Fatty Acid Composition of Oral Isolates of *Selenomonas*

By ERIK JANTZEN¹ AND TOR HOFSTAD²*

¹ National Institute of Public Health, Oslo, Norway

² Department of Microbiology and Immunology, The Gade Institute, University of Bergen, N-5000 Bergen, Norway

(Received 4 July 1984; revised 3 September 1984)

Fatty acids of 16 strains of *Selenomonas* isolated from the human oral cavity were examined by gas-liquid chromatography. The strains showed similar patterns, characterized by the presence of straight-chain fatty acids in the range C₁₁ to C₁₈. Fatty acids of odd-numbered carbon atoms dominated and the major acids were *n*-pentadecanoate and 3-hydroxytridecanoate. The general fatty acid pattern of *Selenomonas* differed distinctly from those of other previously analysed anaerobic or microaerophilic Gram-negative bacilli.

INTRODUCTION

Analysis of cellular fatty acid composition has provided valuable information for the classification of various bacteria including *Fusobacterium* spp. (Jantzen & Hofstad, 1981), *Leptotrichia buccalis* (Hofstad & Jantzen, 1982) and *Bacteroides* spp. (Miyagawa *et al.*, 1979; Shah & Collins, 1983). *Selenomonas* is a group of anaerobic crescent- to spiral-formed organisms having a tuft of flagella that arises on the concave side of the cell (Kingsley & Hoeniger, 1973). Two species are described in Bergey's Manual of Systematic Bacteriology (Bryant, 1984): the rumen organism *S. ruminantium* and the oral organism *S. sputigena*. Recent DNA homology studies have indicated that the genus may comprise more than one oral species (Kiely & Syed, 1984).

In this communication we report the fatty acid characteristics of 16 oral isolates of *Selenomonas*, and compare their fatty acid patterns with those of other anaerobic Gram-negative straight- or spiral-formed rods.

METHODS

Strains and cultivation. The *Selenomonas* strains ATCC 33150, IDB-2, S7-11, B 12, Ray Donut, D-3, D-8, F-1, F-4, F-10, F-11, F-8N, FRY, KFS, PD-11 and Per 3 were supplied by Dr S. A. Syed, University of Michigan, Ann Arbor, Mich., USA. They were all isolated from marginal plaque of human adults or diabetic children suffering from periodontitis. Strains F-1 and F-8N were from the same patient.

Cells were cultivated on chocolate agar plates of the following composition (g l⁻¹): beef extract (Difco), 5.0; proteose peptone (no. 3, Difco), 10.0; NaCl, 5.0; Na₂HPO₄ · 12H₂O, 2.0; agar (Difco), 13.0. The pH of the medium was adjusted to 7.1 with 5 M-NaOH. This basal medium (1 litre) was supplemented with 85 ml human blood and 10 ml Isovitalex Enrichment (BBL Microbiology Systems) containing 10% (w/v) glucose, and heated to 85 °C. The inoculated plates were incubated at 37 °C for 72 h in a deoxygenated CO₂ atmosphere (BBL GasPak jars). Bacterial growth was washed from the plates using a right-angled glass rod and sterile distilled water. Biomass was sedimented by centrifugation (3000 g, 20 min), washed twice with distilled water, freeze-dried and stored under nitrogen in closed vials at -20 °C.

Chemicals. Solvents of analytical grade were distilled before use. The 2 M-HCl in methanol was obtained by bubbling dry HCl gas (Fluka, Buchs, Switzerland) into dried methanol until saturation and subsequent dilution. Fatty acid standards were obtained from Applied Science Laboratories, Oud Beijerland, The Netherlands. Trifluoroacetic anhydride was purchased from Koch-Light Laboratories, and used without further purification.

Chemical procedures and gas chromatography. Anaerobic bacteria commonly contain alk-1-enyl ethers (plasmalogens), which yield fatty aldehyde dimethylacetals upon acidic methanolysis. Primary analyses by GC and GC-MS verified the presence of compounds with retention and fragmentation characteristics corresponding

to dimethylacetals (Farquhar, 1962; McFadden *et al.*, 1964) in the *Selenomonas* methanolysates. A procedure for removal of these constituents was therefore used (Meyer & Meyer, 1971). Dried bacterial cells (1 to 10 mg) were methanolysed by 2 M-HCl in methanol (3 ml) at 85 °C for 18 h (Jantzen & Hofstad, 1981). After cooling, the methanolysates were concentrated to about 1.5 ml by nitrogen, 3 ml of a 50% saturated NaCl solution was added and then the mixture was extracted twice with 4.5 ml hexane. The combined hexane phases were evaporated to near dryness using dry nitrogen at room temperature. The methyl esters were saponified (1 ml of 0.5 M-NaOH in 90% methanol, 85 °C, 2 h) and the dimethylacetals extracted by hexane (2 × 3 ml). The remaining fatty acids (as sodium salts) were re-methylated by adjusting the pH to 2 with 6 M-HCl, subsequent treatment with 14% (w/v) BCl₃ in methanol (Merck; 0.5 ml, 100 °C, 5 min) and extraction with hexane (2 × 3 ml).

Preparative separation of dimethylacetals and methyl esters of hydroxylated and unsubstituted fatty acids for GC-MS analysis was done on thin-layer sheets (0.2 mm silica gel, F₂₅₄; Merck) developed by toluene/diethyl ether (10:1).

Trifluoroacetyl esters of hydroxylated fatty acid methyl esters were formed by heating to boiling for 5 min with equal volumes of trifluoroacetic anhydride (Merck) and acetonitrile (Merck).

The fatty acid methyl esters were analysed using a Hewlett-Packard 5710 gas chromatograph equipped with a flame ionization detector and a fused-silica capillary column (25 m × 0.2 mm, methyl silicone, OV-1) as previously described (Jantzen & Hofstad, 1981). Peak areas and retention times were recorded by a Hewlett-Packard 3390A recorder-integrator. Hydroxylated fatty acid methyl esters were quantified after trifluoroacetylation of the methanolysates (see above).

Peaks were primarily identified by comparison of retention times to those of methyl ester standards obtained from Applied Science Laboratories or from our collection of previously identified bacterial fatty acids. The identities of hydroxy fatty acids were confirmed by determination of their retention times before and after conversion to trifluoroacetyl derivatives. The presence of unsaturation and/or cyclopropane substitution in the alkyl chain was examined by the reduction method of Brian & Gardner (1968).

Generally, the fatty acid identities were confirmed by MS using a Hewlett-Packard 5992 gas chromatograph-mass spectrometer equipped with a fused-silica capillary column (25 m × 0.3 mm, coated with methylphenyl silicone, SE 54), as previously described (Jantzen & Hofstad, 1981).

RESULTS

All strains were cultured, processed and analysed at least twice (most strains three times); the average amounts of fatty acids are given in Table 1. Unusually high quantitative variations in the fatty acid patterns of each strain were observed. In particular, the amount of *n*-tridecanoate (13:0) and *n*-pentadecanoate (15:1) fluctuated considerably from batch to batch of cells of the same strain. Accordingly the quantitative differences among the examined strains have little diagnostic value.

The 16 *Selenomonas* strains exhibited very similar fatty acid patterns characterized by straight-chain acids of chain length in the range C₁₁ to C₁₈. In contrast to most bacteria (Jantzen, 1984), the odd-numbered acids were generally dominant (Table 1). Most abundant in nearly all strains were *n*-pentadecanoate (15:0) and 3-hydroxytridecanoate (3-OH-13:0), whereas strains D-8, PD-11, Per 3 and S7-11 also contained considerable amounts (13:0).

Standards of 3-OH-13:0 and 15:1 were not available. The identities of the two constituents given these labels were based on their gas chromatographic properties and mass spectra. Thus the presence of a hydroxyl group and its 3-position in the 3-OH-13:0 constituent was tentatively identified by the gas chromatographic retention characteristics (including shift in retention times after trifluoroacetylation) in comparison to homologues of hydroxy fatty acid standards. The mass spectrum of this compound showed the distinct fragmentation pattern of 3-hydroxy fatty acid methyl esters, including the abundant and specific *m/z* 103 fragment (Ryhage & Stenhagen, 1960). Thus both the method of log retention time plotting and MS confirmed its identity as 3-OH-13:0. The identity of the presumed 15:1 constituent was strongly supported by the characteristic shift in elution sequence (compared to 15:0), going from a nonpolar to a polar column, and its chromatographic disappearance after hydrogenation or bromination, respectively. A clear distinction from a cyclopropane substituted fatty acid was supported by the observed complete resistance towards methanolic HCl, a reagent which degrades cyclopropane fatty acids almost completely (Vulliet *et al.*, 1974). The identity as 15:1 was further supported by

Table 1. *Fatty acid composition of Selenomonas strains*

Fatty acid*	Fatty acid composition (% w/w, of total) in strain:															Per 3	
	ATCC 33150	IDB 2	S7-11	B 12	Ray	Donut	D-3	D-8	F-1	F-4	F-10	F-11	F-8N	FRY	KFS		PD-11
11:0	3	2	4	4	2	2	2	1	2	1	tr	3	1	6	2	3	3
12:0	2	2	2	3	2	2	2	2	2	2	1	2	2	2	2	1	2
13:0	13	4	23	5	12	4	6	22	4	4	4	5	5	7	5	18	24
3-OH-13:0	21	24	25	25	22	23	23	11	30	23	20	18	20	16	22	23	21
14:0	3	2	4	2	3	4	4	4	3	3	5	4	3	3	3	3	4
3-OH-14:0	2	3	3	5	3	3	5	4	5	7	7	7	5	2	6	2	3
15:1	7	13	9	13	7	7	7	8	8	8	4	5	9	11	8	11	10
15:0	28	22	21	22	34	28	28	29	20	22	25	22	24	32	20	22	23
16:1	2	4	tr	4	1	3	3	4	4	5	4	4	4	3	4	2	2
16:0	8	2	3	3	3	7	7	4	6	6	11	12	5	3	6	4	2
17:1	5	16	3	9	5	6	6	6	7	11	6	6	10	10	11	4	2
17:0	3	4	2	2	4	5	5	2	4	5	6	6	5	4	6	2	2
18:1	1	2	1	2	1	1	1	2	2	3	3	2	2	1	4	3	tr
18:0	2	tr	1	1	1	1	1	1	2	1	3	5	2	tr	2	2	1

tr, Less than 0.5%.

* The figure before the colon indicates the number of carbon atoms in the chain, that after the colon denotes the number of double bonds (the position of the double bonds has not been determined); 3-OH indicates the presence of a 3-hydroxy group.

MS which provided a molecular ion of m/z 254 and the fragmentation pattern of a mono-unsaturated fatty acid methyl ester.

Neither cyclopropane nor methyl-branched fatty acids, could be detected. Fatty aldehyde dimethylacetals were present in the methanolysates. These constituents probably originated from plasmalogens which occur widely among anaerobic bacteria (Kamio *et al.*, 1969; Lchevalier, 1977).

DISCUSSION

The pattern of odd-numbered fatty acids within the oral *Selenomonas* isolates is markedly different from other anaerobic or microaerophilic Gram-negative rods. For example, *Fusobacterium* species (Jantzen & Hofstad, 1981; Calhoon *et al.*, 1983) and *Leptotrichia buccalis* (Hofstad & Jantzen, 1982) share a pattern consisting of unbranched even-numbered fatty acids in the range C_{12} to C_{18} . Similarly, *Campylobacter* species contain unbranched even-numbered fatty acids and no odd-numbered forms (Blaser *et al.*, 1980; Curtis, 1982). The fatty acid pattern of oral *Capnocytophaga* (Collins *et al.*, 1982; Dees *et al.*, 1982) and *Bacteroides* species (Miyagawa *et al.*, 1979; Mayberry *et al.*, 1982) differ by their characteristic methyl-branched (iso and anti-iso) hydroxylated and non-hydroxylated acids. However, a grossly similar, odd-numbered fatty acid pattern has been described for the rumen species *S. ruminantium* (Kamio *et al.*, 1970a, 1971), which supports the placement of the rumen organism in the same genus as the human oral isolates of *Selenomonas*.

Unbranched odd-numbered fatty acids (i.e. n - C_{15} , n - C_{17} , and n - C_{19}) are found as minor constituents in many bacteria (Jantzen, 1984). Their biosynthesis is most probably a function of the availability of the precursor propionyl-CoA (Fulco, 1983). However, for bacteria where odd-numbered acids are dominant (e.g. *S. ruminantium*), the contribution of an α -oxidation reaction has been suggested (Kamio *et al.*, 1970b). Emmanuel (1978), using single and double isotope labelling procedures, demonstrated that both the propionate and the α -oxidation pathway were present in these bacteria, the latter being the most effective.

Although strains ATCC 33150, IDB-2, S7-11, B-12 and Ray Donut form a DNA homology group (S. A. Syed, personal communication) and strains PD-11, FRY and PER 3 have no DNA homology with strain ATCC 33150, these bacteria did not differ significantly in fatty acid patterns.

The results of this study indicate that fatty acid analysis may serve as a useful taxonomic aid for distinguishing *Selenomonas* strains from other anaerobic Gram-negative rods.

We are most grateful to Dr S. A. Syed for supplying us with the strains.

REFERENCES

- BLASER, M. J., MOSS, C. W. & WEAVER, R. E. (1980). Cellular fatty acid composition of *Campylobacter fetus*. *Journal of Clinical Microbiology* **11**, 448-451.
- BRIAN, B. & GARDNER, E. W. (1968). A simple procedure for detecting the presence of cyclopropane fatty acids in bacterial lipids. *Applied Microbiology* **16**, 549-552.
- BRYANT, M. P. (1984). Genus IX *Selenomonas* Von Prowazek 1913, 36. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 650-653. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- CALHOON, D. A., MAYBERRY, W. R. & SLOTS, J. (1983). Cellular fatty acids and soluble protein profiles of oral fusobacteria. *Journal of Dental Research* **62**, 1181-1185.
- COLLINS, M. D., SHAH, H. N., MCKEE, A. S. & KROPPESTEDT, R. M. (1982). Chemotaxonomy of the genus *Capnocytophaga* (Leadbetter, Holt & Socransky). *Journal of Applied Bacteriology* **52**, 409-415.
- CURTIS, M. A. (1982). Cellular fatty acid profiles of campylobacters. In *Campylobacter. Epidemiology, Pathogenesis and Biochemistry*, pp. 234-241. Edited by D. G. Newell. Lancaster, Boston, The Hague: MTB Press.
- DEES, S. B., KARR, D. E., HOLLIS, D. & MOSS, C. W. (1982). Cellular fatty acids of *Capnocytophaga* species. *Journal of Clinical Microbiology* **16**, 779-783.
- EMMANUEL, B. (1978). The relative contribution of propionate, and long-chain even-numbered fatty acids to the production of long-chain odd-numbered fatty acids in rumen bacteria. *Biochimica et biophysica acta* **528**, 239-246.
- FARQUHAR, J. W. (1962). Identification and gas-liquid

- chromatographic behaviour of plasmalogen aldehydes and their acetal, alcohol and acetylated alcohol derivatives. *Journal of Lipid Research* **3**, 21–30.
- FULCO, A. J. (1983). Fatty acid metabolism in bacteria. *Progress in Lipid Research* **5**, 133–166.
- HOFSTAD, T. & JANTZEN, E. (1982). Fatty acids of *Leptotrichia buccalis*: taxonomic implications. *Journal of General Microbiology* **128**, 151–153.
- JANTZEN, E. (1984). Analysis of cellular components in bacterial classification and diagnosis. In *Gas Chromatography/Mass Spectrometry. Applications in Microbiology*, pp. 257–302. Edited by G. Odham, L. Larsson & P.-A. Mårdh. New York, London: Plenum Press.
- JANTZEN, E. & HOFSTAD, T. (1981). Fatty acids of *Fusobacterium* species: taxonomic implications. *Journal of General Microbiology* **123**, 163–171.
- KAMIO, Y., KANEGASAKI, S. & TAKAHASHI, H. (1969). Occurrence of plasmalogens in anaerobic bacteria. *Journal of General and Applied Microbiology* **15**, 439–451.
- KAMIO, Y., KANEGASAKI, S. & TAKAHASHI, H. (1970a). Fatty acid and aldehyde compositions in phospholipids of *Selenomonas ruminantium* with reference to growth conditions. *Journal of General and Applied Microbiology* **16**, 29–37.
- KAMIO, Y., INAGAKI, H. & TAKANASHI, H. (1970b). Possible occurrence of α -oxidation in phospholipid biosynthesis in *Selenomonas ruminantium*. *Journal of General and Applied Microbiology* **16**, 463–478.
- KAMIO, Y., KIM, K. C. & TAKAHASHI, H. (1971). Chemical structure of lipid A of *Selenomonas ruminantium*. *Journal of Biochemistry* **70**, 187–191.
- KIELY, G. M. & SYED, S. A. (1984). Multiple *Selenomonas* species in the human oral activity. Abstract no. 143, 84th Annual Meeting of the American Society for Microbiology.
- KINGSLEY, V. V. & HOENIGER, J. F. M. (1973). Growth, structure and classification of *Selenomonas*. *Bacteriological Reviews* **37**, 479–521.
- LÉCHEVALIER, H. (1977). Lipids in bacterial taxonomy—a taxonomist's view. *CRC Critical Review in Microbiology* **5**, 109–210.
- MAYBERRY, W. R., LAMBE, D. W., JR & FERGUSON, K. P. (1982). Identification of *Bacteroides* species by cellular fatty acid profiles. *International Journal of Systematic Bacteriology* **32**, 21–27.
- MCFADDEN, W. H., WASSERMANN, J., CORSE, J., LUNDIN, R. E. & TERANISHI, R. (1964). Correlation and anomalies in mass spectra: acetals. *Analytical Chemistry* **36**, 1031–1037.
- MEYER, H. & MEYER, F. (1971). Lipid metabolism in the parasitic and free living spirochetes *Treponema pallidum* (Reiter) and *Treponema zuelzeri*. *Biochimica et biophysica acta* **231**, 93–106.
- MIYAGAWA, E., AZUMA, R. & SUTO, T. (1979). Cellular fatty acid composition in Gram-negative obligately anaerobic rods. *Journal of General and Applied Microbiology* **25**, 41–51.
- RYHAGE, R. & STENHAGEN, E. (1960). Mass spectrometric studies: VI. Methyl esters of normal chain-, oxo-, hydroxy-, methoxy- and epoxyacids. *Arkiv för kemi* **15**, 545–569.
- SHAH, H. N. & COLLINS, M. D. (1983). Genus *Bacteroides*. A chemotaxonomical perspective. *Journal of Applied Bacteriology* **55**, 403–416.
- VULLIET, P., MARKEY, S. P. & TORNABENE, T. G. (1974). Identification of methoxyester artifacts produced by methanolic-HCl solvolysis of the cyclopropane fatty acids of the genus *Yersinia*. *Biochimica et biophysica acta* **348**, 299–301.