

Cellular Fatty Acids of *Peptococcus variabilis* and *Peptostreptococcus anaerobius*

C. WAYNE MOSS,* M. A. LAMBERT, AND G. L. LOMBARD

Center for Disease Control, Atlanta, Georgia 30333

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Cellular fatty acids of *Peptococcus variabilis* and *Peptostreptococcus anaerobius* were identified by gas chromatography, mass spectrometry, and associated analytical techniques. Iso- and anteiso-branched-chain acids were major components in both species.

A number of recent studies have shown that various microorganisms can be distinguished on the basis of their cellular fatty acids (3, 4, 10). Closely related species often can be easily differentiated by qualitative or large quantitative differences in their cellular fatty acid contents (5, 8, 9). In preliminary studies of gram-positive anaerobic cocci, we noted that fatty acid profiles of *Peptococcus variabilis* and *Peptostreptococcus anaerobius* differed markedly from each other and from a variety of other bacteria examined in this laboratory. Detailed studies to identify the fatty acids of these two organisms have been completed.

Lyophilized cultures of *P. anaerobius* (CDC-17642; Virginia Polytechnic Institute, VPI, 4329) and *P. variabilis* (CDC-16284; ATCC 14955) were reconstituted and plated onto Trypticase soy agar supplemented with defibrinated sheep blood (5%), yeast extract (0.5%), hemin (0.005%), and vitamin K₁ (0.001%). Well-isolated colonies were picked into thioglycolate broth (BBL, 0135C), and the cultures were checked for purity by the procedures of Dowell and Hawkins (2). Actively growing thioglycolate cultures were inoculated into 200 ml of Schaedler broth (BBL), which was used for producing cells for fatty acid analysis. After incubation in an anaerobic atmosphere (85% N₂, 10% H₂, 5% CO₂) for 24 to 36 h at 35°C, cells were removed by centrifugation and washed three times with distilled water.

After centrifugation, the cells were saponified and the fatty acids were methylated by the procedure described previously (8). Methyl esters were analyzed on a Perkin-Elmer model 900 gas chromatograph (Perkin-Elmer, Norwalk, Conn.) equipped with a flame ionization detector and a disc integrator recorder. Samples were analyzed on a nonpolar 3% OV-1 methyl silicone column and on a polar 15% ethylene glycol adipate column as described previously (7, 8). The ethylene glycol adipate

column was used primarily to separate iso- from anteiso-branched-chain acids (7). Fatty acid methyl ester peaks were tentatively identified by comparing retention times on each column with retention times of methyl ester standards (Applied Science Lab, State College, Pa.; Analabs, North Haven, Conn.; Supelco, Bellefonte, Pa.). Final identification was established by a combination of techniques including hydrogenation (1), bromination (8), and mass spectrometry (12). Combined gas chromatography-mass spectrometry of methyl esters was done with a DuPont instrument type 21-491B equipped with a combination electron impact-chemical ionization source. Isobutane was used as reagent gas for the chemical ionization source.

The chromatograms in Fig. 1 clearly show that there are major differences in the cellular fatty acids of these organisms. Approximately 80% of the total fatty acids of *P. anaerobius* were saturated branched-chain acids, and the remainder were saturated straight-chain acids. This was confirmed by the fact that no change occurred in retention times of any peak in the chromatogram upon acetylation, hydrogenation, or bromination, indicating the absence of hydroxy, unsaturated, and cyclopropane acids (1, 8). The branched methyl group position in the fatty acid chain was first indicated by analysis on the ethylene glycol adipate column, where iso-branched-chain esters are eluted slightly before their anteiso-homologue (7). Retention time data from this column indicated that the major peaks in *P. anaerobius* were iso-branched-chain acids 10:0, 12:0, 14:0, and 16:0; anteiso-branched-chain acids 11:0, 13:0, and 15:0 were also present. The identity of each of these acids was confirmed by comparing its mass spectrum with authentic standards. Electron impact mass spectra of anteiso-branched methyl esters were clearly distinguished from iso-branched and normal methyl esters by com-

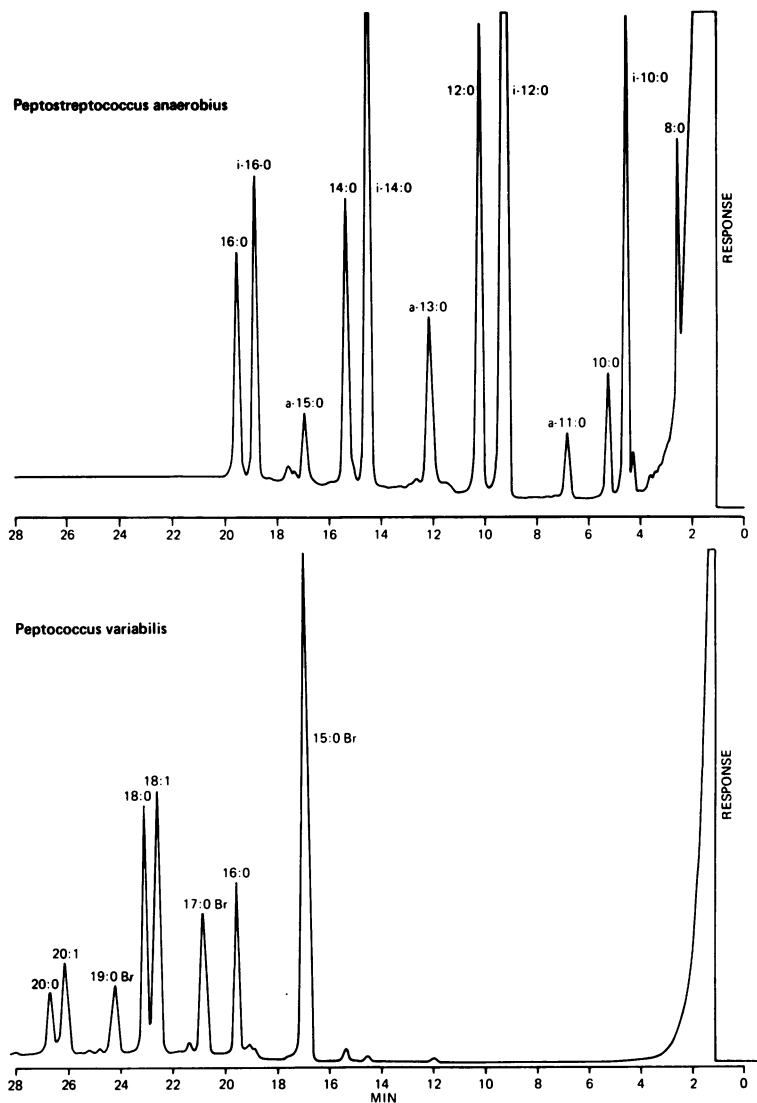


FIG. 1. Gas chromatogram of esterified fatty acids from saponified whole cells of *P. anaerobius* (top) and *P. variabilis* (bottom). Analysis was made on a 0.16-inch (4.06-mm ID) by 12-ft (3.66 m) coiled glass column packed with 3% OV-1 methyl silicone. Helium was used as carrier gas at a flow rate of 50 ml/min. The column temperature was 160°C, and, after injection of the sample, it was programmed to 265°C at a rate of 5°C/min. Sensitivity of the flame ionization detector was 2.5×10^{-12} A; attenuation, 32×10^2 ; peak designation numbers: before colon refers to the number of carbon atoms, to the right refers to the number of double bonds; Br, branched-chain acids; i-, a methyl group at the penultimate carbon atom; and a-, a methyl group at the ante-penultimate carbon.

paring the ratio of $m/e = M-29$ and $m/e = M-31$ peaks in the electron impact spectra. With ante-iso-esters, the M-29 is equal to or greater than the M-31, whereas the M-31 peak is approximately two times the size of the M-29 peak in iso-branched- and normal straight-chain esters (12, 13).

Acids less than 15 carbons in length were absent, or present in only trace amounts, in *P.*

variabilis (Fig. 1). Monounsaturated 18:1 and 20:1 acids present in this organism were confirmed by hydrogenation and mass spectrometry. Relatively large amounts of branched-chain acids (15:0, 17:0, 19:0) were present. Gas-liquid chromatographic analysis on the ethylene glycol adipate column (7) showed both iso- and ante-iso-isomers for each branched-chain acid in a ratio of approximately 2:1 (iso-an-

teiso). Identities of these acids were confirmed by comparing the $m/e = M-29$ and $m/e = M-31$ relationship by mass spectrometry (12, 13).

Although branched-chain fatty acids have been found in other bacteria (6, 11), the presence of large amounts of iso-branched-chain acids 10:0, 12:0, and 14:0, as compared with amounts of other acids present, appears unique to *P. anaerobius*. However, additional strains of this and other species of anaerobic cocci must be studied to establish the usefulness of cellular fatty acids as a means of distinguishing this group of organisms.

ADDENDUM

A report of the cellular fatty acids of various species of anaerobic cocci was recently published (C.L. Wells and C.R. Field, *J. Clin. Microbiol.* 4: 515-521, 1976). Although many of the fatty acids were not identified and different growth media were used, the gas chromatographic profiles for *P. anaerobius* and *P. variabilis* were comparable to those shown in Fig. 1.

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