Reassessment of the Phylogenetic Position of Caulobacter subvibrioides

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Determination of the 16S rRNA gene sequence of Caulobacter subvibrioides ATCC 15264T (T = type strain) confirmed that this species is a member of the alpha subclass of the Proteobacteria and showed that it is phylogenetically most closely related to the Caulobacter group comprising the species Caulobacter bacteroides, Caulobacter crescentus, and Brevundimonas (Pseudomonas) diminuta, for which 16S rRNA sequences of the type strains are currently available. The closest known relative of strain ATCC 15264T among these species is B. diminuta (level of direct pairwise sequence similarity, 95%). On the basis of its previously determined 16S rRNA sequence (accession number M83797), C. subvibrioides is most closely related to Sphingomonas adhaesiva in the alpha-4 subgroup (level of similarity, 97.7%). Analysis of the hydroxy fatty acids of C. subvibrioides ATCC 15264T showed that the 2-hydroxyxyrmyristic acid which is characteristic of the genus Sphingomonas was absent.

In 1992 Stahl et al. (20) reported on the phylogeny of the genus Caulobacter. These authors noted that most of the caulobacters, which belong to the alpha subclass of the Proteobacteria (20, 23), made up a diverse but coherent phylogenetic assemblage based on a comparison of their 16S rRNA sequences; the only exception was Caulobacter subvibrioides, which was only peripherally related to the main Caulobacter assemblage. However, insufficient outgroup reference sequences were used for accurate phylogenetic positioning of C. subvibrioides, and the relationship of this organism with other members of the alpha subclass of the Proteobacteria remained unclear.

Later, Hugenholtz et al. (4) showed that the published 16S rRNA sequence of C. subvibrioides CB81 (nucleotide sequence accession number M83797) was most closely related to the sequences of members of the alpha-4 subgroup of the Proteobacteria and that C. subvibrioides grouped with Blastobacter natatorius (19), Erythrobacter longus (15), and Porphyrobacter neustonensis (3). Subsequently, it was found that the genera Sphingomonas and Rhizomonas were also members of the expanding alpha-4 subgroup (21, 22). Strain CB81 was designated the type strain of the species by Pointecker (12) and was deposited in the American Type Culture Collection as strain ATCC 15264. The culture used by Stahl et al. (20) also originated as strain CB81 but has had a different history since.

In our experience the designated type strain of C. subvibrioides, strain ATCC 15264 (17), is not a typical member of the alpha-4 subgroup and appears to be typical of the caulobacters. Consequently, we decided to examine the hydroxy fatty acid profile of the type strain of C. subvibrioides to see if it contained the signature 2-hydroxyyrsyric acid characteristic of the fatty acids of other members of the alpha-4 subgroup, including Sphingomonas spp. (24), Rhizomonas spp. (21, 22), and B. natatorius (16).

C. subvibrioides ATCC 15264T (T = type strain) was received by us from the American Type Culture Collection in 1982 and was deposited in the Australian Collection of Microorganisms as strain ACM 2483T. Cells from our original freeze-dried ampoules were grown in peptone-yeast extract medium (18) with agitation in an environmental incubator at 28°C, harvested by centrifugation, washed twice in distilled water, and freeze-dried. Hydroxy fatty acids were extracted and methylated by direct acid methanolysis (2 ml of 2 M methanolic HCl, 85°C, 18 h). Fatty acid esters (FAMES) were extracted three times in 1 ml of hexane-chloroform (4:1) after 1 ml deionized water was added to the extract to improve phase separation. The combined extract (3 ml) was dried under nitrogen, 50 μl of chloroform was added, and the hydroxy acids were converted to their corresponding trimethylsilyl (TMS)-ether derivatives with bis(trimethylsilyl)trifluoroacetamide–1% trimethylchlorosilane (50 μl) during incubation for 2 h at 70°C (6). Following cooling to room temperature, the samples were evaporated to dryness with nitrogen gas and then redissolved in chloroform (200 μl). TMS-hydroxy FAMES were analyzed by gas chromatography-mass spectrometry by using a Varian model 3300 gas chromatograph linked directly to a Hewlett-Packard model 5970 mass selective detector. Samples (1 μl) were injected in splitless (1-min) mode. The analytical column used was an Alltech BP5 column (25 m by 0.22 mm [inside diameter]; phase thickness, 0.33 μm), the oven temperature was programmed to increase at a rate of 4°C/min from 150 to 270°C with 0- and 7.5-min hold times, respectively, and the injection temperature was 260°C. The helium carrier gas flow rate was 8 ml/min. The mass spectrometer was run in total-ion chromatograph mode (50 to 500 amu). Constituent FAMES were identified by comparing retention times and mass spectra with the retention times and mass spectra of TMS-derivatized fatty acid standards (4). The results of the analysis showed that no hydroxy fatty acids were present in C. subvibrioides ATCC 15264T. As 2-hydroxyxyrmyric acid was absent in C. subvibrioides but present in Blastobacter natatorius (16; this study), an organism with which the available 16S rRNA sequence of C. subvibrioides (accession number M83797) shows 93.4% sequence similarity (4), the relationship between these two organisms was placed in doubt.

We therefore decided to sequence the 16S ribosomal DNA of C. subvibrioides ATCC 15264T in order to confirm or clarify the phylogenetic position of this organism.

Extraction of genomic DNA and amplification of the 16S rRNA gene were performed as described by Dorsch and Stackebrandt (1). The PCR products were purified by using a Micro-Spin S-300 purification column (Pharmacia Biotech) as...
FIG. 1. Unrooted phylogenetic tree obtained by a neighbor-joining analysis of 16S rRNA sequences, showing the position of C. subvibrioides in the alpha subclass of the Proteobacteria. The scale bar represents 10 nucleotide substitutions per 100 nucleotides of 16S rRNA sequence. Bootstrap values from 100 analyses are shown at the branch points of the tree. The accession numbers of the sequences of the organisms used in the analysis are as follows: Blastobacter natatorius ACM 2507, Z73043; Brevundimonas diminuta ATCC 11568, M59064; Caulobacter bacteroides ATCC 15254, M83796; Caulobacter crescentus CB2 (= ATCC 15252, X52281); Caulobacter subvibrioides ATCC 15264, X94470; Caulobacter subvibrioides CB81, M83797; Porphyromonas neustonensis ACM 2844, L01785; Rhizomonas subterranei IFO 15211, D13737; Rhodopseudomonas capsulata ATCC 11166, D16428; Rhodobacter sphaeroides ATCC 17023, X53855; Rhodogdia centenaria ATCC 45720, D12701; Rhodopseudomonas palustris ATCC 17001, L16664; Rhodospirillum rubrum ATCC 11170, M32920; Sphingomonas adhaesiva IFO 15099 (= Gifu 11458), D18410; Sphingomonas capsulata Gifu 11526 (= ATCC 14466), D16147; Sphingomonas parapaucimobilis IFO 15100, D13724; Sphingomonas paucimobilis Gifu 2395 (= ATCC 29837), D16144; Sphingomonas sanguis ACM 2843, L11664; Sphingomonas tartae IFO 15098, L13727; Zymomonas mobilis, Zym.mobil. (Ribosomal RNA Database Project database). The sequence of Agrobacterium tumefaciens IAM 13129 (accession number D12784) was used as the outgroup.

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REFERENCES


