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Sneathiella chinensis gen. nov., sp. nov., a novel marine alphaproteobacterium isolated from coastal sediment in Qingdao, China

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The taxonomic position of strain LMG 23452^T, which was isolated from coastal sediment from an aquaculture site near Qingdao, China, in 2000, was determined. Strain LMG 23452^T comprised Gram-negative, non-spore-forming, motile rods and was found to be a halotolerant, aerobic, chemoheterotroph that produces catalase and oxidase. Comparative 16S rRNA gene sequence analysis revealed that strain LMG 23452^T shared approximately 89 % sequence similarity with members of the genera *Devosia*, *Hyphomonas*, *Ensifer* and *Chelatococcus*, which belong to two different orders within the *Alphaproteobacteria*. Further phylogenetic analysis of the 16S rRNA gene sequence showed that strain LMG 23452^T formed a separate branch within the order *Rhizobiales*, falling between the genera *Devosia* and *Ensifer* of the families *Hyphomicrobiaceae* and *Rhizobiaceae*, respectively. Strain LMG 23452^T could be differentiated from its closest phylogenetic neighbours on the basis of several phenotypic features, including hydrolysis of the substrates starch and casein and assimilation of the carbohydrates D-glucose, D-mannose, mannitol, maltose and L-arabinose, and chemotaxonomically by the presence of the fatty acids $C_{14:0}$ 3-OH, $C_{16:1}\omega11c$, $C_{16:1}\omega5c$ and $C_{18:1}\omega5c$. The major fatty acids detected in strain LMG 23452^T were $C_{18:1}\omega7c$, $C_{16:0}$, $C_{19:0}$ cyclo $\omega8c$, $C_{16:1}\omega7c$ and $C_{17:1}\omega6c$ and the G+C content of the genomic DNA was 57.1 mol%. Therefore, the polyphasic data support the placement of strain LMG 23452^T within a novel genus and species, for which the name *Sneathiella chinensis* gen. nov., sp. nov. is proposed. The type strain is LMG 23452^T (=CBMAI 737^T).

The class *Alphaproteobacteria* (Garrity *et al.*, 2005a) comprises a large group of Gram-negative bacteria within the phylum *Proteobacteria* and is currently divided into seven orders: *Caulobacterales* (Henrici & Johnson, 1935), *Rhodobacterales* (Garrity *et al.*, 2005b), *Rhodospirillales* (Pfennig & Trüper, 1971), *Rickettsiales* (Gieszczykiewicz, 1939), *Sphingomonadales* (Yabuuchi & Kosako, 2005), *Kordiimonadales* (Kwon *et al.*, 2005) and *Rhizobiales* (Kuykendall, 2005).

Members of the order *Rhizobiales* (Kuykendall, 2005) are morphologically and physiologically diverse and constitute the largest group within the α-2 subgroup of the *Proteobacteria* (Woese *et al.*, 1984; Cho & Giovannoni, 2003). On the basis of 16S rRNA gene sequence analysis, the order currently comprises 11 families with validly published names: *Rhizobiaceae*, *Bartonellaceae*, *Brucellaceae*, *Phyllobacteriaceae*, *Methylocystaceae*, *Beijerinckiaceae*, *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, *Methylobacteriaceae*, *Rhodobiaceae* and *Xanthobacteriaceae* (Bowman, 2006; Garrity *et al.*, 2006a, b, c, d; Mergaert & Swings, 2006; http://www.bacterio.cict.fr). Although these families have

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LMG 23452^T is DQ219355.

been revised and expanded by the inclusion of several new genera in recent years (Garrity *et al.*, 2004; Lee *et al.*, 2005), only a small number of these taxa have been described as coming from marine sources (Satomi *et al.*, 2002; Cho & Giovannoni, 2003; Denner *et al.*, 2003; Labbé *et al.*, 2004; Peix *et al.*, 2005). In the present study, we describe a novel taxon within the order *Rhizobiales*, which was isolated from coastal sediment from an aquaculture site.

In October 2000, strain LMG 23452^T was isolated from a sediment sample from an aquaculture site near Qingdao and was cultivated on marine agar (MA; Difco) at 28 °C. Cultures were maintained on MA slants at room temperature and stock cultures were kept in tryptone soy broth (Oxoid) supplemented with 1 % (w/v) NaCl (TNB) and 20 % (v/v) glycerol and stored at −70 °C. Colony morphology was recorded on MA after 48 h incubation at 28 °C. Cellular morphology was determined by phase-contrast microscopy (Axiophot; Zeiss) at ×1000 magnification. Gram staining was performed using the modified method of Hucker & Conn (1923). Stained cells were observed using a light microscope (Microlux-11; Kyowa) at a magnification of ×1000. Cells of strain LMG 23452^T were found to be Gram-negative rods.

For phenotypic tests, the strain was grown on MA for 48 h at 28 °C and cells were resuspended in saline for use as an inoculum. Tolerance of 3, 5, 7 and 10 % (w/v) NaCl was assessed on appropriately modified tryptone soy agar (Oxoid). Growth in the absence of NaCl was assessed on plate count agar (PCA; Oxoid). Inoculated plates were incubated at 28 °C for up to 5 days. The effects of different temperatures on growth were assessed on tryptone soy agar plates supplemented with 1.0 % (w/v) NaCl (TNA) and incubated at 4, 28, 30, 37, 45 and 50 °C. Anaerobiosis was determined on MA in an anaerobic chamber (Merck) containing the anaerobic catalyst Anaerocult (Merck) prepared according to the manufacturer's instructions. The chamber was incubated at 28 °C and examined after 7 days. Motility was assessed in a semi-solid medium prepared according to MacFaddin (1976). The tube was incubated at 25 °C for 5 days. The reduction of nitrate was assessed in nitrate broth, prepared according to the method of Cowan & Steel (1974), and incubated at room temperature for 10 days. Oxidase and catalase activities were determined by using standard methods. Tests for the hydrolysis of casein, starch, tyrosine, aesculin and Tweens 20, 40, 60 and 80 were performed on TNA plates; the substrate concentrations and incubation conditions were as described by Cowan & Steel (1974). Insoluble-dye-linked polysaccharides (galactan, arabinan, xylan, cellulose or pullulan; Megazyme International) were added to a base medium of MA at a concentration of 0.05 % (w/v) and autoclaved at 115 °C for 15 min. The plates were incubated at 25 °C for 3 days. API 20NE and API ZYM test kits (bioMérieux) were inoculated with strain LMG 23452^T, using the appropriate suspension medium, and incubated according to the manufacturer's instructions.

Antibiotic sensitivity was assessed as follows: a cell suspension ($\sim 10^7$ cells ml^{-1}) was swabbed over the surface of Iso-Sensitest agar (Oxoid) plates supplemented with 1 % (w/v) NaCl to create a uniform lawn before aseptic placement of antibiotic discs (M5 and M27, Mastring; Mast Laboratories) onto the agar surface. The inoculated plates were incubated overnight at 28 °C.

Fatty acid methyl esters of strain LMG 23452^T were obtained from 40 mg cells. Saponification, methylation and extraction were performed according to the procedures of Miller (1982) and Kuykendall *et al.* (1988). Separation and analysis were performed essentially as described by Rivas *et al.* (2003), but with a slight modification to the gas chromatographic parameters (injection-port temperature, 240 °C; detector temperature, 300 °C).

The DNA G+C content (mol%) was determined by HPLC (Tamaoka & Komagata, 1984). The almost-complete 16S rRNA gene sequence (1412 nt) of strain LMG 23452^T was obtained using the universal primers 27f and 1492r (MWG Biotech; Lane, 1991). The PCR amplification mixture (50 µl) comprised 2.5 µl (5 pmol µl^{-1}) each of primers 27f and 1492r, 2.5 µl $MgCl_2$ (50 mM; Bioline), 10 µl *Taq* mix [i.e. 5 µl (10 mM NH_4^+); 10 µl each of dATP, dTTP, dGTP, dCTP (100 mM); 4.6 µl sterile Millipore H_2O], 31.2 µl sterile Millipore water and 1.0 µl DNA template. PCR amplification was carried out on a Bio-Rad iCycler (version 3.021). Template DNA was initially denatured at 95 °C for 5 min and the machine paused for the addition of 0.3 µl *Taq* enzyme (BIOTAQ; Bioline). The PCR was resumed for a further 35 cycles of 95 °C for 1 min, 56 °C for 1 min and 72 °C for 2 min, with a final extension at 72 °C for 10 min. The PCR product was subjected to polyethylene glycol precipitation (Embley, 1991). Purified PCR products (2 µl) were mixed with 1 µl ABI Prism Big Dye Terminator cycle sequencing ready reaction mix V1.1 with AmpliTaq DNA polymerase (Applied Biosystems), 1 µl 5× ABI sequencing buffer (Sigma), 2.4 µl sterile filtered water and 3.2 µl (1 pmol µl^{-1}) of one of the following universal primers designed by Lane (1991), i.e. 27f (5′-AGAGTTTGATCMTGGCTCAG-3′), 519r, 342f, 522f, 926f, 907r, 1114f, 1100r, 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) (MWG Biotech). Reactions were carried out in a thermocycler. The programme consisted of a denaturation step at 98 °C for 5 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. The sequencing products were purified with an ethanol/EDTA/sodium acetate precipitation solution (53 µl 96 % ethanol : 2 µl EDTA : 2 µl sodium acetate) in 96-well reaction plates (Applied Biosystems) and resuspended in 25 µl template suppression reagent (Applied Biosystems), heated at 95 °C for 2 min and then analysed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The resulting forward and reverse sequences were analysed and aligned using the STADEN package (Staden, 1996). A BLASTN (version 2.2.12) search was carried out to compare the corrected 16S rRNA gene sequence with those held in the National Library of

Medicine databases (Bethesda, MD, USA; Altschul *et al.*, 1997). The sequences were aligned, manually cropped using CLUSTAL W (Thompson *et al.*, 1994) and a phylogenetic tree was constructed. Sequences were uploaded into CLUSTAL_X (version 1.81; Jeanmougin *et al.*, 1998) in FASTA format for multiple alignment. A tree was calculated using the neighbour-joining method (Saitou & Nei, 1987), with bootstrapping determined for 1000 replicates. The tree was visualized using TREEVIEW (Page, 1996) and rooted using a suitable outgroup.

A BLASTN search using the almost-complete 16S rRNA gene sequence of strain LMG 23452^T (1412 bp) placed it among members of the class *Alphaproteobacteria*. The closest phylogenetic neighbours were *Chelatococcus asaccharovorans* DSM 6462^T, *Devosia riboflavina* DSM 7230^T and *Hyphomonas polymorpha* DSM 2665^T, which showed 89 % 16S rRNA gene sequence similarity with the novel strain. A neighbour-joining tree (Fig. 1) revealed that strain LMG 23452^T grouped most closely with an uncultured bacterial clone, D101, derived from a deep-sea sediment sample of a western Pacific warm pool in China (GenBank accession number AY375134). The closest cultured relatives of strain LMG 23452^T belonged to two genera, *Devosia* (Nakagawa *et al.*, 1996) and *Ensifer* (Casida, 1982), of the families *Hyphomicrobiaceae* (Babudieri, 1950) and *Rhizobiaceae* (Conn, 1938), respectively. Strain LMG 23452^T formed a separate branch within the order *Rhizobiales*, showing less than 90 % 16S rRNA gene sequence similarity with respect to its neighbours (with high levels of bootstrap support). Clearly this rather low level of similarity suggests that strain LMG 23452^T belongs to a novel taxonomic group. Interestingly, the genus *Chelatococcus* (Auling *et al.*, 1993) was placed within the order *Rhodobacterales* (Fig. 1), which conflicts with the currently accepted taxonomic outline of the *Alphaproteobacteria* (Garrity *et al.*, 2005a). However, a recent phylogenetic analysis of the *Alphaproteobacteria* by Lee *et al.* (2005) placed *Chelatococcus* at an intermediate position within the order *Rhizobiales*, making it difficult to define its taxonomic hierarchy. There are, however, numerous 16S rRNA gene sequence signatures that distinguish the genus *Sneathiella* from the closely related genera *Devosia* and *Ensifer*. Although there are differences in the base sequences of all three genera, e.g. at positions 155, 166, 441, 681 and 709, at various other nucleotide positions the base sequences of *Devosia* and *Ensifer* are identical, whereas those pertaining to strain LMG 23452^T are different. Moreover, at nucleotide positions 1000–1003, (*Escherichia coli* numbering) strain LMG 23452^T contains the signature sequence GTAG, followed by an insertion of the bases TT between position 1003 and 1004, to read GTAGTTT. This sequence (GTAGTTT) is different from the signature sequences of the genera *Devosia* and *Ensifer* (results not shown). At nucleotide positions 1262–1265 and 1270–1273, strain LMG 23452^T contains the signature sequences AGGG and CCCT, respectively, which are also different from those in members of the genera *Devosia* and *Ensifer*. In addition, there is a base deletion at position 1453–1454 (*Escherichia coli* numbering) in strain LMG 23452^T that is not present in members of the genera *Devosia* or *Ensifer*. Clearly there are numerous signature sequences that distinguish strain LMG 23452^T from its closest phylogenetic relatives.

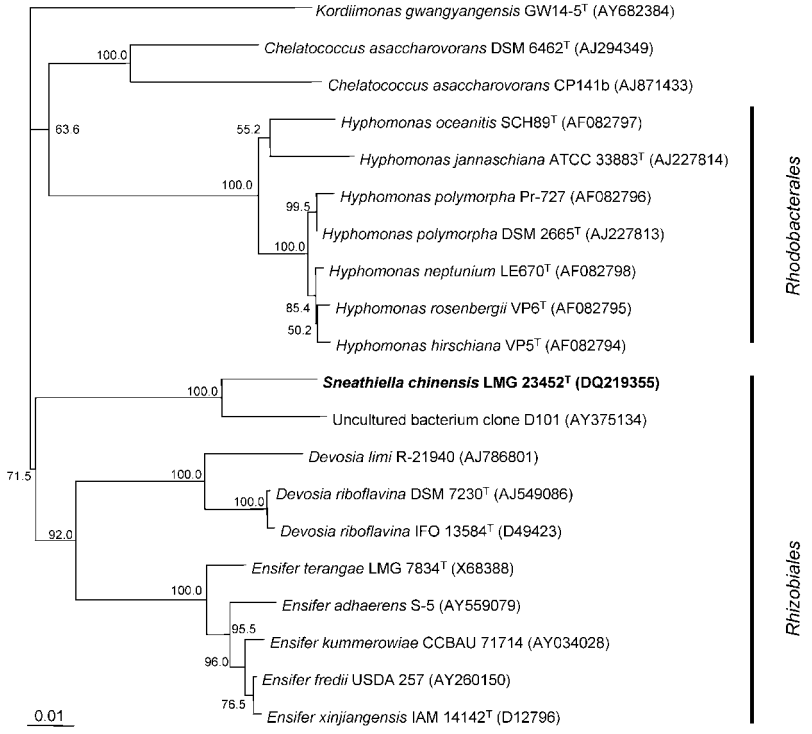

Fig. 1. Neighbour-joining tree, based on almost-complete 16S rRNA gene sequences (~1312 bp) of strain LMG 23452^T and related reference strains. *Kordiimonas gwangyangensis* GW14-5^T was used as the root. Numbers on branches refer to confidence limits (expressed as percentages) estimated from a bootstrap analysis based on 1000 replicates. Accession numbers are shown in parentheses. Bar, 0.01 substitutions per nucleotide.

The complete biochemical and antibiogram data for strain LMG 23452^T are given in the species description. The phenotypic features that differentiate strain LMG 23452^T from its closest phylogenetic relatives are provided in Table 1. Under comparable test conditions, strain LMG 23452^T can be distinguished from members of the genera *Devosia* and *Ensifer* by its ability to hydrolyse starch. In addition, strain LMG 23452^T can be distinguished from the type species of the genus *Devosia* (i.e. *D. riboflavina*) and from members of the genus *Ensifer* by its inability to utilize *N*-acetylglucosamine or to assimilate the carbohydrates D-glucose, L-arabinose, D-mannose, mannitol and maltose. Unlike strain LMG 23452^T, members of the genus *Ensifer*, including the type species of the genus, *Ensifer adhaerens*, are able to utilize malate, but are unable to hydrolyse casein (Young, 2003). Similarly, gentamicin resistance (10 μg), kanamycin resistance (30 μg) and *β*-galactosidase production are observed in strains of *Ensifer adhaerens* (Wang *et al.*, 2002; Willems *et al.*, 2003) and members of the genus *Devosia* (Vanparys *et al.*, 2005), but not in strain LMG 23452^T. Taxa belonging to the genus *Devosia* are able to produce the enzymes *N*-acetyl *β*-glucosaminidase and cystine arylamidase and utilize the substrate caprylate (Vanparys *et al.*, 2005), whereas these traits are not observed in strain LMG 23452^T. Moreover, the type species of the genus *Devosia*, *D. riboflavina* (LMG 2277^T), is able to produce the enzymes α-fucosidase, α-mannosidase and α-galactosidase, unlike strain LMG 23452^T. Clearly, there are several phenotypic features of the novel strain that distinguish it from its closest phylogenetic relatives.

The DNA G+C content of strain LMG 23452^T was 57.1 mol%. In the emended description of the genus *Ensifer* (Young, 2003), the DNA G+C content is 57–66 mol% and that of members of the genus *Devosia* falls within the range 61–63 mol% (Rivas *et al.*, 2003); clearly, the DNA G+C content of strain LMG 23452^T differs markedly from those of its closest phylogenetic relatives (Table 1).

The use of the fatty acid profile and the MIDI database could not provide an accurate identification of LMG 23452^T, which reinforced the notion that this strain belonged to a novel taxonomic group. A comparison of the fatty acid profile of strain LMG 23452^T with the profiles for its closest phylogenetic neighbours, obtained under comparable test conditions (Table 2), revealed that all strains contained $C_{16:0}$, with the majority of strains containing $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$. According to Martínez-Checa *et al.* (2005), the presence of *cis*-11 octadecenoic acid (i.e. $C_{18:1}\omega7c$) as the principal fatty acid is characteristic of taxa within the *Alphaproteobacteria*, whilst the presence of $C_{19:0}$ cyclo $\omega8c$

Table 1. Some phenotypic features useful for differentiating strain LMG 23452^T from its closest phylogenetic neighbours

Taxa: 1, strain LMG 23452^T; 2, *Ensifer fredii* (de Lajudie *et al.*, 1994, 1998; Chen *et al.*, 1988); 3, *Ensifer meliloti* (de Lajudie *et al.*, 1994, 1998); 4, *Ensifer sahelii* (de Lajudie *et al.*, 1994, 1998); 5, *Ensifer terangae* (de Lajudie *et al.*, 1994, 1998); 6, *Ensifer adhaerens* (Wang *et al.*, 2002; Willems *et al.*, 2003); 7, *Devosia limi* LMG 22951^T (Vanparys *et al.*, 2005); 8, *Devosia riboflavina* (Nakagawa *et al.*, 1996; Rivas *et al.*, 2003; Vanparys *et al.*, 2005); 9, *Devosia neptuniae* LMG 21357^T (Rivas *et al.*, 2003; Vanparys *et al.*, 2005). All of the species produce motile, Gram-negative rods, are catalase- and oxidase-positive and are negative for utilization of the substrates citrate, phenylacetate, caprate and adipate. +, Positive result; −, negative result; (+), weakly positive.

Characteristic	1	2	3	4	5	6	7	8	9
N-Acetylglucosamine	−	+	+	+	+	+	−	+	−*
Starch hydrolysis	+	−†	−†	−†	−†	−†	−	−	−
Assimilation of:									
D-Glucose	−	+	+	+	+	+	−	+	+
L-Arabinose	−	+	+	+	+	+	−	+	+
D-Mannose	−	+	+	+	+	+	−	+	+
Mannitol	−	+	+	+	+	+	−	+	+
Maltose	−	+	+	+	+	+	−	+	+
Malate	−	+	+	+	+	+	−	−	−
Caprylate	−	−	−	−	−	ND	(+)	+*	−*
DNA G+C content (mol%)	57.1	59.9–63.8‡	62–63§	65–66§	60.8–61.6§	61.7–62.3‖	61.9*	61.4¶	62.4#

*Data from Vanparys *et al.* (2005).
†Data from Young (2003).
‡Data from Chen *et al.* (1988).
§Data from de Lajudie *et al.* (1994).
‖Data from Willems *et al.* (2003).
¶Data from Nakagawa et al. (1996).
#Data from Rivas *et al.* (2003).

Table 2. Total fatty acid content (%) of strain LMG 23452^T and related species

Taxa: 1, strain LMG 23452^T; 2, *D. riboflavina* (Vanparys *et al.*, 2005); 3, *D. limi* (Vanparys *et al.*, 2005); 4, *D. neptuniae* (Vanparys *et al.*, 2005); 5, *Ensifer fredii* (Tighe *et al.*, 2000); 6, *Ensifer terangae* (Tighe *et al.*, 2000); 7, *Hyphomonas oceanitis* (Weiner *et al.*, 1985); 8, *Hyphomonas jannaschiana* (Weiner *et al.*, 1985). Summed features contain one or more of each fatty acid. Summed features: 1, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 2, $C_{12:0}$ (aldehyde?), unknown (equivalent chain-length 10.928), $C_{16:1}$ iso I/$C_{14:0}$ 3-OH; 3, $C_{18:1}\omega7c/\omega9t/\omega12t$, $C_{18:1}\omega7c/\omega9c/\omega12t$; 4, $C_{16:1}\omega7t$, $C_{15:0}$ iso 2-OH and $C_{16:1}\omega7c$; 5, $C_{17:1}$ iso/anteiso B; 7, $C_{18:1}\omega7c/\omega9t$ and/or $C_{18:1}\omega12t$. −, Fatty acid not detected during analysis; ECL, equivalent chain length. Values in bold signify major fatty acids present in strain LMG 23452^T.

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$ 3-OH	−	4.09	1.68	<1.00	−	−	−	−
ECL 13.97§	0.46	−	−	−	−	−	−	−
$C_{14:0}$	0.83	−	<1.00	<1.00	−	−	−	−
$C_{14:0}$ 3-OH	2.66	−	−	−	−	−	−	−
Unknown (ECL 14.502)	0.59	−	−	−	−	−	−	−
Unknown (ECL 14.952)	−	−	−	−	−	0.15		
$C_{15:1}\omega8c$	0.38	−	−	−	−	−	−	−
$C_{15:0}$ iso	−	−	−	−	0.01	−	−	−
$C_{15:0}$	**2.14**	**−**	**−**	**−**	**−**	**−**	**−**	**5.00**
$C_{15:0}$ anteiso	−	−	−	−	0.10	−	−	−
$C_{16:0}$	**17.16**	**22.56**	**11.34**	**24.65**	**5.12**	**3.30**	**27.00**	**2.00**
$C_{16:1}\omega11c$	1.36	−	−	−	−	−	−	−
$C_{16:1}\omega7c$	**6.95**	**−**	**−**	**−**	**−**	**−**	**6.00**	**−**
$C_{16:1}\omega5c$	1.73	−	−	−	−	−	−	−
$C_{16:0}$ 3-OH	−	−	−	−	0.49	0.18	−	−
$C_{16:0}$ iso	−	−	−	−	0.03	−	−	−
$C_{17:0}$	0.50	−	<1.00	−	0.73	0.24	−	31.00
$C_{17:0}$ 3-OH	−	−	−	−	−	0.02	−	−
$C_{17:1}\omega8c$	0.72	−	−	−	0.11	0.03	−	−
$C_{17:1}\omega6c$	**5.64**	**−**	**−**	**−**	**−**	**−**	**−**	**22.00**
$C_{17:0}$ cyclo	−	1.60	−	3.13	0.20	−	ND	ND
$C_{18:1}\omega7c$	**46.21**	**26.80**	**36.32**	**14.35**	**−**	**−**	**53.00**	**11.00**
$C_{18:1}\omega5c$	0.92	−	−	−	−	−	−	−
$C_{18:1}\omega9c$	−	−	−	−	0.11	0.01	−	−
$C_{18:0}$	−	2.92	9.68	4.95	4.99	3.44	−	−
11-methyl $C_{18:1}\omega7c$	0.31	34.88	−	36.15	−	−	−	−
Unknown ECL 18.794	−	−	−	−	0.19	0.05	−	−
$C_{19:0}$ cyclo $\omega8c$	**9.80**	**4.19**	**<1.00**	**7.85**	**4.37**	**2.92**	**−**	**−**
10-methyl $C_{19:0}$	−	−	−	−	0.41	0.07	−	−
$C_{18:0}$ 3-OH	−	−	4.59	4.79	−	−	−	−
$C_{20:1}\omega7c$	−	<1.00	<1.00	−	−	−	−	−
$C_{20:1}\omega9t$	−	−	−	−	0.12	−	−	−
$C_{20:3}\omega6,9,12c$	−	−	−	−	3.28	2.00	−	−
Summed feature 1	−	1.54	2.96	2.27	0.63	0.42	−	−
Summed feature 2	1.66	−	−	−	7.62	7.70	−	−
Summed feature 3	−	−	−	−	71.45	79.40	−	−
Summed feature 5	−	−	−	−	−	0.08	−	−

in significant amounts is thought to be typical of members of the order *Rhizobiales* (Rivas *et al.*, 2005). However, a 16S rRNA gene sequence comparison of strain LMG 23452^T in BLASTN revealed 89 % similarity with members of the genus *Hyphomonas* (order *Rhodobacterales*) (results not shown). Table 2 shows the absence of the fatty acid $C_{19:0}$ cyclo $\omega8c$ in members of the genus *Hyphomonas*. In spite of the fact that strain LMG 23452^T contained significant amounts of $C_{19:0}$ cyclo $\omega8c$, the fatty acid profile was distinct from those obtained for other members of the *Rhizobiales*, such as the genera *Devosia* and *Ensifer* (Table 2). The fatty acids $C_{20:3}\omega6,9,12c$ and summed feature 3 ($C_{18:1}\omega7c/\omega9t/\omega12t$, $C_{18:1}\omega7c/\omega9c/\omega12t$) (Table 2) are common to all species of *Ensifer* (Tighe *et al.*, 2000), but were not detected in the novel strain, which confirms that LMG 23452^T is not a member of this genus. Similarly, members of the genus *Devosia* contained the fatty acids $C_{10:0}$ 3-OH and $C_{18:0}$, which were not detected in strain LMG 23452^T.

Clearly, the fatty acids $C_{14:0}$ 3-OH, $C_{16:1}\omega11c$, $C_{16:1}\omega5c$ and $C_{18:1}\omega5c$, were present only in strain LMG 23452^T.

On the basis of the polyphasic taxonomic data obtained in this study, we conclude that strain LMG 23452^T is a representative of a hitherto unknown marine taxon of the order *Rhizobiales*, class *Alphaproteobacteria*, for which the name *Sneathiella chinensis* gen. nov., sp. nov., is proposed.

Description of *Sneathiella* gen. nov.

Sneathiella (Sneath.i.el′la. N.L. fem. dim. n. *Sneathiella* honouring the British microbiologist Peter H. A. Sneath for his contributions to bacterial taxonomy).

Gram-negative, motile, aerobic, small, non-spore-forming and rod-shaped. Cells are oxidase- and catalase-positive. Cells grow at temperatures in the range 4–37 °C. NaCl is not required for growth. Colonies are beige, low convex, glossy, smooth, irregular and 0.5–2.0 mm in diameter on MA. Major cellular fatty acids are $C_{18:1}\omega7c$ (46.2 %), $C_{16:0}$ (17.2 %), $C_{19:0}$ cyclo $\omega8c$ (9.8 %), $C_{16:1}\omega7c$ (6.9 %) and $C_{17:1}\omega6c$ (5.6 %). 16S rRNA gene sequence analysis indicates that it is phylogenetically related to members of the α-subgroup of the *Proteobacteria*. The type species is *Sneathiella chinensis*.

Description of *Sneathiella chinensis* sp. nov.

Sneathiella chinensis (chi.nen′sis. N.L. fem. adj. *chinensis* pertaining to China, where the type strain was isolated).

In addition to the properties described for the genus, the following properties apply. After 48 h on MA, colonies (diameter 0.5–2.0 mm) are beige, low convex, glossy, smooth, irregular and butyrous. Cells are Gram-negative and motile. No growth occurs at 45 °C, but growth occurs in 0–3 % (w/v) NaCl. Positive for oxidase, catalase, nitrate reduction, aesculin hydrolysis, casein hydrolysis, starch hydrolysis, hydrolysis of Tweens 20, 40, 60 and 80 and deamination of tyrosine, but negative for haemolysis of horse blood and the acidification of glucose, the production of arginine dihydrolase, the production of urease, the hydrolysis of *p*-nitrophenyl-β-D-galactopyranoside and the assimilation of maltose, glucose, gluconate, arabinose, phenylacetate, mannitol, malate, adipate, *N*-acetylglucosamine, citrate, caprate and mannose. Positive for the production of the enzymes alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase and negative for the production of esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Negative for hydrolysis of the substrates arabinan, cellulose, galactan, gelatin, pullulan and xylan. Sensitive to chloramphenicol (25 μg), gentamicin (10 μg), nalidixic acid (30 μg), streptomycin (25 μg) and tetracycline (100 μg), but resistant to ampicillin (25 μg), penicillin G (1 U), nitrofurantoin (50 μg), carbenicillin (100 μg), sulphatriad (200 μg), cotrimoxazole (25 μg) and sulphamethizole (200 μg). Major fatty acids produced by the type strain are $C_{18:1}\omega7c$ (46.21 %), $C_{16:0}$ (17.16 %), $C_{19:0}$ cyclo $\omega8c$ (9.80 %), $C_{16:1}\omega7c$ (6.95 %) and $C_{17:1}\omega6c$ (5.64 %). Other fatty acids are listed in Table 2. The DNA G+C content of the type strain is 57.1 mol%.

The type strain, LMG 23452^T (=CBMAI 737^T), was isolated from sediment from a coastal aquaculture site at Xianlangzhui, Qingdao, China.

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