositions of *Moraxella* and *Neisseria* species (5, 9, 14, 15) and found that false *Neisseria* or *Branhamella* (i.e., *M. catarrhalis*, *M. caviae*, and *M. ovis*) strains have fatty acid patterns grossly similar to those of *Moraxella* species. This observation is one of the reasons that the false *Neisseria* species were reclassified

in the genus *Moraxella*. Bøvre described the taxonomic problems encountered with the false *Neisseria* species and proposed to divide the genus *Moraxella* into the following two subgenera, based mainly on cell shape: subgenus *Moraxella*, containing rod-shaped organisms, and subgenus *Branhamella*, containing cocci (3).

However, Bøvre et al. did not distinguish positional isomers of 18:1 acid, which is a major fatty acid in *Moraxella* species. Two species of subgenus *Branhamella*, *M. catarrhalis* and *M. ovis*, were clearly separated from species belonging to subgenus *Moraxella* and *M. caviae* by the positional isomer compositions of 18:1 acids in the present study. The positional isomers of 18:1 acid have taxonomic significance in the genus *Moraxella* since this genus has been divided into the oleic acid group and the *cis*-vaccenic acid group. The double bond positions in 18:1 acids were easily deduced from ECL values when a GLC analysis was performed by using a flexible fused capillary column because of its

FIG. 2. Mass spectra of ditrimethylsilyloxy derivatives of monounsaturated fatty acid methyl esters. $\Delta 9$ 16:1 acid (A) and $\Delta 11$ 18:1 acid (B) were detected in *H. equigenitalis*, and $\Delta 9$ 18:1 acid (C) was detected in *M. bovis*.

high resolution and separation capacity, as reported by Moss et al. (25).

In contrast to the previous finding that *M. urethralis* is separated from other *Moraxella* species by cellular fatty acid composition (14, 15), *M. urethralis*, *M. catarrhalis*, and *M. ovis* clustered together in our study. This apparently resulted from differences in the methods used to calculate similarity. The primary logarithmic transformation of the data for each fatty acid used in the previous studies may have given relatively more weight to fatty acids that occur in small quantities. For example, *M. urethralis* contained relatively small amounts of 14:0 and 3OH 14:0 acids but lacked 10:0, 12:0, and 3OH 16:0 acids, in contrast to *M. catarrhalis* and *M. ovis*. Furthermore, the amount of 16:1 acid was relatively smaller than the amount of 16:0 acid in *M. urethralis* compared with the other two species.

It is known that unsaturated fatty acids are synthesized from saturated fatty acids by two pathways (aerobic and anaerobic) (8, 10, 11). The anaerobic pathway of desaturation generally leads to palmitoleic and *cis*-vaccenic acids as the major products, whereas the aerobic pathway yields primarily palmitoleic and oleic acids

(10). The detection of $\Delta 9$ monounsaturated fatty acids in *M. bovis* and *M. osloensis* strongly suggests that these organisms have the aerobic pathway of desaturation at the $\Delta 9$ position. The existence of this pathway in gram-negative bacteria is known only in *Alcaligenes faecalis*, although it is common in gram-positive bacteria (17). On the other hand, the detection of $\omega 7$ monounsaturated fatty acids in *H. equigenitalis* and *M. urethralis* indicates the existence of the anaerobic pathway of desaturation. *H. equigenitalis* synthesizes a trace amount of oleic acid, probably by the anaerobic pathway since the two pathways are mutually exclusive in the sense that no organism which possesses both

FIG. 3. Typical cleavage of ditrimethylsilyloxy derivatives (OTMS) of a monounsaturated fatty acid methyl ester. Δ and ω indicate the numbers of carbon atoms measured from the terminal methyl base and carboxyl base, respectively.

FIG. 4. Dendrogram of the strains tested based on cellular fatty acid composition. The correlation coefficient was calculated by the formula of Bergan (2).

pathways has been found (8, 11).

In our previous study of *H. equigenitalis* (30), we found no significant difference in cellular fatty acid composition among cells cultivated on different media even if we substituted a chemically defined growth supplement, such as IsoVitalX (BBL Microbiology Systems, Cockeysville, Md.), for blood components. However, we cannot rule out the possibility that some species tested in this study incorporate fatty acids from the medium, as indicated by Letts et al. (20). If this is the case, the differences in cellular fatty acid composition result from differences in the ability to incorporate lipid constituents from the medium rather than differences in pathways of desaturation. Because only a few species contain both of oleic and *cis*-vaccenic acids and either of these acids is dominant in amount, the possibility that the dominant positional isomer was incorporated from the medium is unlikely. However, the pathway by which organisms synthesize monounsaturated fatty acids must be proved directly by using labeled precursors.

Taylor et al. proposed that the causative organism of contagious equine metritis should be classified as a member of the genus *Haemophilus* based on the guanine-plus-cytosine content of its DNA and its X-factor requirement, and these authors designated this organism *H. equigenitalis* (31). The X-factor requirement of this organism was reported by Shreeve (28), but Taylor et al. (31), Dabernat et al. (7), and we could not confirm this result. Because the porphyrin test for this organism is positive, like other X-factor-independent *Haemophilus* spe-

cies (16), this organism does not require X-factor. *H. equigenitalis* is a catalase- and oxidase-positive asaccharolytic coccobacillus, is very unreactive in conventional biochemical tests, and shows phenotypic similarity to *Moraxella* and *Legionella* species. In cellular fatty acid composition, *H. equigenitalis* is very similar to the *cis*-vaccenic acid group of *Moraxella* species but distinct from the oleic acid group of *Moraxella* species and *Legionella* species.

When other taxonomic criteria are taken in consideration, *H. equigenitalis* differs from the *Moraxella* species described above in some characteristics. The guanine-plus-cytosine contents of *M. urethralis*, *M. catarrhalis*, *M. ovis*, and *H. equigenitalis* DNAs are 46 to 47, 46.5 to 47.5, 44.5 to 46.5, and 36.1 mol%, respectively (4, 31). Furthermore, *M. urethralis*, which has been tentatively placed in the genus *Moraxella* despite genetic incompatibility with this genus, grows slowly in a simple mineral medium supplemented with ammonium ions as the source of nitrogen and acetate or hydroxybutylate as the carbon source (19). The nutritional properties of *H. equigenitalis*, which have not been reported, seem rather complex.

Further taxonomic studies based on DNA-DNA hybridizations and cellular enzyme profiles are now in progress to clarify the possible relationship of *H. equigenitalis* to the family *Neisseriaceae*.

ACKNOWLEDGMENT

We thank R. Sakazaki, National Institute of Health, Tokyo, Japan, for his advice and stimulating discussions.

LITERATURE CITED

- Bangun, A., D. N. Tripathy, and L. E. Hanson. 1980. Studies on *Pasteurella anatispestifer*: an approach to its classification. *Avian Dis.* 25:326-336.
- Bergan, T. 1972. Survey of numerical techniques for grouping. *Bacteriol. Rev.* 35:379-389.
- Bøvre, K. 1979. Proposal to divide the genus *Moraxella* Lwoff 1939 emend. Henriksen and Bøvre 1968 into two subgenera, subgenus *Moraxella* (Lwoff 1939) Bøvre 1979 and subgenus *Branhamella* (Catlin 1970) Bøvre 1979. *Int. J. Syst. Bacteriol.* 29:403-406.
- Bøvre, K., and N. Hagen. 1981. The family *Neisseriaceae*: rod-shaped species of the genera *Moraxella*, *Acinetobacter*, *Kingella*, and the *Branhamella* group of cocci, p. 1506-1529. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balow, and H. G. Schlegel (ed.), *The prokaryotes*. Springer-Verlag, Berlin.
- Bøvre, K., R. Hytta, E. Jantzen, and L. O. Frøholm. 1972. Gas chromatography of bacterial whole cell methanolytates. III. Group relations of *Neisseriae* and *Moraxellae*. *Acta Pathol. Microbiol. Scand Sect. B* 80:683-689.
- Capella, P., and C. M. Zorzut. 1968. Determination of double bond position in monounsaturated fatty acid esters by mass spectrometry of their trimethylsilyloxy derivatives. *Anal. Chem.* 40:1458-1463.
- Dabernat, H. J., D. Tainturier, C. Delmas, J. Ferney, and M. B. Lareng. 1980. Étude bactériologique de *Haemophilus equigenitalis* Taylor 1978, agent de la métrite contagieuse de la jument. *Ann. Rech. Vet.* 11:289-299.
- Edwin, J., and K. Bloch. 1964. Biosynthesis of unsaturated fatty acids in microorganisms. *Science* 143:1006-1012.

9. Frøholm, L. O., E. Jantzen, R. Hytta, and K. Bøvre. 1972. Gas chromatography of bacterial whole cell methanolysates. II. A taxonomic evaluation of the method for species of *Moraxella*. *Acta Pathol. Microbiol. Scand. Sect. B* 80:672-682.
10. Fulco, A. J. 1974. Metabolic alterations of fatty acids. *Annu. Rev. Biochem.* 43:215-241.
11. Gurr, M. I. 1974. The biosynthesis of unsaturated fatty acids, p. 181-235. In T. W. Goodwin (ed.), *Biochemistry of lipids*. Biochemistry series 1, vol. 4. Butterworth, London.
12. Henriksen, S. D. 1976. *Moraxella*, *Neisseria*, *Branhamella*, and *Acinetobacter*. *Annu. Rev. Microbiol.* 30:63-83.
13. Jantzen, E., B. P. Berdal, and T. Omeland. 1980. Cellular fatty acid composition of *Haemophilus* species, *Pasteurella multocida*, *Actinobacillus actinomycetemcomitans* and *Haemophilus vaginalis* (*Corynebacterium vaginale*). *Acta Pathol. Microbiol. Scand. Sect. B* 88:89-93.
14. 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.
15. 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.
16. Killian, M. 1976. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. *J. Gen. Microbiol.* 93:9-62.
17. Kshirsagar, S. S., and P. Nair. 1979. Isolation and properties of a particulate fraction for the desaturation of palmitic acid from *Alcaligenes faecalis*. *Biochim. Biophys. Acta* 574:369-378.
18. Lambert, M. A., D. G. Hollis, C. W. Moss, R. E. Weaver, and M. L. Thomas. 1971. Cellular fatty acids of nonpathogenic *Neisseria*. *Can. J. Microbiol.* 17:1491-1502.
19. Lautrop, H., K. Bøvre, and W. Frederiksen. 1970. A *Moraxella*-like microorganism isolated from the genitourinary tract of man. *Acta Pathol. Microbiol. Scand. Sect. B* 78:255-256.
20. Letts, V., P. Shaw, L. Shapiro, and S. Henry. 1982. Synthesis and utilization of fatty acids by wild-type and fatty acid auxotrophs of *Caulobacter crescentus*. *J. Bacteriol.* 151:1269-1278.
21. Miyagawa, E., R. Azuma, and T. Suto. 1979. Cellular fatty acid composition in gram-negative obligately anaerobic rods. *J. Gen. Appl. Microbiol.* 25:41-51.
22. Miyagawa, E., and T. Suto. 1980. Cellular fatty acid composition in *Bacteroides oralis* and *Bacteroides ruminalis*. *J. Gen. Appl. Microbiol.* 26:331-343.
23. Moss, C. W. 1981. Gas-liquid chromatography as an analytical tool in microbiology. *J. Chromatogr.* 203:337-347.
24. Moss, C. W., and S. B. Dees. 1979. Cellular fatty acid composition of WIGA, a rickettsia-like agent similar to the Legionnaires disease bacterium. *J. Clin. Microbiol.* 10:390-391.
25. Moss, C. W., Shinoda, T., and J. W. Samuels. 1982. Determination of cellular fatty acid compositions of various yeasts by gas-liquid chromatography. *J. Clin. Microbiol.* 16:1073-1079.
26. Moss, C. W., R. E. Weaver, S. B. Dees, and W. B. Cherry. 1977. Cellular fatty acid composition of isolates from Legionnaires disease. *J. Clin. Microbiol.* 6:140-143.
27. Nishimura, Y., H. Yamamoto, and H. Iizuka. 1979. Taxonomical studies of *Acinetobacter* species. Cellular fatty acid composition. *Z. Allg. Mikrobiol.* 19:307-308.
28. Shreeve, J. E. 1978. Preliminary observations on X-factor requirement of the bacterium responsible for CEM. *Vet. Rec.* 102:20.
29. Sugimoto, C., Y. Isayama, M. Kashiwazaki, T. Fujikura, and K. Mitani. 1980. Detection of *Haemophilus equigenitalis*, the causal agent of contagious equine metritis, in Japan. *Natl. Inst. Anim. Health Q.* 20:118-119.
30. Sugimoto, C., E. Miyagawa, K. Mitani, M. Nakazawa, and Y. Isayama. 1982. Cellular fatty acid composition of *Haemophilus equigenitalis*. *J. Clin. Microbiol.* 15:791-794.
31. Taylor, C. E. D., R. O. Rosenthal, D. F. J. Brown, E. S. P. Lapage, L. R. Hill, and R. M. Legros. 1978. The causative organism of contagious equine metritis 1977: proposal for a new species to be known as *Haemophilus equigenitalis*. *Equine Vet. J.* 10:136-144.