Microbacterium sediminis sp. nov., a psychrotolerant, thermotolerant, halotolerant and alkalitolerant actinomycete isolated from deep-sea sediment

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An aerobic, rod-shaped, Gram-positive, oxidase- and catalase-positive bacterial isolate, strain YLB-01^T, was characterized using phenotypic and molecular taxonomic methods. 16S rRNA gene sequence analysis revealed that the isolate belonged to the genus *Microbacterium* and represented an evolutionary lineage that was distinct from recognized species of the genus *Microbacterium*. The isolate showed <97 % 16S rRNA gene sequence similarity with respect to the type strains of all of the members of the genus *Microbacterium* with validly published names. Cell-wall hydrolysate from the isolate contained the amino acids ornithine, alanine, glycine homoserine and glutamic acid, and the cell-wall sugars consisted of ribose, glucose and galactose. The main respiratory quinones were MK-8, 9, 10 and 11. The major cellular fatty acids were anteiso-C_{15:0} (38.5 %), anteiso-C_{17:0} (23.1 %) and C_{16:0} (18.9 %). The polar lipids contained diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and two unidentified glycolipids. The DNA G + C content of strain YLB-01^T was 71 mol%. On the basis of the morphological, physiological and chemotaxonomic data and the results of comparative 16S rRNA gene sequence analysis, this isolate represents a novel species of the genus *Microbacterium*, for which the name *Microbacterium sediminis* sp. nov. is proposed. The type strain is YLB-01^T (=DSM 23767^T=CCTCC AB2010363^T=MCCC 1A06153^T).

Recent study of the microbial diversity of deep-sea sediments has shown that this environment might contain >1300 different actinobacterial operational taxonomic units, a great proportion of which are predicted to represent novel species and genera (Stach & Bull, 2005). This study focused on isolating new actinomycetes from deep-sea sediments, and one novel strain of the genus *Microbacterium* was found. The genus *Microbacterium* was described by Orla-Jensen (1919) and belongs to the family *Microbacteriaceae*. Strains of the genus *Microbacterium* are widespread and have been isolated from various environmental sources (Collins & Bradbury, 1992), including soil, water, plants, clinical samples and deep-sea sediments. At the time of writing, the genus *Microbacterium* comprises

73 species with validly published names (<http://www.bacterio.cict.fr/>).

Strain YLB-01^T was isolated from a sediment sample collected at 2327 m water depth, at a site (49.8405° E 37.8111° S) in the south-west Indian Ocean in November 2008 during the cruise of 'Da-Yang Yi-Hao'. Two grams of soil was suspended in 18 ml sterile seawater and mixed. Soil particles were allowed to sediment, the liquid phase was diluted 10⁵-fold and 100 µl samples were spread onto the surface of each cultivation plate. FJ agar plates (1 % glucose, 1 % yeast extract, 1.5 % agar, 50 % seawater) with rifampicin (5 mg l⁻¹) and potassium dichromate (50 mg l⁻¹) were used, and were cultured at 28 °C. Biomass for biochemical and chemotaxonomic study was prepared by culturing on trypticase soy agar (TSA) medium at 28 °C followed by cell harvesting (Bakir *et al.*, 2008).

DNA extraction was performed as described earlier (Xu *et al.*, 2007), and the crude DNA obtained was purified by an EZNA. Cycle-Pure kit (Omega Bio-Tek). Universal

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YLB-01^T is HQ219727.

Six supplementary figures are available with the online version of this paper.

primers, PCR conditions and sequencing were as described by Chun & Goodfellow (1995). The almost-complete 16S rRNA gene sequence (1487 bp) of strain YLB-01^T was obtained and analysed using BLAST searches against the GenBank and EzTaxon databases (Altschul *et al.*, 1997; Chun *et al.*, 2007). The 16S rRNA gene sequences of closely related taxa obtained from the GenBank database were aligned using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic trees were constructed by using the neighbour-joining, minimum-evolution and maximum-parsimony methods with MEGA3.1 software (Kumar *et al.*, 2004; Saitou & Nei, 1987). The topologies of the trees were evaluated by performing a bootstrap analysis using 1000 replications (Felsenstein, 1985). Clustering results using the minimum-evolution and maximum-parsimony approaches (Figs S1 and S2 available in IJSEM Online) were similar to that obtained using the neighbour-joining method.

Phylogenetic analysis showed that strain YLB-01^T formed a clade within the genus *Microbacterium* (Fig. 1). Its closest relatives were *Microbacterium hatanonis* DSM 19179^T (96.9 % 16S rRNA gene sequence similarity), *Microbacterium testaceum* DSM 20166^T (96.9 %), *Microbacterium kitamiense* Kitami C2^T (96.9 %), *Microbacterium phyllosphaerae* DSM 13468^T (96.8 %) and *Microbacterium hydrocarbonoxydans* DSM 16089^T (96.8 %); levels of sequence similarity to other species tested were below 96.8 %. 16S rRNA gene sequence divergences between strain YLB-01^T and recognized species of the genus *Microbacterium* were ≥ 3.1 %, and thus the data support the suggestion that strain YLB-01^T represents a novel

species according to the standards proposed by Schumann & Evtushenko (2006).

Morphological observation under a scanning electron microscope (JEM-1230; JEOL) was performed on cultures grown on TSA at 28 °C for 24 h. The Gram-reaction, and catalase and oxidase activities were studied as previously described (Lai *et al.*, 2009). The ability to grow at various NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 %, w/v) and pH values (from 4.0 to 12.0) were determined at 28 °C using TSA medium. Growth at different temperatures (4, 10, 20, 25, 28, 30, 37, 45, 50, 55 and 60 °C) was tested on TSA. Isolate YLB-01^T and reference strains *M. hatanonis* DSM 19179^T, *M. testaceum* DSM 20166^T, *M. phyllosphaerae* DSM 13468^T and *M. hydrocarbonoxydans* DSM 16089^T were characterized biochemically by using the API 20NE, API ZYM and API 50CH systems according to the manufacturer's instructions (bioMérieux).

Cells of strain YLB-01^T were aerobic, rod-shaped, Gram-positive, oxidase-positive, catalase-positive and motile, and with cell size in the range 0.4–0.7 $\times$ 0.8–1.7 μ m (Fig. S3). Colonies were circular, convex with entire margins, moist and white in colour. The isolate grew at 4–50 °C, at a maximum NaCl concentration of 8 % and at pH 5.0–10.0. Optimum growth was observed at 28 °C without the addition of NaCl.

The G+C content of the chromosomal DNA was determined according to the methods described by Mesbah *et al.* (1989) using reversed-phase HPLC. The DNA G+C content of the new isolate YLB-01^T was 71 mol% and was in the range for species of the genus *Microbacterium* (66.5–71.6 mol%; Takeuchi & Hatano, 1998a).

Fatty acids of whole cells of strain YLB-01^T grown on TSA at 28 °C for 48 h were extracted, saponified and esterified; this was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Microbial ID, Inc.; Method TSBA6; version 6.0B; Agilent Technologies 6850) (Sasser, 1990). The fatty acid profiles of strain YLB-01^T were determined in parallel with those of *M. hatanonis* DSM 19179^T, *M. testaceum* DSM 20166^T, *M. phyllosphaerae* DSM 13468^T and *M. hydrocarbonoxydans* DSM 16089^T in this study. The results are shown in Table 1. The major fatty acids were anteiso-C_{15:0}, anteiso-C_{17:0} and C_{16:0}, which accounted for >75 % of the total fatty acids. Strain YLB-01^T (18.9 %) differed from the other strains (2.0–8.3 %) in the proportion of C_{16:0}, which could be used as a distinguishing characteristic. Polar lipids of strain YLB-01^T were analysed in parallel with those of *M. testaceum* DSM 20166^T. Polar lipids were extracted and identified by two-dimensional TLC (Merck silica gel 60 F254, 10 $\times$ 10 cm, aluminium-backed thin-layer plates) according to the method of Minnikin *et al.* (1984). The TLC plates were sprayed with specific reagents for total lipids (molybdatophosphoric acid), phospholipids (Dittmer and Lester reagents) and glycolipids (α -naphthol). The polar lipids of strain YLB-01^T contained diphosphatidylglycerol, phosphatidylglycerol,

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences created using the neighbour-joining method, showing the phylogenetic position of strain YLB-01^T. Numbers at branch points are bootstrap percentages (based on 1000 resamplings). *Arthrobacter globiformis* DSM 20124^T served as the outgroup. Bar, 0.01 accumulated changes per nucleotide. A more complete tree is shown in Fig. S6.

Table 1. Whole-cell fatty acid compositions (%) of strain YLB-01^T and the type strains of related *Microbacterium* species

Strains: 1, YLB-01^T; 2, *M. hatanonis* DSM 19179^T; 3, *M. testaceum* DSM 20166^T; 4, *M. kitamiense* Kitami C2^T (data from Matsuyama *et al.*, 1999); 5, *M. phyllosphaerae* DSM 13468^T; 6, *M. hydrocarbonoxydans* DSM 16089^T; 7, *M. aurantiacum* IFO 15234^T (Takeuchi & Hatano, 1998a); 8, *M. schleiferi* IFO 15075^T (Bakir *et al.*, 2008); 9, *M. barkeri* DSM 20145^T (Young *et al.*, 2010); 10, *M. paraoxydans* CF36^T (Madhaiyan *et al.*, 2010); 11, *M. profundus* Shh49^T (Wu *et al.*, 2008). Data are from this study except where noted. –, Negative; ND, no data available; tr, trace amount (<1.0 %).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
Saturated fatty acids											
C _{14:0}	2.8	1.1	tr	ND	1.3	tr	ND	–	tr	tr	–
C _{16:0}	18.9	tr	2.0	ND	8.3	3.7	7.5	tr	2.4	2.1	–
C _{18:0}	6.0	tr	tr	ND	3.3	1.2	5.0	tr	ND	ND	ND
C _{19:0}	–	–	tr	ND	–	1.7	ND	–	ND	ND	ND
Unsaturated fatty acids											
anteiso-C _{17:1}	–	2.1	–	ND	–	–	ND	–	ND	ND	ND
C _{18:1} ω9c	–	–	–	ND	tr	–	ND	–	ND	ND	ND
Branched fatty acids											
iso-C _{14:0}	1.7	tr	tr	ND	tr	tr	–	tr	–	–	2.5
iso-C _{15:0}	1.0	tr	5.4	ND	4.3	4.7	4.0	tr	1.8	12.5	15.0
anteiso-C _{15:0}	38.5	48.1	43.6	40–47	40.9	44.4	36.1	55.0	42.1	39.5	60.9
iso-C _{16:0}	14.1	12.5	20.5	10–13	21.7	15.2	20.6	11.3	28.4	14.5	11.7
anteiso-C _{17:0}	17.1	36.6	24.8	32–39	16.3	23.1	26.8	31.1	24.5	25.5	8.4

an unidentified phospholipid and two unidentified glycolipids (Fig. S4).

Analyses of quinones, peptidoglycan structure and cell-wall sugars were performed by the identification service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Respiratory lipoquinones were extracted and analysed by the method described by Tindall (1990a, b). Analysis of the peptidoglycan was performed using TLC, two-dimensional TLC, GC and GC/MS (Schleifer, 1985; MacKenzie, 1987; Groth *et al.*, 1996). For analysis of the whole-cell sugars of strain YLB-01^T, cells were hydrolysed in 0.5 M H₂SO₄ (2 h at 100 °C). H₂SO₄ was removed by 20 % *N,N*-dioctylmethylamine in chloroform according to Whiton *et al.* (1985) and sugars in the hydrolysate were analysed by TLC on cellulose plates according to Stanek & Roberts (1974).

The menaquinones were MK-10 (32 %), MK-11 (25 %), MK-8 (20 %), MK-9 (17 %) and MK-12 (3 %) and some unidentified minor components (Fig. S5). The total hydrolysate (4 M HCl, 100 °C, 16 h; Schleifer, 1985) of the peptidoglycans of strain YLB-01^T contained the amino acids ornithine, alanine, glycine homoserine and glutamic acid. The peptidoglycan was of type B2β. The whole-cell sugars of strain YLB-01^T contained ribose, glucose and galactose; the type strains of related species analysed here lacked ribose (Table 2). The cell-wall diamino acid of strain YLB-01^T was ornithine. These traits are in accordance with the properties of its closest relatives (Behrendt *et al.*, 2001; Bakir *et al.*, 2008; Schippers *et al.*, 2005; Schumann *et al.*, 1999; Takeuchi & Hatano, 1998a).

Strain YLB-01^T differed clearly from phylogenetically related species (Table 2): for instance, in contrast to *M. phyllosphaerae*, it utilized D-fructose, α-D-glucose, D-mannose

and D-sorbitol as a sole carbon source, and produced *N*-acetyl-β-glucosaminidase. The major quinones and cell-wall sugars of strain YLB-01^T were different from those of *M. phyllosphaerae*. The novel strain was able to grow under more extreme conditions than its nearest neighbours: at 4 °C, at 50 °C, at a maximum NaCl concentration of 8 % and at pH 10.0. Consequently, strain YLB-01^T is psychrotolerant, thermotolerant, halotolerant and alkali-tolerant. Colonies of strain YLB-01^T were white, whereas those of *M. phyllosphaerae* were yellow. On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons, strain YLB-01^T should be placed in a novel species within the genus *Microbacterium*, for which the name *Microbacterium sediminis* sp. nov. is proposed.

Description of *Microbacterium sediminis* sp. nov.

Microbacterium sediminis (se.di'mi.nis. L. gen. n. *sediminis* of sediment, the environment from where the type strain was isolated).

Cells are aerobic, rod-shaped, Gram-positive, oxidase-positive, catalase-positive and motile, and with cell size in the range 0.4–0.7 × 0.8–1.7 μm. Colonies are circular, convex with entire margins, moist and white in colour. The temperature range for growth is 4–50 °C, with optimal growth at 28 °C. The pH range for growth is 5.0–10.0, with optimal growth at pH 7.0. No growth occurs at NaCl concentrations above 8 % and optimal growth occurs without the addition of NaCl. Demonstrates positive results for catalase, oxidase, D-glucose fermentation, β-glucosidase (aesculin hydrolysis), β-galactosidase, potassium gluconate, trisodium citrate, and assimilation of D-fructose, α-D-glucose, D-mannitol, D-mannose and

Table 2. Differential characteristics between strain YLB-01^T and related *Microbacterium* species

Strains: 1, YLB-01^T; 2, *M. hatanonis* DSM 19179^T; 3, *M. testaceum* DSM 20166^T; 4, *M. kitamiense* Kitami C2^T (data from Matsuyama *et al.*, 1999); 5, *M. phyllosphaerae* DSM 13468^T; 6, *M. hydrocarbonoxydans* DSM 16089^T; 7, *M. aurantiacum* IFO 15234^T (Collins *et al.*, 1983; Takeuchi & Hatano, 1998a, b; Matsuyama *et al.*, 1999); 8, *M. schleiferi* IFO 15075^T (Yokota *et al.*, 1993; Takeuchi & Hatano, 1998a, b; Bakir *et al.*, 2008); 9, *M. barkeri* DSM 20145^T (Komagata & Suzuki, 1984; Takeuchi & Hatano, 1998a, b; Brennan *et al.*, 2001); 10, *M. paraoxydans* CF36^T (Laffineur *et al.*, 2003); 11, *M. profundus* Shh49^T (Wu *et al.*, 2008). Data are from this study except where noted. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colony colour*	WH	LY	O	O	Y	Y	Y	Y	Y	Y	Y
Grown in/at:											
37 °C	+	–	–	+	+	+	+	+	+	+	–
5 % NaCl	+	–	+	+	–	–	+	–		+	+
8 % NaCl	+	–	–	–	–	–	–	–		+	–
DNA G + C content (mol%)	71	69	67.7	69.2	64	ND	70.3	66.5	68.7	69.9	66.8
Major menaquinones (MK)	8, 9, 10, 11	11, 12	10, 11	11, 12	10, 11, 12	11, 12	11, 12	10, 11, 12	11, 12	11, 12	12, 13
Cell-wall diamino acid†	Orn	Orn	Orn	Orn	Orn	Orn	Lys	Orn	Orn	Orn	Orn
Cell-wall sugars‡	Rib, Glc, Gal	Rha, Gal	Rha, Man, Gal	ND	Gal, Rha	ND	Rha, Fuc, Gal,	6dTal, Man, Gal	Rha, Gal, Glc	Glc, Gal	ND
API 50CH results											
D-Fructose	–	–	+	ND	–	–	ND	ND	ND	ND	+
α-D-Glucose	+	–	–	+	–	–	+	w	ND	+	+
D-Mannitol	+	+	–	+	+	+	–	–	–	ND	+
Maltose	+	+	+	ND	w	+	+	+	ND	ND	+
D-Mannose	–	–	+	ND	–	w	+	+	+	ND	+
L-Rhamnose	+	+	–	–	w	+	+	ND	+	+	+
D-Gluconic acid	–	–	+	ND	–	–	ND	ND	ND	ND	ND
L-Alaninamide	w	–	+	ND	–	–	ND	ND	ND	ND	+
D-Alanine	w	–	+	ND	–	–	ND	ND	+	ND	–
D-Galactose	w	–	+	ND	w	w	+	ND	+	+	+
Gentiobiose	w	+	+	ND	–	+	ND	ND	ND	ND	ND
Sucrose	–	–	+	ND	–	–	+	+		–	–
D-Sorbitol	–	–	+	ND	–	+	ND	ND	ND	ND	ND
API ZYM results											
Alkaline phosphatase	+	w	+	ND	w	–	ND	–	ND	ND	ND
Esterase (C8)	+	+	+	ND	w	w	ND	–	+	–	ND
Lipase (C14)	w	+	+	ND	+	–	ND	ND	ND	ND	ND
Trypsin	+	+	+	ND	+	w	ND	w	ND	ND	ND
α-Chymotrypsin	+	–	+	ND	+	–	ND	–	ND	ND	ND
α-Galactosidase	+	–	+	ND	+	w	ND	–	ND	ND	ND
β-Galactosidase	w	+	+	ND	+	+	ND	+	ND	ND	ND
β-Glucuronidase	+	w	+	ND	w	w	ND	–	ND	ND	ND
α-Glucosidase	–	+	+	ND	+	+	ND	+	–	+	ND
β-Glucosidase	–	+	+	ND	+	+	ND	+	+	–	ND
N-Acetyl-β-glucosaminidase	+	–	+	–	–	+	+	–	+	+	–
α-Mannosidase	+	–	+	ND	+	+	ND	–	ND	ND	ND
API 20NE results											
Gelatin hydrolysis	–	–	–	ND	+	–	ND	ND	ND	ND	ND
L-Arabinose	–	+	+	ND	+	+	ND	ND	ND	ND	ND
Malic acid	w	–	+	ND	+	+	ND	ND	ND	ND	ND
Phenylacetic acid	–	–	–	ND	–	w	ND	ND	ND	ND	ND

*WH, white; LY, light yellow; O, orange; Y, yellow.

†Lys, lysine; Orn, ornithine.

‡Rib, ribose; 6dTal, 6-deoxytalose; Fuc, fucose; Gal, galactose; Glc, glucose; Rha, rhamnose; Man, mannose.

D-sorbitol. Weakly positive results in tests for D-gluconic acid, L-alaninamide, D-alanine, L-alanyl glycine and D-galactose. Gives positive results for the production of alkaline phosphatase, esterase lipase (C8), lipase (C14), leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, α -chymotrypsin, acid phosphatase, β -galactosidase, β -glucosidase and N-acetyl- β -glucosaminidase. Shows negative results for nitrate reduction, indole production, arginine dihydrolase, urease, utilization of L-arabinose, capric acid, adipic acid and phenylacetic acid, and for production of naphthol-AS-BI-phosphamidase, β -glucuronidase, α -glucosidase, α -mannosidase and α -fucosidase. Cell-wall hydrolysate contains the amino acids ornithine, alanine, glycine homoserine and glutamic acid, and the cell-wall sugars are ribose, glucose and galactose. The menaquinones are MK-10, MK-11, MK-8, MK-9 and MK-12 and some unidentified minor components. The major cellular fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0} and C_{16:0}. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and two unidentified glycolipids.

The type strain, YLB-01^T (=DSM 23767^T=CCTCC AB2010363^T=MCCC 1A06153^T), was isolated from sediment of the Indian Ocean. The DNA G+C content of the type strain is 71 mol%.

Acknowledgements

This study was supported by Science and Technology project of Xiamen (3502Z20111051) and the R&D Special Fund for Public Welfare Industry (Oceanography) (201005022-1, 201005032-1).

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