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CFA-FAME. The CFA-FAME technique is based on the conversion of cellular fatty acids to methyl esters by mild alkaline methanolysis, followed by GLC analysis. Isolates were cultivated on a standard medium: Columbia II agar base (BBL 4397596) with 5% horse blood, aerobically for 16-48h. at 37°C. Fastidious organisms were cultivated on Chocolate agar (complex formulation), either in CO_2, microaerophilic or anaerobic for 16h to 72h at the optimum temperature. Cells were removed from the plate using a plastic inoculating loop, carefully scraped to avoid including medium in the sample. 50-100 mg of cells were then transferred to glass tubes. In the first step, cells were saponified; 1 ml methanolic base (45 g NaOH, 150 ml methanol, 150 ml distilled water) was added before vortexing for 5-10 s and heating to 100 °C for 5 min. After vortexing again, tubes were heated for a further 25 min at 100 °C. Cells were then methylated as follows: after cooling in cold water, 2 ml methylation reagent (240 ml 6.0M hydrochloric acid, 205 ml methanol) was added and the tubes were vortexed for 5-10 s, heated at 80 °C for 10 min, and then rapidly cooled. Finally, fatty acids were extracted by addition of 1.25 ml of a 50/50 mixture of analytical grade hexane and methyl-*tert*-butyl ether to each tube. After 10 min on a rocking shaker, the aqueous phase was discarded, 3 ml NaOH (0.3 M) was added and the combination was mixed before centrifugation. The upper phase was carefully removed and used for analysis. Analysis was carried out with a Hewlett Packard HP 5890 Gas Chromatograph equipped with a phenyl methyl silicone fused silica capillary column (HP-5 25m x 0.2 mm x 0.33 mm film thickness) and a flame ionization detector. Hydrogen was used as the carrier gas. The temperature programme was initiated at 170 °C and increased at 5 °C min^{-1} to a final temperature of 270 °C.
Integration of peaks and further calculations was performed by a HP 3396A integrator. After correction of the area%, retention time data were converted to Equivalent Chain Length (ECL) values. Correlating calculated ECL's with known ECL values in the Peak Naming Table identifies peaks. The Agilent MIS FAME standard was used as reference in the analysis. The electronic data was stored on a computer hard disk and the fatty acid methyl ester composition of the sample was compared to a stored database using the Eerola-Lehtonen-formula for probabilistic calculations.

References:

Sasser, M. ***Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids.*** Technical Note #101. 2001: MIDI Inc, Newark, DE.

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Text basically adopted from:

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