Asticcacaulis taihuensis sp. nov., a novel stalked bacterium isolated from Taihu Lake, China

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A novel stalked bacterium, designated strain T3-B7^T, was isolated from sediment of Taihu Lake, Jiangsu Province, China, and its taxonomy was studied by using a polyphasic approach. Cell morphology, physiological and biochemical properties, and polar lipids indicated that strain T3-B7^T represented a member of the genus *Asticcacaulis*. Based on 16S rRNA gene sequence similarity analysis, strain T3-B7^T was found to be phylogenetically related to *Asticcacaulis biprosthecium* DSM 4723^T (98.5%) and *Asticcacaulis excentricus* DSM 4724^T (95.0%), but could be differentiated from these two species on the basis of the number and position of prosthecae, assimilation of sugars, nitrate reduction and tolerance to NaCl. Levels of DNA–DNA relatedness of strain T3-B7^T to *A. biprosthecium* DSM 4723^T and *A. excentricus* DSM 4724^T were 37.1 and 18.0%, respectively. The G + C content of strain T3-B7^T was 59 mol% (T_m). It is concluded that strain T3-B7^T represents a novel species of the genus *Asticcacaulis*, for which the name of *Asticcacaulis taihuensis* sp. nov. is proposed. The type strain is T3-B7^T (= AS 1.3431^T = JCM 12463^T).

The genus *Asticcacaulis* was established by Poindexter (1964) to accommodate isolates that were regarded as representing *Caulobacter* species but with prosthecae that were not adhesive. Subsequent studies on 16S rRNA gene sequence similarities and chemotaxonomic features indicated that members of the genus *Asticcacaulis* were distantly related to members of the genera *Caulobacter* and *Brevundimonas* (Abraham *et al.*, 2001; Sly *et al.*, 1999). *Asticcacaulis* comprises only two species (Poindexter, 1989), *Asticcacaulis excentricus* Poindexter 1964 and *Asticcacaulis biprosthecium* Pate *et al.* 1973. All strains representative of this genus have been isolated from freshwater environments.

During ecological surveys on the freshwater Taihu Lake (31° 27' 10.7" N 120° 02' 16.8" E), which is located in Jiangsu Province, China, strain T3-B7^T was isolated from lake sediment by plating 10-fold dilutions of samples on diluted LB agar (0.5 g yeast extract, 1 g peptone, 1 g NaCl, 15 g Difco agar, 1 litre distilled water). Sediment samples were obtained from 10 cm beneath the sediment surface. Routine cultivation was conducted with LB agar or LB broth at 30 °C.

Gram reactions were determined by staining cells grown on LB agar at 30 °C for 24 h according to the method described by Gerhardt *et al.* (1994). Endospore formation was determined after malachite green staining of cells grown on LB agar. Flagellation and stalk generation were examined by transmission electron microscopy (H-600; Hitachi) at 100 kV after negative staining with 1% (w/v) phosphotungstic acid. Denitrification capacity was determined by the method of Stanier *et al.* (1966). For assimilation of carbon sources, the standard mineral base of Stanier *et al.* (1966) was used. After the mineral base was autoclaved, each compound was added at a concentration of 0.2% (w/v). Growth was examined after incubation at 30 °C for 1, 3, 7 and 14 days. Aerobic and anaerobic production of acids (OF reaction) from carbohydrates was determined in OF basal medium (Hugh & Leifson, 1953). Carbohydrate solution sterilized by filtration was added at a concentration of 1% (w/v), and acid production was recorded after 7 and 14 days of incubation. Cellular fatty acids were extracted and analysed by using the Sherlock system (MIDI Inc.) following the recommendations of the manufacturer. Polar lipids were separated with one- and two-dimensional TLC and were characterized with spreading reagents specific for α -glycols (periodate-Schiff), sugars (α -naphthol-H₂SO₄, anisaldehyde-H₂SO₄), phosphate (Zindzadze), free amino groups (ninhydrin) and quaternary nitrogen compounds (Dragendorff) (Ventosa *et al.*, 1993). The developed spots were identified by comparison with those of *A. biprosthecium* DSM 4723^T.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T3-B7^T is AY500141.

Polar lipid profiles of *A. taihuensis* T3-B7^T and *A. biprosthecium* DSM 4723^T are shown in a supplementary figure available in IJSEM Online.

Fig. 1. Transmission electron micrographs of cells of strain T3-B7^T showing a single polar flagellum (a) and the typical stalk near the cell pole (b). Bars, 1 μm.

DNA base composition was determined by thermal denaturation (Marmur & Doty, 1962) using DNA from *Escherichia coli* DH-5α as a control. DNA–DNA hybridizations were carried out according to the method of De Ley *et al.* (1970). Renaturation rates and levels of relatedness were calculated as described by Jahnke (1992). 16S rRNA genes were amplified and sequenced as described by Zhang *et al.* (2003), and 16S rRNA gene sequence alignments were performed with the CLUSTAL X program (version 1.64b; Thompson *et al.*, 1997). A phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with Kimura's two-parameter calculation model in TREECON W version 1.3b.

Cells of strain T3-B7^T are Gram-negative, aerobic, non-spore-forming rods. They are motile with a single polar flagellum (Fig. 1a). A single stalk was located near the cell pole and the stalk possessed no adhesive material (Fig. 1b). 16S rRNA gene sequence analysis indicated that strain T3-B7^T was phylogenetically related to members of the genus *Asticcacaulis*, with similarities of 95.0% to *A. excentricus* DSM 4724^T and 98.5% to *A. biprosthecium* DSM 4723^T. Based on these 16S rRNA gene sequence analyses, a neighbour-joining tree was constructed (Fig. 2); the results showed that strain T3-B7^T and the two recognized species of the genus *Asticcacaulis* were grouped together to form a cluster with 100% bootstrap support. Investigation of polar lipids revealed that strain T3-B7^T contained phosphatidylglycerol, 1,2-diacyl-3-*O*-α-D-glucopyranosyl glycerol, 1,2-diacyl-3-*O*-α-D-glucuronopyranosyl glycerol and three unidentified phospholipids (see Supplementary Fig. A in IJSEM Online). Comparison with *A. biprosthecium* DSM 4723^T reported by Abraham *et al.* (2001) showed that both strains contained phosphatidylglycerol, 1,2-diacyl-3-*O*-α-D-glucopyranosyl glycerol and 1,2-diacyl-3-*O*-α-D-glucuronopyranosyl glycerol, but that the two strains differed in their unidentified polar lipids (compare Supplementary Figs A and B). Strain T3-B7^T also differed from *A. excentricus* and *A. biprosthecium* with regard to the number and position of its stalks, assimilation of sugars (L-arabinose, D-mannose, D-ribose and sucrose), tolerance to NaCl and nitrite reduction (Table 1). Cellular fatty acid

Fig. 2. Phylogenetic tree constructed with the neighbour-joining method based on 16S rRNA gene sequence analysis of strain T3-B7^T and related bacteria. Numbers at nodes indicate percentages of bootstrap support based on 1000 resampled datasets. *Phenylobacterium immobile* was used as the outgroup. Bar, evolutionary distance (K_{nuc}) of 0.1.

Table 1. Characteristics used to differentiate strain T3-B7^T from other *Asticcacaulis* species

+, Positive; −, negative; D, 11–89% of strains positive. Data for reference species were taken from Poindexter (1989) unless stated.

Characteristic	T3-B7 ^T	<i>A. excentricus</i>	<i>A. biprosthecium</i>
Assimilation of:			
L-Arabinose	+	D	−
D-Mannose	+	+	−
Sucrose	+	+	−
Growth on NaCl at:			
10 g l ^{−1}	+	+	−
25 g l ^{−1}	−	+	−
Nitrate reduction	+	D	−
Number of prosthecae	1	1	2
Position of prosthecae	Subpolar	Subpolar	Lateral

*Data for *A. excentricus* and *A. biprosthecium* were taken from Abraham *et al.* (2001).

profiles of strains T3-B7^T, *A. biprosthecium* DSM 4723^T and *A. excentricus* DSM 4724^T are given in Table 2. Levels of DNA–DNA relatedness of strain T3-B7^T to *A. excentricus* DSM 4724^T and *A. biprosthecium* DSM 4723^T were 18.0 and 37.1%, respectively. Based on the above results we conclude that strain T3-B7^T represents a novel species of the genus

Asticcacaulis, for which the name *Asticcacaulis taihuensis* is proposed.

Description of *Asticcacaulis taihuensis* sp. nov.

Asticcacaulis taihuensis (tai.hu.en'sis. N.L. masc. adj. *taihuensis* pertaining to Taihu Lake, the source of the sediment from which the type strain was isolated).

Gram-negative, aerobic, non-spore-forming rods. Motile with a single polar flagellum. Forms a single typical stalk near its cell pole and the stalk possesses no adhesive material. Assimilates L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, D-melibiose, raffinose, L-rhamnose, lactose, sucrose, D-trehalose, D-xylose, melezitose, L-alanine and L-proline but not D-ribose, D-mannitol, D-sorbitol, β-alanine, L-arginine, L-phenylalanine, L-lysine or citrate. Acids are produced oxidatively from L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, L-rhamnose, lactose, D-xylose and melezitose but not from raffinose, D-ribose, sucrose, trehalose, D-mannitol or D-sorbitol. Cells grow well at NaCl concentrations below 20 g l^{−1}. Starch and aesculin are hydrolysed, but gelatin is not. Nitrate is reduced to nitrite. Does not denitrify. Arginine dihydrolase and urease are negative. Major cellular fatty acids are octadecanoic acid (18:1), hexadecanoic acid (16:0), 2-hydroxy-hexadecanoic acid (16:0 2-OH) and hexadecanoic acid (16:1). Contains phosphatidylglycerol, 1,2-diacyl-3-O-α-D-glucopyranosyl glycerol, 1,2-diacyl-3-O-α-D-glucuronopyranosyl glycerol and three unidentified phospholipids. The G+C content of the DNA is 59 mol% (T_m).

The type strain, T3-B7^T (= AS 1.3431^T = JCM 12463^T), was isolated from sediment of Taihu Lake, Jiangsu Province, China.

Table 2. Cellular fatty acid compositions of strain T3-B7^T and the type strains of *Asticcacaulis* species

Only fatty acids accounting for more than 1.0% in one of the strains studied are indicated. −, Not detected. Data for reference species were taken from Abraham *et al.* (2001).

Fatty acid	T3-B7 ^T	<i>A. excentricus</i>	<i>A. biprosthecium</i>
12:0	−	−	2.5
12:0 3-OH	2.32	−	−
12:1 3-OH	2.61	4.2	7.5
14:0	1.08	−	1.1
15:0	1.53	2.3	2.9
anteiso-15:0	2.58	−	−
16:0	28.69	17.3	20.8
16:0 2-OH	10.4	−	−
Summed feature 4*	7.41	7.4	13.8
iso-16:0	−	1.8	−
17:0	−	1.8	−
17:1ω6c	−	1.5	1.0
17:1ω8c	−	1.1	−
Summed feature 7*	41.86	57.7	40.7
cyclo 19:0ω8c	1.46	−	−

*Summed features consist of one or more fatty acids that could not be separated. Summed feature 4 contained iso-15:0 2OH, 16:1ω7c and/or 16:1ω7t; summed feature 7 consisted of 18:1ω7c, 18:1ω9t and/or 18:1ω12t.

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