Asticcacaulis benevestitus sp. nov., a psychrotolerant, dimorphic, prosthecate bacterium from tundra wetland soil

Lina V. Vasilyeva,¹ Marina V. Omelchenko,¹ Yulia Y. Berestovskaya,¹ Anatolii M. Lysenko,¹ Wolf-Rainer Abraham,² Svetlana N. Dedysh¹ and George A. Zavarzin¹

Correspondence

Svetlana N. Dedysh
dedysh@mail.ru

¹S. N. Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-let Otyabrya 7/2, Moscow 117312, Russia

²German Research Centre for Biotechnology, Chemical Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany

A Gram-negative, aerobic, heterotrophic, non-pigmented, dimorphic prosthecate bacterium was isolated from tundra wetland soil and designated strain Z-0023^T. Cells of this strain had a dimorphic life cycle and developed a non-adhesive stalk at a site not coincident with the centre of the cell pole, a characteristic typical of representatives of the genus *Asticcacaulis*. A highly distinctive feature of cells of strain Z-0023^T was the presence of a conical, bell-shaped sheath when grown at low temperature. This prosthecate bacterium was a psychrotolerant, moderately acidophilic organism capable of growth between 4 and 28 °C (optimum 15–20 °C) and between pH 4.5 and 8.0 (optimum 5.6–6.0). The major phospholipid fatty acid was 18:1 ω 7c and the major phospholipids were phosphatidylglycerols. The G + C content of the DNA was 60.4 mol%. On the basis of 16S rRNA gene sequence similarity, strain Z-0023^T was most closely related to *Asticcacaulis biprosthecium* (98 % similarity), *Asticcacaulis taihuensis* (98 %) and *Asticcacaulis excentricus* (95 %). However, low levels of DNA–DNA relatedness to these organisms and a number of distinctive features of the tundra wetland isolate indicated that it represented a novel species of the genus *Asticcacaulis*, for which the name *Asticcacaulis benevestitus* sp. nov. is proposed. The type strain is Z-0023^T (= DSM 16100^T = ATCC BAA-896^T).

Dimorphic prosthecate bacteria (DPB) are present in almost any sample of freshwater or seawater and in many types of soils (Henrici & Johnson, 1935; Poindexter, 1964, 1981a, 1999; Nikitin *et al.*, 1966; Belyaev, 1968; Staley, 1968; Staley *et al.*, 1987; Lapteva, 1987). An important characteristic of DPB is their ability to metabolize organic materials available in extremely low quantities and to tolerate prolonged periods of nutrient scarcity (Larson & Pate, 1975; Poindexter, 1981b; Vasilyeva & Zavarzin, 1995).

DPB are characterized by asymmetric cell reproduction. Each reproductive event in these bacteria produces two siblings: a sessile cell with a cellular stalk (a prostheca) and a motile cell with a polar flagellum. On the basis of the

morphology of the reproductive stage (fission or budding), two fundamentally different types of DPB are distinguished: caulobacteria (*Caulobacter*, *Brevundimonas*, *Maricaulis* and *Asticcacaulis*) and hyphomicrobia (*Hyphomicrobium*, *Pedomicrobium* and *Hyphomonas*) (Poindexter, 1999; Abraham *et al.*, 1999). Caulobacteria are especially widespread in environments where nutrient concentrations and ambient temperatures are low (Poindexter, 1981a, 1999; Staley *et al.*, 1987). The taxonomy of caulobacteria has for many years relied primarily upon morphological criteria. Recently, a polyphasic approach, comprising 16S rRNA gene sequencing, lipid analysis and NaCl tolerance characterization, was used to analyse a large set of strains of these bacteria (Abraham *et al.*, 1999, 2001). It has been shown that caulobacteria form two different phylogenetic lineages within the *Alphaproteobacteria*, one comprising the freshwater and brackish water representatives of the group *Caulobacter*–*Brevundimonas*–*Asticcacaulis*, and the other comprising the marine species of the genus *Maricaulis*.

All known representatives of the genus *Asticcacaulis* have been isolated from freshwater samples. Sessile cells of these

Abbreviations: DPB, dimorphic prosthecate bacteria; FAMES, fatty acid methyl esters.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Z-0023^T is AM087199.

Tables showing data for utilization of carbon compounds, glycolipid and phospholipid fatty acid content, and polar lipid content of *Asticcacaulis* species are available as supplementary material in IJSEM Online.

bacteria develop subpolar or lateral prosthecae, which do not possess adhesive material at their tips. Instead, the cells bear holdfast material directly on their surfaces. At the time of writing, the genus comprised three recognized species, *Asticcacaulis excentricus* (Poindexter, 1964), *Asticcacaulis biprosthecium* (Pate *et al.*, 1973) and *Asticcacaulis taihuensis* (Liu *et al.*, 2005). Representatives of the genus *Asticcacaulis* are rarely observed and even more rarely isolated. In this report, we describe a novel species of the genus that has been isolated from tundra wetland peat.

The peat sample was collected from a depth of 3–6 cm of a shrub tundra wetland, north-east of Vorkuta in the Polar Urals of Russia (68° N 52° E). One gram of peat was homogenized in 10 ml sterile water, and 1 ml of this suspension was used to inoculate 100 ml of a liquid dilute peptone–yeast extract (PY) medium containing 0.005 % peptone and 0.005 % yeast extract, pH 6.8, supplemented with 1 % (v/v) vitamin stock solution and 2 % (v/v) modified Hutner's basal salts as described by Staley (1968). For enrichment of psychrophilic bacteria, incubation was carried out for 2 months at 6 °C. The resultant enriched cell suspension was spread-plated onto the surface of the PY medium supplemented with sodium acetate (0.1 %, w/v) and solidified with Difco agar (2 %, w/v), and the plates were then further incubated at 6 °C. As colonies became visible, they were successively restreaked onto fresh plates with the same agar medium until a culture with uniform colonies was obtained. This isolate, designated Z-0023^T, was maintained both on agar slants and in liquid cultures using the above-described PY medium with acetate (PY-A) and PYG medium (0.1 % peptone, 0.1 % yeast extract, 0.1 % glucose, pH 6.7). Cell morphology and cell life cycle, as well as culture purity, were examined by using phase-contrast and electron microscopy. In the latter case, the samples were stained with 1 % (w/v) uranyl acetate. For preparation of thin sections, cells were collected from agar plates and prefixed with 1.5 % (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 6.5) for 1 h at 4 °C and then fixed in 1 % (w/v) OsO₄ in the same buffer for 4 h at 20 °C. After dehydration in a graded series of ethanol, the samples were embedded in a Spurr epoxy resin. Thin sections were cut on an LKB-4800 microtome, and stained with 3 % (w/v) uranyl acetate in 70 % (v/v) ethanol. The specimen samples were examined with a JEM-100C transmission electron microscope. Growth of the isolate was monitored by nephelometry at 600 nm for 2–4 weeks in PY-A and PYG liquid media under a variety of conditions, including temperatures of 4–37 °C, pH 4.5–8.3 and NaCl concentrations of 0.1–5.0 % (w/v). Variations in acidity level were achieved by mixing 0.05 M solutions of Na₂HPO₄ and KH₂PO₄ to create media with the same ionic strength. To determine the range of potential growth substrates of strain Z-0023^T, the following carbon sources were tested using liquid PY medium with addition of the respective compounds at a concentration of 0.1 % (w/v): D-glucose, L-arabinose, D-ribose, D-xylose, D-galactose, D-fructose, lactose, D-maltose, sucrose, D-cellobiose, D-mannose,

D-melibiose, raffinose, L-rhamnose, D-trehalose, D-mannitol, D-sorbitol, starch, acetate, propionate, butyrate, pyruvate, malate, fumarate, succinate, citrate, methanol, ethanol, propanol, butanol, L-arginine, DL-alanine, L-phenylalanine, L-glutamate, L-lysine, L-proline, L-hydroxyproline, L-serine and L-tryptophan. Growth was examined after 8 days incubation at 20 °C. Sensitivity to antibiotics was tested by spreading 2-day-old cell suspension onto PYG agar medium and applying filter discs containing the following antibiotics: polymixin M (300 U), benzylpenicillin (10 U), ristomycin (30 µg), canomycin (30 µg), monomycin (30 µg), tetracycline (30 µg), erythromycin (15 µg), streptomycin (30 µg) and levomycetin (30 µg). Growth was assessed after 2 days incubation at 20 °C.

For fatty acid analyses, cells of strain Z-0023^T were grown on PYG agar plates at 10 and 20 °C. 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 on a gas chromatograph equipped with a flame-ionization detector and an autosampler. Separation of FAMES was achieved using a fused-silica capillary column (25 m × 0.2 mm) with cross-linked 5 % phenyl methyl silicone (film thickness 0.33 µm; HP Ultra 2). Injection temperature was 250 °C and detector temperature was 300 °C. The oven programme was 150 °C for 2 min, then increased from 150 to 289 °C at 4 °C min⁻¹, followed by an isothermal period of 11 min. The instrument was equipped with a flame-ionization detector and an autosampler; H₂ served as the carrier gas. FAMES were identified by comparison with standards or by GC-MS (Abraham *et al.*, 1998). For polar lipid fatty acid analysis, lipids were extracted using a modified Bligh–Dyer procedure (Bligh & Dyer, 1959) as described previously by Abraham *et al.* (1997). The extract of the total lipids was analysed by electrospray ionization using a quadrupole time-of-flight mass spectrometer (Yakimov *et al.*, 2004).

Genomic DNA from strain Z-0023^T was extracted using an SDS-based assay as described by Dedysh *et al.* (1998). The DNA base composition of strain Z-0023^T was determined by thermal denaturation using a Unicam SP1800 spectrophotometer at a heating rate of 0.5 °C min⁻¹. The G + C value was calculated by using the equation of Owen *et al.* (1969): $G + C (\%) = T_m \times 2.08 - 106.4$. The DNA of *Escherichia coli* K-12 was used as the standard. DNA–DNA hybridization experiments between strain Z-0023^T and two species of the genus *Asticcacaulis*, i.e. *A. excentricus* (DSM 4724^T) and *A. biprosthecium* (DSM 4723^T), were carried on nitrocellulose membrane filters (Hybond-N; Amersham International) according to Lysenko *et al.* (1988). The genome size of strain Z-0023^T was calculated by using the equation of De Ley *et al.* (1970): $M = [98.37 - 0.91 \times G + C (\%)]/k$, where k is the DNA reassociation rate. PCR-mediated amplification of the 16S rRNA gene from positions 28 to 1491 (numbering according

to the International Union of Biochemistry nomenclature for *E. coli* 16S rRNA) was performed using primers Eub9f and Eub1492r and reaction conditions described by Lane (1991). The 16S rRNA gene amplicons were purified using QIAquick spin columns (Qiagen) and sequenced on an ABI Prism 377 DNA sequencer using BigDye terminator chemistry, as specified by the manufacturer (PE Applied Biosystems). Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al.*, 2004).

Following growth on PY-A agar for 2 weeks at 6 °C, small (1–2 mm in diameter), smooth, circular, convex with an

entire edge, opaque, white colonies were observed. Single colonies were successively selected from plates for restreaking until a pure culture, designated Z-0023^T, was obtained. Microscopic examination revealed that cells of strain Z-0023^T were Gram-negative, motile rods, 0.5–0.7 µm in width and 1.4–2.0 µm in length (Fig. 1a). These cells reproduced by binary fission and underwent a dimorphic life cycle during which a sessile cell with a cellular stalk (a prostheca) divided to give rise to a motile cell with a polar flagellum (Fig. 1b). A single, non-adhesive prostheca of these bacteria was 0.10–0.15 µm in diameter and had an excentral, subpolar location on the cell (Fig. 1c, d), which is

Fig. 1. (a) Phase-contrast micrograph of cells of strain Z-0023^T grown on PY-A medium for 3 weeks at 6 °C. (b–e) Electron micrographs of cells of strain Z-0023^T. (f) Electron micrograph of a section taken through the area of juncture of cell and prostheca of strain Z-0023^T. Bars: (a), 10 µm; (b)–(f), 0.5 µm. p, Prostheca; f, flagellum; sh, sheath; sb, stalk bands.

characteristic of representatives of the genus *Asticcacaulis* (Poindexter, 1964; Pate *et al.*, 1973). Prostheca length ranged from 0.5 to 5.0 µm depending on cultivation conditions; maximal length was attained in defined minimal media. In cells grown on rich complex media (e.g. PYG medium), prosthecae were short or were not observed, and the cells themselves appeared significantly elongated (up to 10-fold) and misshapen.

The major distinctive feature of cells of strain Z-0023^T was the presence of a conical, bell-shaped sheath attached to the sessile cell at the point of juncture of prostheca and cell (Fig. 1d–f). Cell division occurred within this sheath (Fig. 1d). Interestingly, these clothes-like sheaths appeared on cells grown at low temperatures (below 10 °C) but not on cells grown at moderate temperatures (15–25 °C).

In stationary liquid cultures, most of the growth of strain Z-0023^T occurred in the form of a pellicle that developed at the surface of the medium. In liquid cultures incubated on a rotary shaker, cells were evenly dispersed throughout the culture.

Similar to other known representatives of the genus *Asticcacaulis*, strain Z-0023^T was not capable of growth in a mineral medium with glucose or another compound as the only growth substrate. It had an absolute requirement for the presence of growth factors in cultivation media. Thus, utilization of a given carbon compound was assumed to have occurred when growth was distinctly improved in its presence compared with on basal PY medium alone. The carbon compounds tested and the results are presented in Supplementary Table S1 (available in IJSEM Online). Most sugars, ethanol and several organic acids (acetate, malate, fumarate and succinate) were the preferred growth substrates. Strain Z-0023^T differed from *A. excentricus* and *A. biprosthecium* by its inability to utilize pyruvate, and it could also be differentiated from *A. taihuensis* by its inability to utilize D-cellobiose, D-mannose and D-melibiose.

Strain Z-0023^T grew in the pH range 4.5–8.3 with the optimum at pH 5.6–6.0. The temperature range for growth was 4–28 °C with the optimum at 15–20 °C. The culture generation time under optimal temperature conditions, calculated from increases in OD₆₀₀ in the exponential phase of growth, was in the range 30–35 h. Both the specific growth rate and the growth yield of the culture at 25–28 °C were significantly lower than those attained at 15–20 °C. No growth occurred at 37 °C.

Compared with *A. excentricus* DSM 4724^T and *A. biprosthecium* DSM 4723^T (Abraham *et al.*, 2001), NaCl was not required for growth of strain Z-0023^T and this isolate had less tolerance of dissolved salts. Growth inhibition of 50 % was observed in the presence of NaCl in the medium at concentrations of 1–1.5 % (w/v), whereas NaCl at concentrations above 2.0 % completely inhibited growth. Strain Z-0023^T was susceptible to antibiotics that inhibit prokaryotic protein synthesis, i.e. streptomycin, tetracycline, erythromycin, levomycetin and monomycin.

Whole-cell fatty acid content and comparison of glycolipid and phospholipid fatty acid profiles of strains Z-0023^T, *A. biprosthecium* DSM 4723^T, *A. excentricus* DSM 4724^T and *A. taihuensis* T3-B7^T are shown in Table 1 and in Supplementary Table S2 in IJSEM Online, respectively. As with recognized members of the genus *Asticcacaulis*, 18:1ω7c was the major fatty acid in strain Z-0023^T. The distinguishing feature of the fatty acid profile of strain Z-0023^T was the absence of 12:1 3-OH, which was considered to be a common feature of the FAME profiles in *Asticcacaulis* (Abraham *et al.*, 2001). Glycolipids present in recognized *Asticcacaulis* species are α-D-glucopyranosyl- and α-D-glucopyranuronosyl-diacylglycerols, which are also common in *Caulobacter*, *Brevundimonas* and some other members of the *Alphaproteobacteria*. In strain Z-0023^T, four different glycolipids were detected, among them a compound of 796 Da previously detected in *A. biprosthecium* DSM 4723^T but lacking in *A. excentricus* DSM 4724^T. The electrospray ionization mass spectra of the phospholipids of strain Z-0023^T showed clear differences from those of the other type strains (Supplementary Table S3 in IJSEM Online). Phospholipids of this bacterium were of the phosphatidylglycerol type and six different phospholipids could be identified, analogous to *A. biprosthecium* DSM 4723^T and *A. excentricus* DSM 4724^T. Interestingly, most of the phospholipids detected in strain Z-0023^T were also found in *A. excentricus* DSM 4724^T but not in *A. biprosthecium* DSM 4723^T.

Comparative sequence analysis of the 16S rRNA gene revealed that strain Z-0023^T belonged to the *Alphaproteobacteria* and,

Table 1. Whole-cell fatty acid content of the novel prosthecae isolate Z-0023^T and other representatives of the genus *Asticcacaulis*

Taxa: 1, Z-0023^T; 2, *A. biprosthecium*; 3, *A. excentricus*; 4, *A. taihuensis*. Values shown are percentage of total fatty acids.

FAME	1	2	3	4
12:0	—	2.5	—	—
12:0 3-OH	—	—	—	2.3
12:1 3-OH	—	7.5	4.2	2.6
14:0	—	1.1	—	1.1
15:0a	—	—	—	2.6
15:0	3.4	2.9	2.3	1.5
16:1ω7/ω9	14.8	13.8	7.4	7.4
16:0	10.6	20.8	17.3	28.7
16:0 iso	—	—	1.8	—
16:0 2-OH	—	—	—	10.4
17:0 cyclo 7,8	2.2	—	—	—
17:0	1.7	—	1.8	—
17:1ω6c	—	1.0	1.5	—
17:1ω8c	—	—	1.1	—
18:1ω7	57.5	40.7	57.7	41.9
18:0	0.9	—	—	—
19:0 cyclo 8,9	—	—	—	1.5

more precisely, that it could be included in the phylogenetic lineage comprising the freshwater and brackish water representatives of the group *Caulobacter*–*Brevundimonas*–*Asticcacaulis* (Fig. 2). The novel isolate from tundra wetland peat was most closely related to the three known representatives of the genus *Asticcacaulis*, i.e. *A. excentricus* DSM 4724^T (95 % 16S rRNA gene sequence similarity), *A. taihuensis* T3-B7^T (98 %) and *A. biprosthecium* DSM 4723^T (98 %). The DNA G+C content of strain Z-0023^T was 60.4 mol% and the genome size was 2.5×10^9 Da. Levels of DNA–DNA hybridization between strain Z-0023^T and *A. biprosthecium* DSM 4723^T and *A. excentricus* DSM 4724^T were 40 and 35 %, respectively.

The combined morphological and genotypic characteristics reported above indicate that strain Z-0023^T belongs to the genus *Asticcacaulis*. However, compared with *A. biprosthecium* and *A. excentricus*, the novel strain has greater tolerance to low temperatures and low pH, but has higher sensitivity to dissolved salts. We were not able to compare temperature and pH growth ranges of strain Z-0023^T with those of *A. taihuensis*, as these characteristics have not been reported for the latter bacterium. The unique morphological feature of strain Z-0023^T was the presence of bell-shaped sheaths that appeared on cells grown at low temperatures. Strain Z-0023^T also differed from all three recognized species of the genus *Asticcacaulis* with regard to the assimilation of several substrates (Table 2) and the absence of fatty acid 12:1 3-OH in the FAME profile. Thus we propose a novel species, *Asticcacaulis benevestitus* sp. nov., to accommodate this prosthecate bacterium from tundra wetland peat. The major characteristics differentiating this novel species from recognized species of the genus *Asticcacaulis* are summarized in Table 2.

Table 2. Major characteristics that distinguish strain Z-0023^T from other species of the genus *Asticcacaulis*

Taxa: 1, Z-0023^T; 2, *A. excentricus* (data taken from Poindexter, 1964); 3, *A. biprosthecium* (data taken from Pate *et al.*, 1973); 4, *A. taihuensis* (data taken from Liu *et al.*, 2005). v, Variable; ND, not determined.

Characteristic	1	2	3	4
Number of prosthecae per cell	1	1	1–2	1
Presence of a sheath	+	–	–	–
Utilization of:				
Pyruvate	–	+	+	ND
L-Arabinose	+	v	–	+
Sucrose	+	+	–	+
D-Mannose	–	+	–	+
D-Cellobiose	–	ND	ND	+
D-Melibiose	–	ND	ND	+
Temperature optimum (°C)	15–20	30	30	ND
pH optimum	5.6–6.0	6.5	7.2	ND
NaCl requirement for growth	–	+	+	ND
Growth at >2 % (w/v) NaCl	–	+	+	–
Presence of 12:1 3-OH fatty acid	–	+	+	+
DNA G+C content (mol%)	60.4	55	61	59

Description of *Asticcacaulis benevestitus* sp. nov.

Asticcacaulis benevestitus (be.ne.ves'ti'tus. L. adv. *bene* well; L. part. adj. *vestitus* clothed/clad; N.L. masc. part. adj. *benevestitus* well clad).

Cells are rod-shaped, Gram-negative, 0.5–0.7 by 1.4–2.0 µm. Reproduces by binary fission. Undergoes a

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain Z-0023^T in relation to *Asticcacaulis biprosthecium*, *Asticcacaulis excentricus*, *Asticcacaulis taihuensis*, prosthecate bacteria of the genera *Caulobacter*, *Brevundimonas* and *Maricaulis*, and some other representative members of the *Alphaproteobacteria*. Bootstrap values (1000 data resamplings) of >50 % are shown. The 16S rRNA gene sequence of the gammaproteobacterium *Pseudomonas stutzeri* (AF143245) was used as an out-group (not shown). Bar, 0.1 substitutions per nucleotide position.

dimorphic life cycle during which a sessile cell with one excentral, subpolar prostheca divides to give rise to a motile cell with a single, polar flagellum. A single, non-adhesive prostheca 0.10–0.15 µm in diameter arises from a subpolar site. At temperatures below 10 °C most prosthecae cells are embedded in a conical bell-shaped sheath, which is attached to the cell at the point of juncture of prostheca and cell. Colonies are opaque, white, smooth, circular, convex with an entire edge, and 1–2.5 mm in diameter after 7 days growth at 20 °C on PYG medium. Chemo-organotrophic aerobe. Carbon sources include glucose, sucrose, xylose, maltose, galactose, arabinose, lactose, fructose, ethanol, acetate, malate, fumarate, succinate, raffinose, rhamnose and trehalose. Yeast extract is required for growth. Temperature range for growth is 4–28 °C (optimum at 15–20 °C). Growth occurs between pH 4.5 and 8.3, with an optimum at pH 5.6–6.0. NaCl is not required for growth and is tolerated up to a concentration of 2.0% (w/v). Susceptible to antibiotics that inhibit prokaryotic protein synthesis. The major fatty acid is 18:1 ω 7c and the major phospholipids are phosphatidylglycerols. The DNA G+C content is 60.4 mol%. Genome size is 2.5×10^9 Da.

The type strain, Z-0023^T (=DSM 16100^T=ATCC BAA-896^T), was isolated from a tundra wetland in the Vorkuta region, northern Russia.

Acknowledgements

This research was supported by the Federal Scientific-Technical Program 'Biogenic sinks, sources, and reservoirs of the greenhouse gases' no. 43.016.11.1622, the Russian Science Support Foundation and the programme 'Molecular and Cell Biology' of the Russian Academy of Sciences.

References

- Abraham, W.-R., Meyer, H., Lindholm, S., Vancanneyt, M. & Smit, J. (1997). Phospho- and sulfolipids as biomarkers of *Caulobacter*, *Brevundimonas* and *Hyphomonas*. *Syst Appl Microbiol* **20**, 522–539.
- Abraham, W.-R., Hesse, C. & Pelz, O. (1998). Ratios of carbon isotopes in microbial lipids as an indicator of substrate usage. *Appl Environ Microbiol* **64**, 4202–4209.
- Abraham, W.-R., Strömpl, C., Meyer, H. & 8 other authors (1999). Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *Int J Syst Bacteriol* **49**, 1053–1073.
- Abraham, W.-R., Strömpl, C., Vancanneyt, M., Lünsdorf, H. & Moore, E. R. B. (2001). Determination of the systematic position of the genus *Asticcacaulis* Poindexter by a polyphasic analysis. *Int J Syst Evol Microbiol* **51**, 27–34.
- Belyaev, S. S. (1968). Distribution of the caulobacter group of bacteria in the Volga-Don reservoirs. *Microbiology* (English translation of *Mikrobiologiya*) **36**, 130–133.
- Bligh, E. G. & Dyer, W. J. (1959). A rapid method for total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
- Dedysh, S. N., Panikov, N. S. & Tiedje, J. M. (1998). Acidophilic methanotrophic communities from *Sphagnum* peat bogs. *Appl Environ Microbiol* **64**, 922–929.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Henrici, A. T. & Johnson, D. E. (1935). Studies on fresh water bacteria. II. Stalked bacteria, a new order of schizomycetes. *J Bacteriol* **30**, 61–93.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lapteva, N. A. (1987). Ecological characteristics of *Caulobacter* incidence in fresh-water basins. *Microbiology* (English translation of *Mikrobiologiya*) **56**, 677–684.
- Larson, R. J. & Pate, J. L. (1975). Growth and morphology of *Asticcacaulis biprosthecum* in defined media. *Arch Microbiol* **106**, 147–157.
- Liu, Z.-P., Wang, B.-J., Liu, S.-J. & Liu, Y.-H. (2005). *Asticcacaulis taihuensis* sp. nov., a novel stalked bacterium isolated from Taihu Lake, China. *Int J Syst Evol Microbiol* **55**, 1239–1242.
- Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Lysenko, A. M., Gal'chenko, V. F. & Chernykh, N. A. (1988). Taxonomic study of obligate methanotrophic bacteria using the DNA-DNA hybridization technique. *Microbiology* (English translation of *Mikrobiologiya*) **57**, 653–658.
- Nikitin, D. I., Vasilyeva, L. V. & Lohmacheva, R. A. (1966). *New and Rare Forms of Soil Microorganisms*. Moscow: Nauka.
- Osterhout, G. J., Shull, V. H. & Dick, J. D. (1991). Identification of clinical isolates of Gram-negative nonfermentative bacteria by an automated cellular fatty acid identification system. *J Clin Microbiol* **29**, 1822–1830.
- Owen, R. J., Lapage, S. P. & Hill, L. R. (1969). Determination of base composition from melting profiles in dilute buffers. *Biopolymers* **7**, 503–516.
- Pate, J. L., Porter, J. S. & Jordan, T. L. (1973). *Asticcacaulis biprosthecum* sp. nov. Life cycle, morphology and cultural characteristics. *Antonie van Leeuwenhoek J Microbiol Serol* **39**, 569–583.
- Poindexter, J. S. (1964). Biological properties and classification of the *Caulobacter* group. *Bacteriol Rev* **28**, 231–295.
- Poindexter, J. S. (1981a). The Caulobacters: ubiquitous unusual bacteria. *Microbiol Rev* **45**, 123–179.
- Poindexter, J. S. (1981b). Oligotrophy. Fast and famine existence. *Microb Ecol* **5**, 63–89.
- Poindexter, J. S. (1999). Dimorphic prosthecae bacteria: the genera *Caulobacter*, *Asticcacaulis*, *Hyphomicrobium*, *Pedomicrobium*, *Hyphomonas*, and *Thiodendron*. In *The Prokaryotes*, 3rd edn. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer. <http://link.springer-ny.com/link/service/books/10125>.
- Staley, J. T. (1968). New prosthecae freshwater bacteria. *J Bacteriol* **95**, 1921–1942.
- Staley, J. T., Konopka, A. E. & Dalmasso, J. P. (1987). Spatial and temporal distribution of *Caulobacter* spp. in two mesotrophic lakes. *FEMS Microbiol Ecol* **45**, 1–6.
- Vasilyeva, L. V. & Zavarzin, G. A. (1995). Dissipatrophs in the microbial community. *Microbiology* (English translation of *Mikrobiologiya*) **64**, 198–202.
- Yakimov, M. M., Giuliano, L., Denaro, R., Crisafi, E., Chernikova, T. N., Abraham, W.-R., Luensdorf, H., Timmis, K. N. & Golyshin, P. N. (2004). *Thalassolituus oleivorans* gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons. *Int J Syst Evol Microbiol* **54**, 141–148.