Marisediminicola antarctica gen. nov., sp. nov., an actinobacterium isolated from the Antarctic

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Strain ZS314^T was isolated from a sandy intertidal sediment sample collected from the coastal area off the Chinese Antarctic Zhongshan Station, east Antarctica (69° 22' 13" S 76° 21' 41" E). The cells were Gram-positive, motile, short rods. The temperature range for growth was 0–26 °C and the pH for growth ranged from 5 to 10, with optimum growth occurring within the temperature range 18–23 °C and pH range 6.0–8.0. Growth occurred in the presence of 0–6 % (w/v) NaCl, with optimum growth occurring in the presence of 2–4 % (w/v) NaCl. Strain ZS314^T had MK-10 as the major menaquinone and anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0} as major fatty acids. The cell-wall peptidoglycan type was B2β with ornithine as the diagnostic diamino acid. The major polar lipids were diphosphatidylglycerol and phosphatidylglycerol. The genomic DNA G + C content was approximately 67 mol%. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain ZS314^T represents a new lineage in the family *Microbacteriaceae*. On the basis of the phylogenetic analyses and phenotypic characteristics, a new genus, namely *Marisediminicola* gen. nov., is proposed, harbouring the novel species *Marisediminicola antarctica* sp. nov. with the type strain ZS314^T (=DSM 22350^T =CCTCC AB 209077^T).

The family *Microbacteriaceae* was proposed by Park *et al.* (1993) and emended by Stackebrandt *et al.* (1997) and Zhi *et al.* (2009) successively. At the time of writing, the family *Microbacteriaceae* includes 30 genera. The cell wall peptidoglycan type of the genera *Agromyces*, *Cryobacterium*, *Curtobacterium*, *Frigoribacterium*, *Fronidhabitans*, *Mycetocola*, *Microbacterium*, *Microcella*, *Rathayibacter*, *Subtercola* and *Yonghaparkia* within the family *Microbacteriaceae* is B. Recently, a few psychrophilic or psychrotolerant actinobacterial strains belonging to the genera *Cryobacterium* (Suzuki *et al.* 1997), *Frigoribacterium* (Kämpfer *et al.* 2000) and *Subtercola* (Männistö *et al.* 2000) have been described. In this study, we report the characterization of a novel actinobacterium isolated from a sandy intertidal sediment sample collected from the coastal area off the Chinese Antarctic Zhongshan Station, east Antarctica (69° 22' 13" S 76° 21' 41" E). Based on our results, we propose a new genus *Marisediminicola* gen. nov. The type species, *Marisediminicola antarctica* sp. nov., is described.

In the laboratory, the sediment samples were dried at 8 °C in a refrigerator for 2 weeks. Dried sediment (approx. 2 g) was diluted with 10 ml sterile (autoclaved) seawater. The diluted sample was vortexed, allowed to settle for 30 min, and 100 µl of the resulting solution was further diluted

(1:10) and spread onto Gause Mineral agar 1 plates (Bredholdt *et al.* 2007) supplemented with 100 µg cycloheximide ml⁻¹, 50 µg nystatin ml⁻¹ and 10 µg nalidixic acid ml⁻¹. The plates were incubated at 10 °C for two months. Strain ZS314^T was obtained in pure culture after three successive transfers to fresh Bacto marine 2216 agar and stored at –80 °C in 20 % (v/v) glycerol.

The cell morphology of strain ZS314^T was examined with a Nikon eclipse 80i microscope. The colonial morphology was observed on Bacto marine 2216 agar after 4 days incubation at 15 °C. The effect of different growth temperatures was determined on Bacto marine 2216 agar incubated at 0, 4, 10, 15, 20, 26, 30 and 37 °C. Plates were incubated for 4 weeks and growth was indicated by visible colonies. The pH range for growth was determined in Bacto marine 2216E broth at various pH, adjusted with HCl or NaOH (1 M). The requirement for NaCl was tested in a broth medium containing (l⁻¹) 5 g MgCl₂, 2 g MgSO₄, 0.5 g CaCl₂, 1 g KCl, 5 g peptone and various concentrations of NaCl at pH 7.5, adjusted with KOH (Smibert & Krieg, 1994). The pH-range and NaCl-tolerance tests were incubated at 12 °C for 7 days. Amylase production on Bacto marine 2216E agar after 7 days incubation at 20 °C was determined as described by Smibert & Krieg (1994). Except for amylase, substrate utilization patterns and other enzymic activities were tested by using the commercial API 50CH, API 20E, API 20NE and API ZYM strips (bioMérieux). Cells for inoculation of the API systems were suspended in 3 % sea salts (Sigma).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ZS314^T is GQ496083.

The cellular fatty acid composition of strain ZS314^T is available with the online version of this paper.

Acid production from carbohydrates was determined with API 50 CH strips [3 % (w/v) NaCl was added to the API 50 CHB/E medium]. The API strips were incubated at 12 °C for 3 weeks. All of the tests were performed in duplicate. Morphological, physiological and biochemical characteristics of strain ZS314^T are given in the species description (see below).

Respiratory lipoquinone, polar lipid, fatty acid methyl ester, peptidoglycan structure and DNA G + C content analyses were carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989). The cellular polar lipids were extracted and analysed by TLC (Kates, 1986). Cell-wall peptidoglycan was isolated and analysed by the methods of Schleifer & Kandler (1972), Schleifer (1985), MacKenzie (1987) and Groth *et al.* (1996). For fatty acid methyl ester analysis, the cell mass of strain ZS314^T was harvested from Bacto marine 2216 agar plates after incubation at 20 °C for 1 day. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI (Microbial Identification) system (Sasser, 1990). Strain ZS314^T contained MK-10 as the major menaquinone. Diphosphatidylglycerol, phosphatidylglycerol and four unknown glycolipids were detected in the polar lipid extracts of strain ZS314^T. The peptidoglycan of the isolate belonged to the B2β group (Schleifer & Kandler, 1972), containing a cell-wall type of {Gly} [L-Hsr] D-Glu(Hyg)-Gly-D-Orn (type B6; {}), position 1; [], position 3). The amino acids found in the cell wall hydrolysate were ornithine, alanine, glycine and homoserine in a molar ratio of approximately 0.8:2.0:2.6:1.0. The predominant cellular fatty acids of strain ZS314^T were anteiso-C_{15:0} (44.44 %), iso-C_{16:0} (26.93 %), anteiso-C_{17:0} (18.73 %) and anteiso-C_{15:1} A (6.97 %). The fatty acid profile of strain ZS314^T is available as supplementary material in IJSEM Online (Supplementary Table S1). The DNA G + C content was determined by the HPLC method (Cashion *et al.* 1977; Mesbah *et al.* 1989; Tamaoka & Komagata, 1984) with non-methylated Lambda-DNA (Sigma), *Bacillus subtilis* DSM 402, *Xanthomonas campestris* pv. *campestris* DSM 3586^T and *Streptomyces violaceoruber* DSM 40783 as references. The DNA G + C content of strain ZS314^T was 67.3 mol%.

DNA was extracted and purified by using a ColumnMate Bacteria gDNA Mini kit (Watson) following the manufacturer's protocol. The 16S rRNA gene was amplified by PCR with two universal primers (Li *et al.*, 2009). Sequencing reactions were carried out using an ABI BigDye 3.1 Sequencing kit (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied BioSystems). The nearly complete 16S rRNA gene sequence (1480 bp) of strain ZS314^T was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. The sequence was aligned by using CLUSTAL X1.8 (Thompson *et al.* 1997) with sequences retrieved from the databases. Alignments were manually checked by using the SequentiX alignment editor of

Hepperle (2004). The phylogenetic tree was reconstructed by using the neighbour-joining method with Kimura's two-parameter analysis implemented in the program PHYLIP v. 3.6 (J. Felsenstein, University of Washington, Seattle). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates. Phylogenetic analysis (Fig. 1) including representative members of all 30 genera in the family *Microbacteriaceae* showed that strain ZS314^T grouped with the members of the genus *Mycetocola* supported by relatively low bootstrap values (≤26 %). This separate phylogenetic position indicated that strain ZS314^T deserved the status of a novel genus. The new isolate showed the highest levels of 16S rRNA gene sequence similarity with species of the genera *Frigoribacterium* (96.1–96.5 %), *Fronidhabitans* (96.3–96.4 %), *Mycetocola* (96.2–96.4 %), *Subtercola* (96.2 %) and *Cryobacterium* (91.7–94.8 %). The bootstrap analysis indicated no significance in the branching pattern of the lineage comprising the new isolate ZS314^T and any previously described generic lineage of this family. Similar topologies were found in the trees generated with the maximum-parsimony and maximum-likelihood algorithms (Felsenstein, 1981).

The characteristics that differentiate strain ZS314^T from 11 other genera of the family *Microbacteriaceae* with B-type peptidoglycan are shown in Table 1. Based on the phenotypic and phylogenetic evidence, strain ZS314^T cannot be placed in any of the 30 genera of the family *Microbacteriaceae* described at the time of writing and we propose to create a new genus, namely *Marisediminicola* gen. nov. Strain ZS314^T represents the type strain of the type species, *Marisediminicola antarctica* sp. nov.

Description of *Marisediminicola* gen. nov.

Marisediminicola [ma.ri.se.di.mi.ni'co.la. L. neut. n. *mare* the sea; L. n. *sedimen* -inis sediment; L. suff. -cola (from L. fem. n. *incola*) inhabitant, dweller; N.L. fem. n. *Marisediminicola* marine sediment dweller].

Cells are Gram-positive, motile, short rods. Neither substrate mycelium nor aerial mycelium is formed. Catalase is produced but oxidase is not produced. The cell-wall peptidoglycan belongs to the B2β group with ornithine as diamino acid. MK-10 is the major menaquinone. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and some glycolipids. The main cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. The G + C content of the DNA is approximately 67 mol%. The type species is *Marisediminicola antarctica*.

Description of *Marisediminicola antarctica* sp. nov.

Marisediminicola antarctica (ant.arc'ti.ca. L. fem. adj. *antarctica* southern, of the Antarctic, the geographical origin of the type strain).

The morphology of the species and its chemotaxonomic characteristics are the same as described for the genus. Cells

Fig. 1. Phylogenetic relationships of strain ZS314^T and other members of the family *Microbacteriaceae* based on 16S rRNA gene sequence similarity. The tree was reconstructed by using the neighbour-joining algorithm. The strain characterized in this study is shown in bold type. Numbers at nodes represent levels of bootstrap support (%) based on maximum-likelihood analysis of 100 resampled datasets, and neighbour-joining and maximum-parsimony analysis of 1000 resampled datasets, respectively (values ≥ 50 are shown). GenBank accession numbers are given in parentheses. The affiliation of strain ZS314^T to the genera *Mycetocola*, *Frigoribacterium* and *Frondihabitans* is indicated by a shaded grey rectangle. *Leucobacter komagatae* JCM 9414^T (D45063) was used as an outgroup. Bar, 1 % sequence divergence.

are short rods with sizes of 0.2–0.3 $\mu\text{m} \times 0.7$ –0.9 μm . Colonies are light-yellow, smooth, circular and convex with entire margins after 4 days at 15 °C on Bacto marine 2216 agar. Produces catalase, and is negative for oxidase activity. Growth occurs at 0–26 °C and pH 5.0–10.0, with optimum growth occurring in the temperature range 18–23 °C and pH range 6.0–8.0. Growth occurs in the presence of 0–6 % (w/v) NaCl, with optimum growth occurring in the presence of 2–4 % (w/v) NaCl. Positive for hydrolysis of *para*-nitrophenyl- β -D-galactopyranoside and aesculin. Neither reduction of nitrate nor production of hydrogen sulfide occurs. Indole production and the Voges–Proskauer reaction are negative. Tests for leucine arylamidase, valine

arylamidase and naphthol-AS-BI-phosphohydrolase are positive. Cells are weakly positive for the activities of cystine arylamidase, esterase (C4) and esterase lipase (C8). Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, gelatinase, trypsin, amylase, alkaline phosphatase, acid phosphatase, lipase (C14), α -galactosidase, *ortho*-nitrophenyl- β -D-galactopyranosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase and α -chymotrypsin are negative. Only aesculin is utilized as a sole carbon source (as determined using API 50CH strips). Acid is not produced from a wide variety of carbohydrates other than aesculin. The predominant

Table 1. Differentiating characteristics of the genera of the family *Microbacteriaceae* with B-type peptidoglycan

Genera: 1, *Marisediminicola* sp. nov. (data from this study); 2, *Frigoribacterium* (data taken from Kämpfer *et al.*, 2000); 3, *Frondihabitans* (Zhang *et al.*, 2007b); 4, *Subtercola* (Männistö *et al.*, 2000); 5, *Yonghaparkia* (Yoon *et al.*, 2006); 6, *Cryobacterium* (Suzuki *et al.*, 1997; Zhang *et al.*, 2007a); 7, *Curtobacterium* (Alzawa *et al.*, 2007); 8, *Rathayibacter* (Zgurskaya *et al.*, 1993); 9, *Mycetocola* (Tsukamoto *et al.*, 2001); 10, *Agromyces* (Dorofeeva *et al.*, 2003; Jurado *et al.*, 2005); 11, *Microbacterium* (Behrendt *et al.*, 2001; Takeuchi & Hatano, 1998); 12, *Microcella* (Tiago *et al.*, 2005, 2006). PG, Phosphatidylglycerol; DPG, disphosphatidylglycerol; GL, glycolipids; PL, unidentified phospholipids; Orn, ornithine; Lys, lysine; DAB, diamminobutyric acid; a, anteiso-branched acids; i, iso-branched acids; ch, ω -cyclohexyl acids; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Peptidoglycan type	B2 β	B2 β	B2 β	B2 γ	B2 γ	B2 γ	B2 β	B2 γ	B2 β	B2 γ	B1 α /B1 β /B2 α /B2 β	B2 β
Diamino acid in the peptidoglycan	Orn	D-Lys	Orn	DAB	DAB	DAB	D-Orn	DAB	D-Lys	DAB	L-Lys/D-Orn	D-Lys/D-Orn
DNA G+C content (mol%)	67	72	70–72	64–68	71.1–71.6	65–67	68–75	63–72	64–65	70–72	66–72	67–71
Major fatty acids ($\geq 5\%$)	a-C _{15:0} i-C _{16:0} a-C _{17:0} a-C _{15:1} A	a-C _{15:0} i-C _{16:0} C _{16:0} a-C _{17:0}	C _{18:1} ω 7c C _{14:0} 2-OH	a-C _{15:0} i-C _{16:0} a-C _{17:0}	a-C _{15:0} i-C _{16:0} a-C _{17:0}	a-C _{15:0} a-C _{15:1} b i-C _{15:0} i-C _{16:0}	a-C _{15:0} i-C _{16:0} a-C _{17:0} a-C _{15:1} b ch-C _{17:0}	a-C _{15:0} i-C _{16:0} a-C _{17:0}	a-C _{15:0} i-C _{16:0} a-C _{17:0}	a-C _{15:0} a-C _{17:0} i-C _{16:0}	a-C _{15:0} a-C _{16:0} i-C _{15:0} i-C _{16:0}	i-C _{16:0} a-C _{15:0} i-C _{14:0} i-C _{15:0}
Major menaquinone	MK-10	MK-9	MK-7, 8	MK-9, 10	MK-12	MK-10, 11, 12	MK-9	MK-10	MK-9, 10	MK-12, 13	MK-11, 12, 13	MK-12, 13
Polar lipids	DPG, PG, GL	DPG, PG, GL	DPG, PG, PL	DPG, PG, PL, GL	DPG, PG, PL, GL	DPG, PG, PL, GL	PG, GL	PG, GL	NA	DPG, PG, GL	DPG, PG, GL	NA

cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and anteiso-C_{15:1} A. The DNA G+C content of the type strain is 67.3 mol%.

The type strain, ZS314^T (=DSM 22350^T =CCTCC AB 209077^T), was isolated from a sandy intertidal sediment sample collected from the coastal area off the Chinese Antarctic Zhongshan Station, east Antarctica (69° 22' 13" S 76° 21' 41" E).

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