JOURNAL OF CLINICAL MICROBIOLOGY, June 1980, p. 625-630
0095-1137/80/06-0625/06$02.00/0
Vol. 11, No. 6

Cellular Fatty Acid Composition of *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus*

SABINA D. BRAUNTHAL,[1] S. C. HOLT,[1]* A. C. R. TANNER,[2] AND S. S. SOCRANSKY[2]

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003,[1] and Forsyth Dental Center, Boston, Massachusetts 02115[2]

Strains of *Actinobacillus actinomycetemcomitans* isolated from deep pockets of patients with juvenile periodontitis were analyzed for their content of cellular fatty acids. Oral *Haemophilus* strains, morphologically and biochemically similar to *Haemophilus aphrophilus,* were also examined for their content of cellular fatty acids. The extractable lipids of the actinobacilli represented approximately 10% of the cell dry weight, with the bound lipids representing 2 to 5%. The major fatty acids consisted of myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids and a $C_{16:1}$ acid, possibly palmitoleic acid, accounting for 21, 35, and 31% of the total extractable fatty acids, respectively. *Haemophilus* strains had a similar cellular fatty acid content.

That microorganisms play a dominant role in the development of the various forms of periodontal disease is well established (4, 13, 29, 30, 36). These microorganisms, found either in intimate association with host cell tissue or free within a developing pocket, represent over 100 separate morphological and physiological bacterial types. Many of these microorganisms have already been isolated, and a large body of information is accumulating describing their morphology, biochemistry, and physiology. In oral pathologies involving molar-incisor lesions, several bacterial species have consistently been recovered in cell numbers consistent with their probable participation in the disease process (36, 39). The major bacterial types recovered are members of the genera *Capnocytophaga, Bacteroides,* and several small, gram-negative rods, morphologically and physiologically identical to members of the genus *Actinobacillus* (31); these latter bacteria have been described as *Actinobacillus actinomycetemcomitans* (A. C. R. Tanner and S. S. Socransky, unpublished data). The actinobacilli have been recovered from several human pathologies, and it has only been in recent years that they have been recovered from the gingival pockets of patients diagnosed as having juvenile periodontitis, or periodontosis.

Microorganisms similar to *A. actinomycetemcomitans* have also been isolated from saliva and oral mucous membranes, and are classified in the genus *Haemophilus* (35). *Haemophilus parainfluenzae* was the major species isolated from saliva, and *Haemophilus aphrophilus,* along with *A. actinomycetemcomitans,* was recovered from dental plaques (17). Morphologically these haemophili and actinobacilli are very similar (S. C. Holt, manuscript in preparation), whereas physiologically these two genera are separable (16; A. C. R. Tanner, S. S. Socransky, and S. C. Holt, manuscript in preparation).

This study was undertaken to examine the lipid and fatty acid composition of human isolates of the genera *Actinobacillus* and *Haemophilus* and to compare them to bona fide (i.e., ATCC) strains for use as a taxonomic tool in the identification of these gram-negative bacteria. In addition, we were interested in establishing base-line parameters for lipid and cellular composition studies of cells grown under controlled environmental conditions for future studies involving potentially virulent and avirulent members of both genera.

MATERIALS AND METHODS

Microorganisms. The fresh isolates used in this study were originally isolated by S. S. Socransky at the Forsyth Dental Center. These isolates have been identified as belonging to the genera *Actinobacillus* (31) and *Haemophilus* (41) and are biochemically and morphologically identical to designated (i.e., ATCC strains) *A. actinomycetemcomitans* and *H. aphrophilus.*

The strains and their designations and disease associations are listed in Table 1.

Growth of microorganisms. All strains were grown in Brewer anaerobic jars or in a Coy Anaerobic Chamber under an atmosphere of 10% H_2, 10% CO_2 and 80% N_2. Cultures were grown either on the surface of Trypticase soy-blood plates (BBL Microbiology Systems) or in liquid culture in a Trypticase-peptone medium consisting of (per liter of distilled water) trypticase peptone (20 g), yeast extract (5 g), NaCl (6 g), KNO_3 (1 g), and glucose (2 g). Hemin (10 ml of a 50-mg/100 ml stock solution) and sodium thiosulfite

TABLE 1. *Strains of A. actinomycetemcomitans and H. aphrophilus*

Group	Strain	Disease	Source
A. actinomycetemcomitans	Y4	Periodontosis	FDC[a]
	N27	Periodontosis	FDC
	650	Periodontosis	FDC
	511	Periodontitis	FDC
	2097	Periodontitis	FDC
	2026	Periodontitis	FDC
	2042	Periodontitis	FDC
	2151	Periodontitis	FDC
	2065	Periodontitis	FDC
	2043	Periodontitis	FDC
	2112	Periodontitis	FDC
	2119	Periodontitis	FDC
	29522	Mandibular abscess	ATCC[b]
	29523	Blood	ATCC
	29524	Chest aspirate	ATCC
H. aphrophilus	81	Unknown	Sims-Kilian
	621	Periodontosis	FDC
	626	Periodontosis	FDC
	654	Periodontitis	FDC
	655	Periodontitis	FDC

[a] FDC, Forsyth Dental Center.
[b] ATCC, American Type Culture Collection.

(10 ml of a 20-mg/10 ml stock solution) were also included in the liquid cultures.

Both plate-grown cells and liquid cultures were incubated for 24, 48, and 72 h. For batch culture growth, 48-h plate-grown cells were aseptically scraped from the agar surface with sterile Ringer solution and used as an inoculum for medium in sterile screw-capped tubes (approximately 15 ml per tube). The inoculated tubes were incubated for 48 h under anaerobic conditions (as described above), after which time the growth was inoculated into sterile medium (in 125-ml flasks) at a ratio of 1:10, inoculum-medium. The inoculated culture was incubated statically, at 37°C, for 24, 48, and 72 h. No differences in lipid and cellular fatty acid composition were observed in cells grown at 37°C for 24, 48, or 72 h. In most instances, and under the growth conditions described, 0.3 to 0.4 mg (dry weight) of *Actinobacillus* and *Haemophilus* per liter of medium was obtained.

Lipid extraction. Lipids were extracted from fresh packed cells and from lyophilized cells (2). Cells were routinely harvested by centrifugation at 12,000 × *g*, 10 min, 4°C. The resulting pellets were washed at least two times with cold Ringer solution and centrifuged, and butylated hydroxytoluene was added to the cell pellets to a final concentration of 0.1% (wt/vol) to prevent lipid oxidation. For lyophilized cells, butylated hydroxytoluene was added before lipid extraction. All cells were either used immediately or stored under N_2 at −20°C. Lipid extraction was carried out by stirring with a magnetic stirrer in closed flasks under an N_2 atmosphere, in the dark, in solvent-rinsed glassware. Cells were extracted two times each with 50 ml of chloroform-methanol (2:1) and 50 ml of chloroform-methanol (1:1), and once with 50 ml of absolute methanol, for at least 30 min each. All extracts were combined, reduced to dryness by flash evaporation, and dried at 55°C in vacuo to constant weight. This crude lipid fraction was purified by the procedure of Wells and Dittmer (40). Briefly, the crude lipid fraction was eluted through a G25 Sephadex fine chromatography column prepared in chloroform-methanol-water (60:30:4.5). The purified lipids were eluted with chloroform-methanol-water (60:30:4.5) and chloroform-methanol (2:1), and dried at 55°C in vacuo to constant weight. The dried material represented the purified extractable cellular lipids.

Bound lipids were extracted two times from the solvent-extracted cell residue (above) with diethyl ether after acid hydrolysis for 2 h in 2 N HCl at 120°C. The extracted bound lipids were dried in vacuo to constant weight.

Fatty acid analysis. *Actinobacillus* and *Haemophilus* strains were grown and washed as described above. Lyophilized or fresh packed cells were routinely used in the analysis of fatty acid methyl esters. In most instances, whole-cell fatty acids were extracted and methylated by the procedure of Moss and Dees (23). The procedure of Kaneshiro and Marr (15) was used as a control of fatty acid methylation. The methylated esters were purified by thin-layer chromatography as described by Holt et al. (12). Catalytic hydrogenation was performed by the method of Poukka et al. (32).

All samples were stored in hexane under an N_2 atmosphere at −20°C. The methyl esters were analyzed by flame-ionization detection in Varian models 1200 and 3700 gas-liquid chromatographs; 10% SP-2330 and 10% SP-2100 on 100/200 Supelcoport (Supelco, Inc., Bellafonte, Pa.) served as polar and nonpolar columns, respectively.

For analysis of the methyl esters, samples were routinely programmed between 160 and 200°C. The initial temperature was maintained for 5 min, then programmed at a rate of 5°C/min to the final temperature. For isothermal analysis, the column temperature was maintained at 200°C. The other chromatographic analysis parameters included a nitrogen flow rate of 30 ml/min, an injection temperature of 250°C, and a detector temperature of 220°C.

All data were recorded by graphic display and on a Varian CDC-111 Data Processor.

Identification of fatty acid methyl esters was made by comparison of retention time of authentic standards (Applied Science Labs, State College, Pa.) with unknown peaks, by semilogarithmic plots of retention time versus C-number, and by gas-liquid chromatography-mass spectrometry (23). Percent distribution of each fatty acid was determined by Varian CDC-111 data processing and by the method of Carroll (5).

RESULTS AND DISCUSSION

Lipid content. Cells of all the *Actinobacillus* strains studied contained approximately 11 to 16% of their dry weight as lipid (Table 2). The extractable lipids represented between 8 and 14% of the total cell dry weight, whereas the bound lipids (those extracted after acid hydrolysis) accounted for only 1 to 5%. Both exponential- (24 h) and late-stationary-phase cells (48 to 72 h) were essentially similar in their distribu-

TABLE 2. *Lipid composition of representative A. actinomycetemcomitans and H. aphrophilus strains*

Lipid class	Composition (% dry wt) of strain:						
	511	N27	2097	Y4	81	621	655
Extractable	11.6	10.8	13.6	9.2	6.4	8.2	8.5
Bound	4.6	4.8	3.6	2.1	1.6	2.1	1.3
Total	16.2	15.6	17.2	11.3	8.0	10.3	9.8

tion of total lipid content.

Fatty acid composition. The fatty acid profile of the *Actinobacillus* and *Haemophilus* strains was similar, and a representative gas-liquid chromatographic scan is seen in Fig. 1. Characteristic of the fatty acids in these two genera was the presence of only three major acids, $C_{14:0}$, $C_{16:0}$, and $C_{16:1}$, which accounted for almost 90% of the total cellular fatty acids in *Actinobacillus* (Table 3). Minor amounts of $C_{12:0}$, $C_{18:0}$, $C_{18:1}$, $C_{20:0}$, and $C_{20:1}$, as well as some β-OH-$C_{14:0}$, were also observed in several of the strains. The presence of $C_{16:1}$ was confirmed by catalytic hydrogenation.

The relative percent distribution of these fatty acids is seen in Tables 3 and 4. In *A. actinomycetemcomitans*, $C_{14:0}$ comprised 21 to 27% of the total cellular fatty acids, whereas $C_{16:0}$ and $C_{16:1}$ comprised 33 to 48% and 23 to 34%, respectively. The remainder of the extractable fatty acids comprised less than 15% of the total identifiable compounds.

H. aphrophilus strains contained a similar distribution of cellular fatty acids (Table 4). Large amounts of $C_{16:0}$ (46 to 60%) were consistently found, with lesser amounts of $C_{14:0}$ and $C_{16:1}$.

Both *H. aphrophilus* and *A. actinomycetemcomitans* have a cellular lipid distribution similar to that observed for other gram-negative bacteria (1, 6–12, 14, 20–28, 33, 34, 37, 38). However, the majority of gram-negative procaryotes possess a broader distribution of fatty acids. For example, members of the genus *Pseudomonas* possess $C_{16:0}$ and $C_{16:1}$, as well as $C_{18:1}$, and an array of hydroxy-fatty acids (11, 14, 23, 24, 27, 34). The actinobacilli and haemophili possess a limited distribution of extractable fatty acids, consisting of only two to three major acids, $C_{14:0}$, $C_{16:0}$, and $C_{16:1}$. Other long-chain fatty acids ($C_{18:1}$, $C_{18:0}$, $C_{20:1}$, $C_{20:0}$) are observed, but these are routinely only found in trace amounts (<2%). Furthermore, the content of hydroxy-fatty acids was either much reduced or absent in the actinobacilli and haemophili, unlike that observed for a large number of other gram-negative microorganisms.

Interestingly, a similar pattern of cellular fatty acid distribution was observed in other host-derived gram-negative bacteria. *Salmonella minnesota* (7), for example, contained large amounts of $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$; the enterobacteria *Escherichia coli, Salmonella typhimurium, Shigella flexneri,* and *Proteus vulgaris* exhibited large amounts of $C_{16:0}$ (22 to 48%), β-hydroxy-$C_{14:0}$ (35 to 45%), and some $C_{14:0}$ (6 to 15%) (38).

Kramzer and Lynch (18) examined the fatty acid distribution of a wide array of gram-positive and gram-negative bacteria and found larger amounts of saturated fatty acids (i.e., 56 to 78% of the total extractable acids) in gram-negative bacteria, including *Serratia* sp., *Enterobacter,* and *Escherichia.* The major fatty acids included $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{19:0}$, and $C_{20:0}$. Branched-chain fatty acids, as well as cyclopropane acids, have also been reported (12). They have not been observed in any of the *Actinobacillus* or *Haemophilus* strains that we have examined in this study. *Neisseria* species (1, 3, 19, 20, 26, 37) displayed a cellular fatty acid distribution similar to *Actinobacillus* and *Haemophilus;* however, *Neisseria,* in addition to possessing large amounts of $C_{16:1}$ and $C_{16:0}$ (approximately 65% of the total), also possessed significant amounts of $C_{18:1}$ (25%) and $C_{14:0}$ (7 to 13%).

Several microorganisms, some of which are common inhabitants of periodontal lesions, also displayed a fatty acid distribution similar to *A. actinomycetemcomitans* and *H. aphrophilus.* Prefontaine and Jackson (33) found that *Eikenella corrodens* ATCC 23834, *E. corrodens* 53P, *Bacteroides corrodens* 3936, and *Pasteurella multocida* (all sputum isolates) possessed $C_{16:0}$, while the amounts of $C_{16:1}$ and $C_{18:1}$ varied between genera. A large number of the fatty acids were unidentified, but their reported retention times make them appear very similar to branched-chain fatty acids found in several oral and nonoral *Bacteroides.* Recently, Holt et al. (12) and Miyagawa et al. (22) have reported a similar distribution of branched-chain fatty acids (predominantly iso- and anteiso-branched $C_{15:0}$) in oral *Capnocytophaga* and several *Bacteroides* species.

The most striking feature of *Actinobacillus* and *Haemophilus* strains examined in this study was the observation that these microorganisms

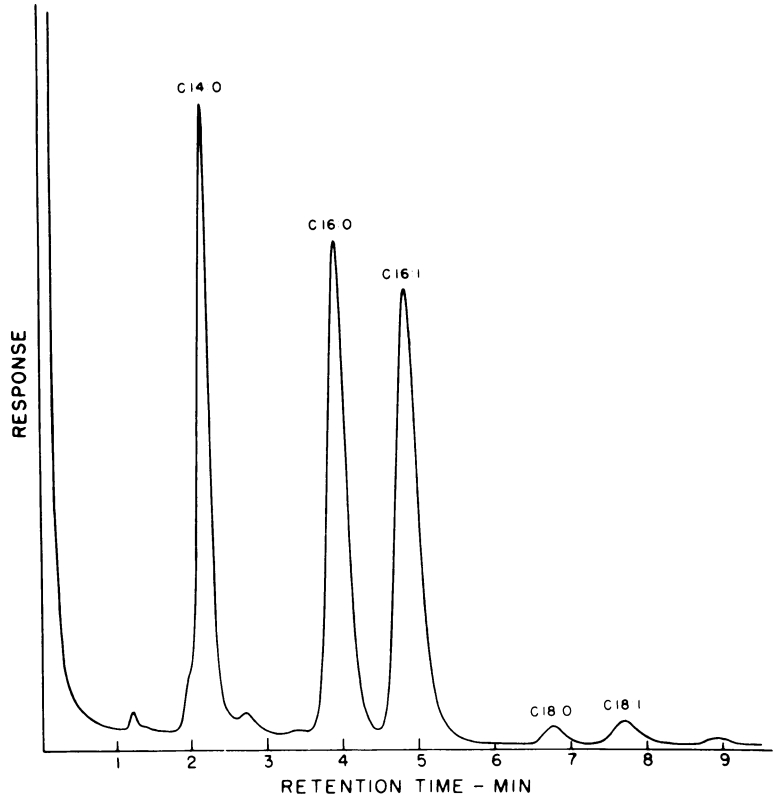

FIG. 1. *Representative gas chromatogram of esterified fatty acids from whole cells of A. actinomycetemcomitans. Analysis was made on 10% SP-1200, 100/200 Supelcoport. Operating parameters in text. Some strains of H. aphrophilus do not contain* $C_{16:1}$ *(see Table 4).*

TABLE 3. *Cellular fatty acid composition of A. actinomycetemcomitans*

Strain	% Extractable lipid[a]								
	12:0[b]	14:0	β-OH-14:0	16:0	16:1	18:0	18:1	20:0	20:1
2026	T	20.8		35.0	23.0	4.0			
2042		32.5		33.1	28.2	T	T		
2151		26.6		36.5	30.5				
2065		26.1		39.7	23.6				
2043		23.8		30.1	37.7	3.4	2.5		
2112		23.2	T	47.8	27.9				
2075		21.0		35.0	33.8				
2097		23.0	T	34.8	28.7	4.8	T	2.3	T
2119		30.4		33.4	32.0				
N27		17.2	2.7	41.8	34.4	6.7		4.1	T
Y4		23.6	2.0	41.2	32.7	3.7		T	T
511		21.2	3.1	37.1	27.8	4.6	T	T	T
650		3.9		42.0	39.4	8.3	3.6	5.1	
ATCC 29522		12.2		41.4	40.3	6.1			
ATCC 29523	11.0	12.2		25.3	21.3	7.2	9.3	T	
ATCC 29524		13.7		39.9	31.8	6.7	4.5		
Avg		20.75		34.56	30.81	5.6	5.25		

[a] Numbers refer to percentage of total fatty acids. T, Less than 2%.
[b] Number of carbon atoms:number of double bonds.

TABLE 4. *Cellular fatty acid composition of H. aphrophilus*

Strain	% Extractable lipid[a]					
	12:0	14:0	16:0	16:1	18:0	18:1
81		12.0	60.4		5.4	13.9
621		20.5	46.5		8.1	5.2
626	T	27.2	37.9	31.2	T	
654	12.0	34.9	36.2	15.8	T	
655		20.4	51.0	14.8	5.5	8.0
Avg		23	46.2	20.6	6.3	9

[a] Percentage of total fatty acids. T, Less than 2%. No strain contained $C_{20:0}$.

possess a restricted qualitative distribution of extractable fatty acids. A similar distribution has been observed in other gram-negative bacteria of periodontopathic potential (12, 22); however, in these latter microorganisms there was a preponderance of branched-chain fatty acids. It is unclear whether these fatty acid distributions, both in *Capnocytophaga* (14) and *Bacteroides* (29) and now here in *Actinobacillus* and *Haemophilus,* can be employed as taxonomic markers at the present time. It is clear that many more strains will have to be examined before a definitive conclusion can be reached. However, the determination of these rather narrow and selective fatty acid distributions should provide an excellent first step in taxonomic identification of gram-negative microorganisms of disease-producing potential.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant DE-05123 from the National Institute for Dental Research.

We thank Linda Slakey for the use of her gas-liquid chromatograph in the initial stages of this research, and C. Wayne Moss for his kindness in confirming our fatty acid analyses. Special thanks to Patricia Kiley for growing cultures of *Actinobacillus* and *Haemophilus.*

LITERATURE CITED

1. **Alley, C., B. Brooks, and S. Kellog, Jr.** 1979. Electron capture gas-liquid chromatographic-mass spectral identification of acids produced by *Neisseria meningitidis* in a defined medium. J. Clin. Microbiol. **9**:97-102.
2. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911-917.
3. **Bøvre, K., R. Hytta, E. Jantzen, and L. O. Frøholm.** 1972. Gas chromatography of bacterial whole cell methanolysates. III. Group relations of *Neisseria* and *Moraxellae.* Acta Pathol. Microbiol. Scand. Sect. B **80**:683-689.
4. **Carranza, F. A., Jr.** 1979. The role of microorganisms in the etiology of gingival and periodontal disease, p. 374-404. *In* F. A. Carranza (ed.), Glickman's clinical periodontology, 5th ed. W. B. Saunders Co., Philadelphia.
5. **Carroll, K. K.** 1961. Quantitative estimation of peak areas in gas-liquid chromatography. Nature (London) **191**: 377-378.
6. **Cullen, J., M. C. Phillips, and G. G. Shipley.** 1971. The effects of temperature on the compositive and physical properties of the lipids of *P. fluorescens.* Biochem. J. **125**:733-743.
7. **Ferber, E., S. Schlect, and I. Fromme.** 1976. Investigations on the fatty acid composition of lipids from *Salmonella minnesota* S and R forms. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **236**:275-287.
8. **Finnerty, W. R., and R. A. Makula.** 1975. Microbial lipid metabolism. Crit. Rev. Microbiol. **4**:1-40.
9. **Finnerty, W. R., and R. A. Makula.** 1970. Cellular lipids of the Legionnaires' disease bacterium. Ann. Intern. Med. **90**:631-634.
10. **Goldfine, H.** 1972. Comparative aspects of bacterial lipids. Adv. Microbiol. Physiol. **8**:1-58.
11. **Hancock, I. C., G. O. Humphreys, and P. M. Meadow.** 1970. Characterization of the hydroxyacids of *Pseudomonas aeruginosa* 8602. Biochem. Biophys. Acta **202**: 389-391.
12. **Holt, S. C., G. Forcier, and B. J. Takacs.** 1979. Fatty acid composition of gliding bacteria: oral isolates of *Capnocytophaga* compared with *Sporocytophaga.* Infect. Immun. **26**:298-304.
13. **Irving, J. T., M. G. Newman, S. S. Socransky, and J. D. Heeley.** 1975. Histological changes in experimental periodontal disease in rats monoinfected with a gram-negative organism. Arch. Oral Biol. **20**:219-220.
14. **Kaltenbach, C. M., C. Moss, and E. Weaver.** 1975. Cultural and biochemical characteristics and fatty acid composition of *Pseudomonas diminuta* and *Pseudomonas vesiculare.* J. Clin. Microbiol. **1**:339-344.
15. **Kaneshiro, T., and A. G. Marr.** 1963. Hydroxy fatty acids of *Azotobacter agilis.* Biochim. Biophys. Acta **70**: 271-277.
16. **Kilian, M.** 1976. A taxonomic study of the genus *Haemophilus,* with the proposal of a new species. J. Gen. Microbiol. **93**:9-62.
17. **Kilian, M., and C. R. Schiott.** 1975. Haemophili and related bacteria in the human oral cavity. Arch. Oral Biol. **20**:791-796.
18. **Kramzar, G. R., and D. C. Lynch.** 1976. A qualitative and quantitative study of the fatty acid composition of selected microorganisms. Microbios **17**:7-16.
19. **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.
20. **Lewis, V. J., R. E. Weaver, and D. G. Hollis.** 1968. Fatty acid composition of *Neisseria* species as determined by gas chromatography. J. Bacteriol. **96**:1-5.
21. **Lindner, J. G. E. M., and J. H. Marcelis.** 1978. Quantitative gas chromatography of *Bacteroides* species under different growth conditions. Antonie van Leeuwenhoek J. Microbiol. Serol. **44**:1-14.
22. **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.
23. **Moss, C. W., and S. B. Dees.** 1975. Identification of microorganisms by gas chromatographic-mass spectrometric analysis of cellular fatty acids. J. Chromatogr. **112**:595-604.
24. **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.
25. **Moss, C. W., and S. B. Dees.** 1979. Further studies of the cellular fatty acid composition of Legionnaires disease bacteria. J. Clin. Microbiol. **9**:648-649.
26. **Moss, C. W., P. S. Kellog, Jr., D. C. Farshy, M. S. Lambert, and J. O. Thayer.** 1970. Cellular fatty acids of pathogenic *Neisseria.* J. Bacteriol. **104**:63-68.

27. **Moss, C. W., S. B. Samuels, and R. E. Weaver.** 1972. Cellular fatty acid composition of selected *Pseudomonas* species. Appl. Microbiol. **24**:596–598.
28. **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.
29. **Newman, M. G., and S. S. Socransky.** 1977. Predominant cultivable microbiota in periodontosis. J. Periodont. Res. **12**:120–128.
30. **Newman, M. G., S. S. Socransky, E. Savitt, D. Propas, and A. Crawford.** 1976. Studies on the microbiology of periodontosis. J. Periodontol. **47**:373–379.
31. **Phillips, J. E.** 1974. Genus *Actinobacillus* Brumpt 1910, 849, p. 373–377. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.
32. **Poukka, R., L. Vasenius, and O. Turpeinen.** 1962. Catalytic hydrogenation of fatty acid methyl esters for gas-liquid chromatography. J. Lipid Res. **3**:128–129.
33. **Prefontaine, G., and F. C. Jackson.** 1972. Cellular fatty acid profiles as an aid to the classification of "corroding bacilli" and certain other bacteria. Int. J. Syst. Bacteriol. **22**:210–217.
34. **Samuels, B., C. W. Moss, and R. E. Weaver.** 1973. The fatty acids of *Pseudomonas multivorans* and *Pseudomonas kingii*. J. Gen. Microbiol. **74**:275–279.
35. **Sims, W.** 1970. Oral haemophili. J. Med. Microbiol. **3**: 615–625.
36. **Slots, J.** 1976. The predominant cultivable organisms in juvenile periodontitis. Scand. J. Dent. Res. **84**:1–10.
37. **Sud, I. J., and D. S. Feingold.** 1975. Phospholipids and fatty acids of *Neisseria gonorrhoeae*. J. Bacteriol. **124**: 713–714.
38. **Vaczi, L., A. Rethy, and I. Redai.** 1964. Fatty acids in enteric bacteria. Acta Microbiol. Acad. Sci. Hung. **11**: 375–382.
39. **Van Palenstein-Helderman, W. H.** 1975. Total viable counts and differential count of *Vibrio* (*Campylobacter*) *sputorum*, *Bacteroides ochcraceus*, and *Veillonella* in the inflamed and non-inflamed human gingival crevice. J. Periodont. Res. **10**:294–305.
40. **Wells, M. A., and J. C. Dittmer.** 1963. The use of Sephadex for the removal of nonlipid contaminants from lipid extracts. Biochemistry **2**:1259–1263.
41. **Zinnemann, K., and E. L. Biberstein.** 1974. Genus *Haemophilus* Winslow, Broadhurst, Buchanan, Krumwiebe, Rogers and Smith 1917, 561, p. 364–368. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.