Determination of the systematic position of the genus *Asticcacaulis* Poindexter by a polyphasic analysis

Wolf-Rainer Abraham,¹ Carsten Strömpl,¹ Marc Vancanneyt,² Heinrich Lünsdorf¹ and Edward R. B. Moore¹

Author for correspondence: Wolf-Rainer Abraham. Tel: +49 531 6181 419. Fax: +49 531 6181 411. e-mail: wab@gbf.de

¹ GBF – German Research Centre for Biotechnology, Dept Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany

² Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium

The genus *Asticcacaulis*, to date, comprises two species of unicellular, stalked bacteria, developing a stalk at a site which is not coincidental with the centre of the pole of the cell. Multiplication is similar to that demonstrated by the prosthecate species of the genera *Caulobacter*, *Brevundimonas* and *Maricaulis*. A polyphasic approach, comprising 16S rRNA gene sequencing, lipid analysis and NaCl tolerance characterizations, was used to clarify the taxonomy of the two *Asticcacaulis* species. From the analysis of the 16S rRNA gene sequences, a close phylogenetic relationship between the species that comprise the genera *Asticcacaulis*, *Caulobacter* and *Brevundimonas* could be deduced wherein the three genera form three distinct branches. The individual genera could also be discerned by different characteristic polar lipids. The species of *Asticcacaulis* differed from species of *Caulobacter* and *Brevundimonas* by the lack of 1,2-diacyl-3-O-[6'-phosphatidyl- α -D-glucopyranosyl]glycerol. They also did not contain 1,2-di-O-acyl-3-O-[D-glucopyranosyl-(1 $\rightarrow$ 4)- α -D-glucuronopyranosyl]-glycerol, which is found in most *Brevundimonas* species but not in strains of the genus *Caulobacter*. The morphological differences seen between the two species *Asticcacaulis* *excentricus* and *Asticcacaulis* *biprosthecium* are mirrored by the observed 16S rDNA sequence similarity value of 95.3%, which is relatively low compared to the interspecies similarity values observed within the genera *Brevundimonas* or *Caulobacter*.

Keywords: *Asticcacaulis* *excentricus*, *Asticcacaulis* *biprosthecium*, *Caulobacter*, *Brevundimonas*, polyphasic approach

INTRODUCTION

The genus *Asticcacaulis*, to date, comprises two species of unicellular, stalked bacteria, developing a stalk at a site which is not coincidental with the centre of the pole of the cell. The stalk does not possess adhesive material; this is produced instead by the cell at or near the pole of the cell adjacent to the stalk. Multiplication is similar to that demonstrated by species of the genus *Caulobacter*, i.e. by division of the stalked cell, giving rise to a swarmer cell which is motile by means of a

single flagellum arising at an excentric position on the pole of the cell. This cell subsequently develops a stalk and enters the immotile vegetative phase. All strains have been isolated from freshwater samples.

The genus was named *Asticcacaulis* (Gr. *privative* without; Anglo-Saxon *sticca* stick; L. *caulis* stalk) because of the non-sticking stalks of the cells of the first described species, *Asticcacaulis* *excentricus* (Poindexter, 1964). A second species, *Asticcacaulis* *biprosthecium*, was described by Pate *et al.* (1973). Since then, few reports on strains of this genus have been published and molecular data on the two type strains are scarce. Recently, Sly *et al.* (1999) reported on the 16S rRNA gene sequences of the two *Asticcacaulis* type strains. In the course of our study of caulobacteria (Abraham *et al.*, 1997, 1999), an interest in the lipids of the *Asticcacaulis* spp. and the phylogeny

Abbreviations: CID, collision-induced dissociation; FAB-MS, fast atom bombardment-MS; PG, phosphatidylglycerol.

The EMBL accession numbers for the 16S rRNA gene sequences of *Asticcacaulis* *excentricus* DSM 4724^T and *Asticcacaulis* *biprosthecium* DSM 4723^T are AJ247194 and AJ247193, respectively.

of this genus was generated. The 16S rDNA sequences, which do not coincide with the sequences reported by Sly *et al.* (1999), and the polar lipids of *A. excentricus* and *A. biprosthecium* and their comparison with those of other caulobacteria are reported in this study.

METHODS

Organisms and culture conditions. *A. excentricus* DSM 4724^T and *A. biprosthecium* DSM 4723^T were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). The type strains of the type species of the genera *Caulobacter* (*Caulobacter vibrioides* DSM 9893^T) and *Brevundimonas* (*Brevundimonas diminuta* LMG 2089^T) were obtained from the DSMZ and the Laboratorium voor Microbiologie, Universiteit Gent, Belgium, respectively. All strains were grown in PYEM medium (2 g peptone, 2 g yeast extract, 0.5 g NH₄Cl, 1 l deionized water). After autoclaving and cooling, 5 ml riboflavin (0.2 mg ml⁻¹, sterile filtered), 2 ml 50% glucose (sterile), 1 ml 20% MgSO₄ (sterile) and 1 ml 10% CaCl₂ (sterile) were added. The strains were incubated in 2 l flasks at 30 °C, shaken at 100 r.p.m.; the biomass was harvested by centrifugation, at late exponential growth phase, after 72 h.

Electron microscopy. Cells of the late growth phase were negatively stained with uranyl acetate (2%, pH 4.5) for analysis by transmission electron microscopy as described in detail by Yakimov *et al.* (1998).

16S rDNA sequencing and analysis. Cell pellets from lipid analysis were suspended in 100 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and boiled for 5 min. The lysate was centrifuged briefly and 1 µl supernatant, containing DNA, was used for PCR; the 16S rRNA genes were targeted using a forward primer, hybridizing at positions 8–27, and a reverse primer, hybridizing at the complement of positions 1525–1541 (*Escherichia coli* 16S rRNA gene sequence numbering). PCR was carried out using conditions described previously (Karlson *et al.*, 1993). Amplified 16S rDNA was purified using Microcon 100 microconcentrators (Amicon) and the nucleotide sequences were determined using an Applied Biosystems 373A DNA sequencer and the protocols recommended by the manufacturer (Perkin Elmer) for *Taq* polymerase-initiated cycle sequencing with fluorescent dye-labelled dideoxynucleotides. The sequencing primers have been described previously (Lane, 1991). The nucleotide sequences were aligned with reference 16S rRNA and 16S rRNA gene sequences using evolutionarily conserved primary sequence and secondary structure (Gutell *et al.*, 1985) as references. Evolutionary distances (Jukes & Cantor, 1969) were calculated from sequence-pair dissimilarities using only homologous, unambiguously determined nucleotide positions (ambiguous nucleotide positions were excluded from sequence pair comparisons). Phylogenetic trees were constructed using the programs of the PHYLIP package (Felsenstein, 1989).

Whole-cell fatty acid analysis. Cells were grown for 48 h on PYEM agar plates (1.5% agar). Cells were saponified [15% (w/v) NaOH, 30 min, 100 °C], methylated to fatty acid methyl esters (FAMES) (methanolic HCl, 10 min, 80 °C) and extracted [hexane/methyl-*tert*-butyl ether (1:1, v/v)] as described in detail by Osterhout *et al.* (1991). FAMES were analysed by GC. Separation of FAMES was achieved using fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenyl-methyl silicone (film thickness 0.33 µm; HP Ultra2). The computer-controlled parameters were the

same as those described by Osterhout *et al.* (1991). The instrument was equipped with an FID and an autosampler. H₂ served as the carrier gas.

Polar lipid fatty acid analysis. Lipids were extracted, using a modified Bligh–Dyer procedure (Bligh & Dyer, 1959) and FAMES were generated and analysed by GC, as described previously (Abraham *et al.*, 1997).

Fast atom bombardment (FAB)-MS. FAB-MS in the negative mode was performed on the first of two mass spectrometers of a tandem high-resolution instrument of E₁B₁E₂B₂ configuration (JMS-HX/HX110A; JEOL Tokyo) at 10 kV accelerating voltage. Resolution was set to 1:1500. The JEOL FAB gun was operated at 6 kV with xenon as the FAB gas. A mixture of triethanolamine and tetramethylurea (2:1, v/v) was used as matrix. In tandem MS, negative daughter ion spectra were recorded using all four sectors of the tandem mass spectrometer. High energy collision-induced dissociation (CID) took place in the third field-free region. Helium served as the collision gas at a pressure sufficient to reduce the precursor ion signal to 30% of the original value. The collision cell was operated at ground potential. Resolution of MS2 was set to 1/1000. FAB-CID spectra (linked scans of MS2 at constant B/E ratio) were recorded at 300 Hz filtering with a JEOL DA 7000 data system.

Salt-tolerance characterization. Type strains were grown in 20 ml PYEM medium amended with 0, 5, 10, 20, 30, 40, 60, 80 or 100 g NaCl l⁻¹. The OD of the cell suspension (measured at 600 nm) was set to 0.3 at the beginning of the experiment and determined at time intervals over 2 d. The differences between these measurements were used to determine salt tolerances.

RESULTS

16S rRNA gene sequences

The nucleotide sequences of the nearly complete 16S rRNA genes have been determined for the type strains of the two species of *Asticcacaulis*. A primer pair annealing at the 5′- and 3′-termini of the 16S rRNA genes was used allowing the amplification and sequence determination of 1416 nucleotide positions (approx. 97% of the complete gene as estimated by comparison with the 16S rRNA gene sequence of *E. coli*). The sequence data have been deposited with the EMBL database (Stoesser *et al.*, 1998) under accession nos AJ247194 (*A. excentricus* DSM 4724^T) and AJ247193 (*A. biprosthecium* DSM 4723^T). The 16S rRNA gene sequences of *A. excentricus* and *A. biprosthecium* had 95.3% sequence similarity and comprised a distinct phylogenetic branch separate from *Caulobacter* and *Brevundimonas* species. Fig. 1 presents a dendrogram depicting the estimated phylogenetic/taxonomic positions of *Asticcacaulis* species, with respect to each other and the described species of the *Caulobacter* and *Brevundimonas* genera within the α -Proteobacteria.

Gas chromatographic analysis of FAMES

The cellular fatty acid compositions of both *Asticcacaulis* type strains are shown in Table 1. Dominant fatty acids for both species (the relative

Fig. 1. Unrooted dendrogram showing the estimated phylogenetic relationships based on 16S rDNA sequence comparisons of the species of the genera *Asticcacaulis*, *Caulobacter* and *Brevundimonas* within the context of the α -Proteobacteria. The dendrogram was generated using the FITCH algorithm in the PHYLIP package. The scale bar represents evolutionary distance as the mean number of fixed mutations per nucleotide position since the point of branching. *Rickettsia typhi* is included as an outgroup. The sequence data not resulting from this study were obtained from the DDBJ/EMBL/GenBank and/or RDP databases under the following accession numbers: *Rickettsia typhi* str. Wilmington (L36221), *Sphingomonas paucimobilis* DSM 1098^T (X72722), *Rhodospirillum rubrum* str. ATH 1.1.1 (RDP), *Agrobacterium tumefaciens* bv.1 NCPPB 2437 (D14500), *Rhodobacter sphaeroides* IL 106 (D16424), *Paracoccus denitrificans* LMG 4218^T (X69159), *Caulobacter vibrioides* DSM 9893^T (AJ227754), *Caulobacter henricii* ATCC 15253^T (AJ227758), *Caulobacter fusiformis* ATCC 15257^T (AJ227759), *Brevundimonas diminuta* LMG 2089^T (AJ227778), *Brevundimonas vesicularis* LMG 2350^T (AJ227780), *Brevundimonas bacteroides* LMG 15096^T (AJ227782), *Brevundimonas variabilis* ATCC 15255^T (AJ227783), *Brevundimonas subvibrioides* LMG 14903^T (AJ227784), *Brevundimonas alba* DSM 4736^T (AJ227785), *Brevundimonas intermedia* ATCC 15262^T (AJ227786), *Brevundimonas aurantiaca* DSM 4731^T (AJ227787), [*Caulobacter*] *leidy* ATCC 15260^T (AJ227812), *Hyphomonas polymorpha* DSM 2665^T (AJ227813), *Maricaulis maris* ATCC 15268^T (AJ227802), *Asticcacaulis excentricus* DSM 4724^T (AB016610, AJ247194) and ACM 1263^T (AJ007800), *Asticcacaulis biprosthecium* DSM 4723^T (AB014055, AJ247193) and ACM 2498^T (AJ007799).

percentages for *A. biprosthecium* and *A. excentricus*, respectively, are given in parentheses) are: 12:1 3-OH (7.5 and 4.2%); 15:0 (2.9 and 2.3%); 16:0 (20.8 and

17.3%); summed feature 4 (13.8 and 7.4%); 17:1 ω 6c (1.0 and 1.5%); 18:1 ω 7c (40.7 and 57.7%); and 11-methyl 18:1 ω 7c (3.3 and 3.6%). *A. biprosthecium*

Table 1. Percentage whole-cell fatty acid composition in *Asticcacaulis* species and related taxa

Only the fatty acids counting for more than 1.0% in one of the strains studied were indicated. Abbreviations: —, not detected; tr, trace amount (less than 1.0%); ECL, equivalent chain-length (the identity of the fatty acid is not known); SF, summed feature.

Species	ECL 11:798	12:0	12:0 3-OH	12:1 3-OH	14:0	15:0	16:0	SF 4*	16:1 2-OH	16:0 iso	17:0	17:1 ω 6c	17:1 ω 8c	ECL 17:897	SF 7†	ECL 18:080	ECL 18:140	ECL 18:797	19:0	19:0 cyclo ω 8c
<i>A. biprosthecium</i> DSM 4723 ^T	tr	2.5	—	7.5	1.1	2.9	20.8	13.8	—	—	—	1.0	—	—	40.7	3.3	3.2	—	—	—
<i>A. excentricus</i> DSM 4724 ^T	—	—	—	4.2	—	2.3	17.3	7.4	—	1.8	1.8	1.5	1.1	—	57.7	3.6	—	—	—	—
<i>B. diminuta</i> LMG 2089 ^T	—	—	1.5	tr	tr	7.6	10.1	1.0	—	—	8.5	6.2	10.8	1.1	38.7	—	—	3.1	—	6.2
<i>C. vibrioides</i> DSM 9893 ^T	1.6	—	tr	1.2	1.4	5.8	6.0	9.4	6.1	—	8.6	3.0	2.4	—	35.8	4.3	—	3.7	1.5	—

* Summed feature 4 consisted of one or more of the following fatty acids which could not be separated by the Microbial Identification System: 15:0 iso 2-OH, 16:1 ω 7c and 16:1 ω 7t.

† Summed feature 7 consisted of one or more of the following fatty acids: 18:1 ω 7c, 18:1 ω 9t and 18:1 ω 12t.

DSM 4723^T could be differentiated from *A. excentricus* DSM 4724^T by the presence of small amounts of 12:0, 14:0, ECL 18:140, and the absence of 16:0 iso, 17:0 and 17:1 ω 8c.

Analysis of the polar lipids

Glycolipids present in all *Asticcacaulis* spp. were α -D-glucopyranosyl- and α -D-glucuronopyranosyl-diacylglycerols, which are also common in *Caulobacter*, *Brevundimonas* and some other α -Proteobacteria. Using CID-MS, the structures of twelve of the predominant glycolipids listed in Table 2 were identified. The (–)-FAB mass spectra of the phospholipids of both *Asticcacaulis* species showed clear differences to those of the type species of *Caulobacter* and *Brevundimonas* (Fig. 2). Phospholipids of the *Asticcacaulis* spp. were of the phosphatidylglycerol (PG) type and five phospholipids could be identified.

Salt-tolerance characterization

A. biprosthecium DSM 4723^T and *A. excentricus* DSM 4724^T showed reduced growth without salt, optimal growth with 5 g NaCl l^{–1} and no growth at salt concentrations of 30 g l^{–1} or more (Fig. 3). *Caulobacter vibrioides* DSM 9893^T, the type species of the genus, showed the same reduced growth without NaCl, optimal growth with 5 g NaCl l^{–1} and no growth above 10 g l^{–1}. In contrast, the type strain of *Brevundimonas*, *B. diminuta* LMG 2089^T, grew without NaCl, tolerated NaCl concentrations up to 40 g l^{–1} and did not grow above 80 g NaCl l^{–1}.

DISCUSSION

The 16S rRNA gene sequence similarities of the species of *Asticcacaulis* ranged from 91.6 to 92.4% with species of *Caulobacter* and a 91.9% sequence similarity was

observed between the 16S rRNA genes of *A. excentricus* (the type species of the genus) and *C. vibrioides* (the type species of *Caulobacter*). Additionally, the 16S rRNA gene sequence similarities of the species of *Asticcacaulis* ranged from 92.3 to 94.4% with species of *Brevundimonas* and 93.1% sequence similarity was observed between the 16S rRNA genes of *A. excentricus* and *B. diminuta* (the type species of *Brevundimonas*).

A high overall similarity was observed between the fatty acid content of *Asticcacaulis* type strains and those of *Caulobacter* and *Brevundimonas* reference strains (Table 1; Abraham *et al.*, 1999). All members of the *Asticcacaulis*–*Caulobacter*–*Brevundimonas* phylogenetic branch are characterized by the dominant fatty acids 16:0 and 18:1 ω 7c (summed feature 7 in Abraham *et al.*, 1999) and the majority of the reference strains of this group of organisms contain significant amounts of the fatty acids 15:0, summed feature 4, 17:1 ω 6c and 11-methyl 18:1 ω 7c (ECL 18:080 in Abraham *et al.*, 1999). Furthermore, a common feature for *A. biprosthecium* and *A. excentricus* was the presence of 12:1 3-OH, a feature that was also observed to be characteristic for *Caulobacter* species, but not for *Brevundimonas* taxa. *A. biprosthecium* could be differentiated from *A. excentricus* by some marked quantitative differences in the dominant fatty acids, by the presence of low amounts of 12:0, 14:0 and ECL 18:140, and by the absence of 16:0 iso, 17:0 and 17:1 ω 8c (see above). No 14:0 2-OH was present, a characteristic feature for [*Caulobacter*] *leidyi* and members of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990). The fatty acid data are consistent with the phylogenetic position of the genus *Asticcacaulis* near to the genera *Caulobacter* and *Brevundimonas* and not in the genus *Sphingomonas* as indicated by Sly *et al.* (1999).

The species of *Asticcacaulis* differ from *Caulobacter* and *Brevundimonas* by the lack of 1,2-diacyl-3-O-

Table 2. Polar lipids identified in *Asticcacaulis* spp. using CID-MS

Glycolipid			Phospholipid		
Mass	Type*	Fatty acid	Mass	Type†	Fatty acid
730	MGDOx	16:0-15:0	734	PG	18:1-15:0
742	MGD	18:1-15:0	746	PG	18:1-16:1
742	MGDOx	16:0-16:1	748	PG	18:1-16:0
744	MGD	17:0-16:0	762	PG	18:1-17:0
744	MGDOx	16:0-16:0	774	PG	18:1-18:1
768	MGDOx	18:1-16:1			
768	MGDOx	18:2-16:0			
770	MGDOx	18:1-16:0			
782	MGD	18:1-18:1			
782	MGD	19:1-17:1			
784	MGD	19:1-17:0			
784	MGDOx	18:1-17:0			

* MGD, 1,2-di-*O*-acyl-3-*O*- α -D-glucopyranosylglycerol;

MGDOx, 1,2-di-*O*-acyl-3-*O*- α -D-glucuronopyranosylglycerol.

† PG, phosphatidylglycerol.

[6'-phosphatidyl- α -D-glucopyranosyl]glycerol (PGL). They also did not contain 1,2-di-*O*-acyl-3-*O*-[D-glucopyranosyl-(1 $\rightarrow$ 4)- α -D-glucuronopyranosyl]glycerol (DGL) found in most *Brevundimonas* strains but not in strains of the genus *Caulobacter*. Sphingolipids, characteristic for strains of the genus *Sphingomonas*, were not detected in any of the *Asticcacaulis* species. There were also differences in the lipid composition within the different lipid types detected. From the lipids found, (17:0-16:0)- and (19:1-17:0)- α -D-glucopyranosyl-diacylglycerol, and (16:0-15:0)- and (18:2-16:0)- α -D-glucuronopyranosyl-diacylglycerol were not detected in *Caulobacter* or *Brevundimonas* strains (70 strains analysed). Phospholipids were PG and, from the five identified lipids, (18:1-17:0)-PG was not found in *Caulobacter* or *Brevundimonas* strains (Table 3).

The heterogeneity observed in the 16S rDNA sequences and lipid data of the *Asticcacaulis* spp. was mirrored in the tolerance to NaCl, resulting in a broad range of tolerances by the individual species to NaCl concentrations between 10 and 20 g l⁻¹ (Fig. 3). While *A. biprosthecium* DSM 4723^T showed almost the same NaCl tolerance as *C. vibrioides* DSM 9893^T, *A. excentricus* DSM 4724^T tolerated higher NaCl concentrations. *B. diminuta* LMG 2089^T differed from both *Asticcacaulis* spp. in that it grew well without salt and tolerated much higher NaCl concentrations than even *A. excentricus*.

The sequence similarity of 95.3% between the two described species of *Asticcacaulis*, which is rather low compared to the similarity values observed between the species of *Brevundimonas* or the species of *Caulobacter* (Abraham *et al.*, 1999), suggests that they may be members of separate genera. While bacterial

taxonomy cannot be based solely upon 16S rRNA gene sequence data, in many cases sequence comparisons and cluster analysis are able to recognize cases wherein the phylogeny of organisms does not agree with the described taxonomy. However, the level of 16S rRNA gene sequence similarity observed between the two species of *Asticcacaulis* does not allow one to evaluate confidently whether the organisms should be separated in two distinct genera or retained within the same genus. This is supported by the different morphologies and, in part, by the lipid data and the different salt tolerances. However, although the two species, ultimately, may be separated taxonomically, due to the fact that only a single strain for each species has been analysed to date, it is prudent to retain the current classification of the two species within the genus *Asticcacaulis* until other, more definitive, data provide evidence for reclassification.

Taxonomic implications

During the course of this study, sequence data for the 16S rRNA genes of the two *Asticcacaulis* species were submitted, by two separate groups (Sly *et al.*, 1999; Hamada *et al.*, 1998) to the DDBJ/EMBL/GenBank databases. The sequence data determined in the current study were compared with these sequences. The 16S rRNA gene sequence determined in this study for *A. excentricus* (DSM 4724^T $\leftarrow$ ATCC 15261^T) was observed to be identical for all 1416 homologous nucleotide positions for the sequence determined by Hamada *et al.* (1998) (strain ATCC 15261^T, accession no. AB016610), yet only 85.1% similar to the sequence determined by Sly *et al.* (1999) (strain ACM 1263^T = ATCC 15261^T, accession no. AJ007800). The 16S rRNA gene sequence determined in this study for *A. biprosthecium* (DSM 4723^T $\leftarrow$ ATCC 27554^T) was observed to be identical over all 1406 homologous nucleotide positions for the sequence determined by Hamada *et al.* (1998) (strain ATCC 27554^T, accession no. AB014055), yet only 84.6% similar to the sequence determined by Sly *et al.* (1999) (strain ACM 2498^T = ATCC 27554^T, accession no. AJ007799). Cluster analysis of the sequences of the strains of this study (as well the sequences determined by Hamada *et al.*, 1998) indicates a phylogenetic relationship of *Asticcacaulis* species to *Caulobacter* and *Brevundimonas* species (Fig. 1). This was corroborated by fatty acid data, which were closely related to those of the species of the genera *Caulobacter* and *Brevundimonas* while the 2-hydroxy fatty acids, characteristic for the *Sphingomonas paucimobilis* branch, were absent. However, the sequences determined by Sly *et al.* (1999) cluster with the sequences of species of *Sphingomonas* and [*Caulobacter*] *leidy*. These observations suggest that the strains which have been sequenced have been misidentified in at least one case. In the course of this study, the strains were obtained from the DSMZ, cultivated and examined by microscopy to confirm the development of the characteristic prosthecate appendages described for the two species of *Asticcacaulis*.

Fig. 2. (—) FAB mass spectrum of the phospholipid fraction of *A. biprosthecium* DSM 4723^T (a), *Brevundimonas diminuta* LMG 2089^T (b), *Caulobacter vibrioides* DSM 9893^T (c) and *Asticcacaulis excentricus* DSM 4724^T (d). In the range m/z 250–300 the acyl ions, around m/z 750 phosphatidylglycerol (PG) ions, between m/z 930–990 1,2-di-*O*-acyl-3-*O*-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)- α -D-glucuronopyranosyl]glycerol (DGL) and finally around m/z 1410–1465 1,2-diacyl-3-*O*-[6'-phosphatidyl- α -D-glucopyranosyl]glycerol (PGL) are seen. Note that PGL is only seen in the *Caulobacter* and *Brevundimonas* strains, but is absent in the *Asticcacaulis* strains, and that DGL is only present in the *Brevundimonas* strain.

Fig. 3. Comparison of the growth of *Asticcacaulis biprosthecium* DSM 4723^T ▲ and *A. excentricus* DSM 4724^T ◆ and the type strains of the species *Caulobacter* (*C. vibrioides* DSM 9893^T; ●) and *Brevundimonas* (*B. diminuta* LMG 2089^T; ■) in the presence of different concentrations of NaCl.

Fig. 4. Morphology of negatively stained late-log cells from *Asticcacaulis excentricus* DSM 4724^T (a) and *Asticcacaulis biprosthecium* DSM 4723^T (b). Arrowheads indicate the offshoots of the prostheca relative to the cell body. Bars, 1.1 µm.

(Fig. 4). The cell morphologies of the two strains of this study conformed with the formal descriptions of the respective species. From our examinations, it is concluded that the strains received from DSMZ match, in all aspects, the descriptions given by Poindexter (1964) and Pate *et al.* (1973). It would be very helpful if ACM and DSMZ would check the authenticity of their *Asticcacaulis* type strains in order to find out the origin of the differences in the 16S rRNA gene sequences reported by Sly *et al.* (1999) and in this study.

From the data presented here, a close relationship between the species that comprise the genera *Asticcacaulis*, *Caulobacter* and *Brevundimonas* can be deduced, as was proposed more than three decades ago (Poindexter, 1964). The three genera form three distinct branches within the phylogenetic tree derived from the comparison of the 16S rRNA gene sequences. The individual genera can also be discerned by their characteristic polar lipids. It is noteworthy that the phylogenetic branch including the genera *Caulobacter*,

Brevundimonas and *Asticcacaulis* is comprised predominantly, albeit not exclusively, of organisms capable of developing cellular prosthecate 'holdfast' appendages, although this morphological characteristic is now recognized to occur within diverse phylogenetic lineages. Further, within the phylogenetic branch including the genera *Caulobacter*, *Brevundimonas* and *Asticcacaulis*, species capable of developing prosthecae are recognized to cluster with organisms (i.e. *Brevundimonas* species, *Caulobacter segnis*) which have not been observed to produce them (Abraham *et al.*, 1999). Thus, it is clear, from observations of the phylogenetic relationships of 'appendaged bacteria', as derived from 16S rRNA gene sequence comparisons, that the expression of the prosthecate-forming phenotype in bacteria is widespread and does not, in itself, comprise a taxonomic signature. However, a specific type of appendage, e.g. the characteristic, subpolar, pseudostalk of *Asticcacaulis* species, may be confined to species of defined phylogenetic lineages. The genus *Asticcacaulis* clearly deserves more

Table 3. Polar lipids as biomarkers in *Asticcacaulis* spp., *Brevundimonas* spp. and *Caulobacter* spp. (Abraham *et al.*, 1999)

+, +, +, Present in all strains; +, +, present in most strains (> 80%); blank, absent. PG, Phosphatidylglycerol; SQDG, 1,2-diacyl-3-*O*-sulfoquinovosylglycerol; DGL, 1,2-di-*O*-acyl-3-*O*-[D-glucopyranosyl-(1 → 4)-α-D-glucuronopyranosyl]glycerol; PGL, 1,2-diacyl-3-*O*-[6'-phosphatidyl-α-D-glucopyranosyl]glycerol; MGD, 1,2-di-*O*-acyl-3-*O*-α-D-glucopyranosylglycerol; MGDOx, 1,2-di-*O*-acyl-3-*O*-α-D-glucuronopyranosylglycerol.

Genus	PG	SQDG	DGL	PGL	MGD	MGDOx
<i>Asticcacaulis</i>	+++				+++	+++
<i>Caulobacter</i>	+++			+++	+++	+++
<i>Brevundimonas</i>	+++	++	++	+++	+++	+++

attention from microbiologists which, hopefully, will result in the characterization of more isolates and a deeper insight into the intra- and intergeneric taxonomy of this interesting group of bacteria as well as its ecological role in the environment.

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