Cellular Fatty Acid Compositions of an Unidentified Organism and a Bacterium Associated with Cat Scratch Disease

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The cellular fatty acid composition of a gram-negative bacterium associated with cat scratch disease was determined by capillary gas chromatography-mass spectrometry (GC-MS). The fatty acid profile of this organism was distinct from those of other bacteria we have tested and was characterized by an unknown acid which was identified as 11-methyloctadec-12-enoic acid. The position of the branched methyl group in this acid was established by GC-MS of the reduced acid, and the location of the double bond was confirmed by GC-MS analysis of dimethyl disulfide derivatives. Another clinical isolate with no known relationship to cat scratch disease but with similar morphological and biochemical features had a similar fatty acid profile, including 11-methyloctadec-12-enoic acid.

In 1989, an organism was isolated from a tibial biopsy by Geraldine S. Hall, Cleveland Clinic, Cleveland, Ohio, and subsequently received by the Special Bacteriology Reference Laboratory of the Centers for Disease Control through the Ohio State Health Department. This culture, which was designated G1849 by the Special Bacteriology Reference Laboratory, yielded very faint opaque colonies on chocolate agar at 35°C in 48 h and punctate, smooth, gray-white colonies on buffered charcoal-yeast extract agar at 30°C in 72 h. The cells were gram-negative, very delicate, pale-staining, thin, slightly curved, short- to medium-length rods and were motile, with a single polar, subpolar, or lateral flagellum. The organism could not be identified by our conventional cultural and biochemical testing scheme (3). The cellular fatty acid composition of G1849 determined by gas-liquid chromatography (GLC) also differed from those of a large number of diverse bacteria we have tested and contained about 12% unknown acid which had not been observed previously.

During testing of strain G1849, another isolate, received from D. J. Wear, Armed Forces Institute of Pathology, Washington, D.C., was observed to have morphological and biochemical characteristics essentially identical to those of strain G1849. This organism (or its cell wall-deficient mutant) was observed earlier by English et al. (4) in lymph node specimens obtained from 10 patients with cat scratch disease (CSD). Bacteriological culturing of these specimens showed faint growth of small, delicate, pleomorphic, colonial forms (4). Upon subsequent passage, 9 of 10 isolates yielded only minute growth; however, 1 isolate, which is thought to have spontaneously reverted to a vegetative form, was readily cultivable (4). A transfer of this culture was kindly provided to the Centers for Disease Control and designated as G1492 by the Special Bacteriology Reference Laboratory. The cellular fatty acids of G1492 were similar to those of G1849 and also contained moderate amounts (10 to 15%) of the unknown acid. In this report, we describe the fatty acid

compositions of these two isolates and the identification of the unknown acid.

Cells for fatty acid analysis were grown on heart infusion agar supplemented with 5% rabbit blood and on buffered charcoal-yeast extract agar plates at 30°C. After 48, 72, and 96 h of incubation, the cells were removed and processed for cellular fatty acid analysis as described previously (8). The resulting fatty acid methyl ester (FAME) samples were analyzed with the Hewlett-Packard 5898A microbial identification system (Hewlett-Packard Co., Avondale, Pa.), which consists of the following: an HP5890 gas chromatograph with a flame ionization detector and a methyl phenyl silicone fused silica capillary column (25 m [length] by 0.2 mm [inner diameter]), an HP7373 automatic sampler, an HP3392 integrator, an HP9133 computer, and a printer. Tentative identification of FAMES was accomplished by comparing computer-calculated equivalent chain length (ECL) values with ECL values of authentic standards; confirmation was accomplished by hydrogenation of unsaturated FAMES and combined gas chromatography-mass spectrometry (GC-MS). The GC-MS analyses were performed on an HP5890A gas chromatograph interfaced to an HP5970 mass selective detector (Hewlett Packard). Electron impact spectra were recorded at 70 eV. Separation was achieved on a fused silica capillary column (25 m by 0.2 mm [inner diameter]) coated with 0.33 µm of methylphenylpolysiloxane (HP-5; Hewlett Packard). The column was operated from 120 to 300°C at 8°C/min and with helium at 7 lb/in². Location of the double-bond position of monounsaturated fatty acids was accomplished by GC-MS analysis of dimethyl disulfide derivatives of the FAMES (10).

The GLC fatty acid profile of strain G1492 is shown in Fig. 1. Seven acids were present in the organism, with G_{18:1ω7c} being the major component (35%), followed by C_{16:1ω7c} (16%), C_{17:0cyc} (15%), C_{16:0} (5%), C_{18:0} (5%), C_{19:0cyc} (11%), and about 11% unknown acid. No branched-chain acids were present, but small amounts (1 to 3%) of 2- and 3-hydroxydodecanoic acids (2-OH-C_{12:0}, 3-OH-C_{12:0}) and 3-hydroxyoctadecanoic acid (3-OH-C_{18:0}) were released with acid hydrolysis, indicating that these acids were amide

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FIG. 1. Gas chromatogram of methylated fatty acids of strain G1492. Fatty acids are represented by shorthand designations: the number before the colon represents the number of carbon atoms; the number after the colon represents the number of double bonds; $\omega 7c$ represents the double-bond position from the hydrocarbon end of the *cis* isomer; and cyc represents the cyclopropane ring. UN, Unknown.

linked within the cell. Separate preparations of G1492 cells grown for 48, 72, and 96 h on either heart infusion agar or buffered charcoal-yeast extract agar medium gave similar fatty acid results. The cellular fatty acid composition of strain G1849 was essentially identical to that of G1492 and contained about 15% unknown acid.

The FAME of the unknown acid (ECL, 18.082 ± 0.003)

eluted from the GLC capillary column about 8 s after $C_{18:0}$ (ECL, 18.000 ± 0.003). The mass spectrum of this peak showed a molecular ion at m/z 310, together with peaks at m/z 279, m/z 278 (loss of OCH_3 , CH_3OH), and m/z 194 (loss of 116 atomic mass units), suggesting a monounsaturated 19-carbon FAME. Upon hydrogenation, the unknown FAME was completely converted to its saturated homolog and a new peak with an ECL of 18.407. Compared with the ECL values for several other saturated 19-carbon FAME standards, this ECL value most closely matched that of tuberculostearic (10-methyloctadecanoic) acid, suggesting the presence of one or more branched methyl groups within the hydrocarbon chain of the unknown acid.

The mass spectrum of the hydrogenated (reduced) unknown peak at an ECL of 18.407 is shown in Fig. 2. The large molecular ion (M^+) at m/z 312, a base peak ion at m/z 74, and the $M^+ - 29$, $M^+ - 31$, and $M^+ - 43$ ions are consistent with a saturated 19-carbon FAME (6, 9). The enhanced peaks at m/z 213 and m/z 185 indicated a methyl branch at carbon-11. The m/z 213 ion [$+CH(CH_3)-(CH_2)_9COOCH_3$] decomposed further, losing methanol (m/z 213 minus 32 = m/z 181) or methanol and water (m/z 213 minus 32 minus 18 = m/z 163), characteristic of the methyl branch-containing fragment (11). Further indication of the position of the methyl branch was obtained by complete hydrogenation of the unknown FAME to its corresponding alkane, thereby converting the carboxyl group into a methyl group containing one deuterium atom (1). The resulting mass spectrum of the alkane showed a molecular ion at m/z = 269 and major fragment ions at m/z 126 and m/z 169 (Fig. 3). The cleavage at the methyl branch produced a deuterium-containing 12-carbon fragment at m/z 170 and a nonlabeled 9-carbon fragment at m/z 127. The corresponding olefin peaks from m/z 170 (at m/z 169) and from m/z 127 (at m/z 126) were enhanced because of the length of the carbon chain (7). This fragmentation pattern is consistent with the structure of

FIG. 2. Electron impact mass spectrum of the unknown $C_{19:1}$ FAME (ECL, 18.082 [Fig. 1]) after catalytic hydrogenation to its reduced form ($C_{19:0}$).

FIG. 3. Electron impact mass spectrum of 1-deutero-11-methyloctadecane derived from the unknown $C_{19:1}$ FAME after complete reduction to the alkane. D, Deuterium.

1-deutero-11-methyloctadecane and confirms the structure of the hydrogenated (reduced) unknown peak as 11-methyloctadecanoic acid methyl ester.

Having established the position of the branched methyl group in the unknown acid, we located the double bond by GC-MS analysis of the dimethyl disulfide derivative prepared from the original FAME sample. The resulting mass spectrum of the FAME-dimethyl disulfide adduct showed a molecular ion at m/z 404 and two major ions at m/z 273 and m/z 131 which were attributable to fragmentation between the two CH_3S groups located at the original site of unsaturation. The m/z 131 ion is the aliphatic fragment (ω) and the m/z 273 ion is the fragment containing the carboxyl end (Δ) of the molecule. A fourth major ion was observed at m/z 241 and resulted from the loss of methanol (CH_3OH) from the Δ fragment (m/z 273 minus 32 = m/z 241). This fragmentation clearly showed the location of the double bond to be at the ω_6 (or Δ_{12}) position. Thus, the combined GLC and GC-MS data firmly established the identity of the unknown peak (ECL, 18.082; Fig. 1) as 11-methyloctadec-12-enoic acid. To our knowledge, this acid has not been found previously in bacteria.

The unique fatty acid profile of strain G1492 is easily distinguished from those of other gram-negative bacteria and other bacteria such as mycobacteria and *Rothia* species, which have also been proposed as the cause of CSD (2, 5). The mycobacteria contain tuberculostearic (10-methyloctadecanoic) acid, and *Rothia dentocariosa* has large amounts of branched-chain acids, which are absent in G1492. Thus, the GLC fatty acid analysis provides a rapid means for comparing the chemical relatedness of suspect CSD isolates; its sensitivity will allow testing of isolates that produce only faint growth on initial culturing (2, 4). The presence of 11-methyloctadec-12-enoic acid may prove to be a useful chemical marker of these bacteria.

There is no apparent clinical relationship between strains

G1492 and G1849. Strain G1492 was from a patient with clinically diagnosed CSD, while strain G1849 was isolated from a subcutaneous abscess which developed in the pretibial region of a 69-year-old man during hospitalization following surgery. However, on the basis of cellular fatty acid compositions, morphological features, biochemical characteristics, and recent DNA studies (D. J. Brenner, personal communication), these two strains appear to be significantly related. The pathogenic potential, if any, of strain G1849 should be studied further. Additional evidence to support G1492 as the etiological agent of CSD requires the isolation, in vitro cultivation, and subsequent bacteriological, chemical, and genetic testing of other isolates from patients with clinically or histologically proved CSD.

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