Sediminimonas qiaohouensis gen. nov., sp. nov., a member of the *Roseobacter* clade in the order *Rhodobacterales*

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Two aerobic bacterial strains, YIM B024^T and YIM B025, were isolated from a salt mine in Yunnan, south-west China. Both strains showed almost the same physiological properties. Cells were Gram-negative, non-motile, non-spore-forming rods. The novel strains grew at 15–37 °C, pH 6.5–9.0 and 0.25–20 % (w/v) NaCl; optimum growth was observed at 28–30 °C, pH 7.0–8.5 and 1.5–10 % NaCl. Oxidase, catalase and nitrate-reducing activities were detected. The two strains were closely related to each other with a 16S rRNA gene sequence similarity of 100 %. DNA–DNA hybridization experiments revealed high relatedness values (90 ± 0.4 %) between strains YIM B024^T and YIM B025, which suggested that these two new strains constituted a single species. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the two isolates formed a loose cluster with members of the genus *Roseivivax* in the *Roseobacter* clade, but were clearly separated from this genus. The levels of 16S rRNA gene sequence similarity between the two isolates and members of the genus *Roseivivax* ranged from 92.4 to 93.9 %. The major polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and four unknown phospholipids. The major cellular fatty acids were C_{18:1}ω7c, C_{16:0}, C_{18:1}ω9c, 11-methyl C_{18:1}ω7c and C_{19:0} cyclo ω8c. The sole respiratory quinone was Q-10 and the genomic DNA G + C content was 63.0–64.1 mol%. The distinct phylogenetic position and a combination of phenotypic and chemotaxonomic characteristics supported the proposal of the new isolates as representing a novel species in a new genus, for which the name *Sediminimonas qiaohouensis* gen. nov., sp. nov. is proposed. The type strain of the type species is YIM B024^T (=KCTC 22349^T=CCTCC AA 208033^T).

The *Roseobacter* clade (also known as the *Roseobacter–Sulfitobacter–Silicibacter* group; Wagner-Döbler *et al.*, 2003) in the order *Rhodobacterales* (Garrity *et al.*, 2005) of the subclass *Alphaproteobacteria* is the second most abundant 16S rRNA gene-clone type in marine environments (Giovannoni & Rappé, 2000; Rappé *et al.*, 2000).

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Abbreviations: EPS, exopolysaccharide; PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM B024^T and YIM B025 are EU878003 and EU878004, respectively.

A supplementary figure showing the polar lipid profile of strain YIM B024^T is available with the online version of this paper.

According to Garrity *et al.* (2004), the *Roseobacter* clade contained 33 genera. Recently some novel genera, including *Oceanicola* (Cho & Giovannoni, 2004), *Loktanella* (Van Trappen *et al.*, 2004), *Salipiger* (Martínez-Cánovas *et al.*, 2004), *Roseisalinus* (Labrenz *et al.*, 2005), *Thalassobacter* (Macián *et al.*, 2005), *Palleronia* (Martínez-Checa *et al.*, 2005), *Yangia* (Dai *et al.*, 2006), *Pelagibaca* (Cho & Giovannoni, 2006), *Phaeobacter* (Martens *et al.*, 2006), *Shimia* and *Citreimonas* (Choi & Cho, 2006a, b), *Maribius* (Choi *et al.*, 2007), *Maritimibacter* (K. Lee *et al.*, 2007), *Wenxinia* (Ying *et al.*, 2007), *Thalassococcus* (O. O. Lee *et al.*, 2007) and *Ponticoccus* (Hwang & Cho, 2008) have been established, giving a total of 49 genera in this clade. Members of the group show diverse physiological and morphological features (e.g. phototrophy, aerobic sulfite

oxidation, organic sulfur compound degradation, methylotrophy, gas vacuoles, poly- β -hydroxybutyrate (PHB) granules, rosette formation and production of exopolysaccharide) (Arahal *et al.*, 2005; Buchan *et al.*, 2005; Martínez-Checa *et al.*, 2005).

Strains YIM B024^T and YIM B025 were isolated during an investigation of the cultured microbial diversity of three ancient salt mines in Yunnan, south-west China. The two novel strains were isolated from ancient salt sediment collected from the Qiaohou salt mine using a standard dilution-plating technique at 28 °C on Difco marine agar 2216 (MA; pH 7.2). The strains were stored as 20 % (v/v) glycerol suspensions at -80 °C. To investigate their morphological, physiological and biochemical characteristics, strains YIM B024^T and YIM B025 were routinely cultivated at 28–30 °C, their optimal growth temperature range, on MA or in Difco marine broth 2216 (MB) unless specified otherwise.

Growth of the novel strains at various temperatures (4–50 °C) and over a range of pH values (4.5–10.5) was determined in MB. Tolerance of NaCl (0–25 %, w/v) was measured on MA by supplementing the medium with various concentrations of NaCl. Growth on trypticase soy agar (TSA; Difco), nutrient agar (NA; Difco) and MY medium (Quesada *et al.*, 1993) was tested at 28 °C. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (GasPak Anaerobic systems, BBL) on MA. The morphology of the cells and the presence of flagella were studied by light microscopy (BH-2; Olympus) after staining. For exopolysaccharide (EPS) recovery, cells in the stationary growth phase were harvested by centrifugation at 15 000 r.p.m. at 4 °C and the supernatants were treated and analysed as described by Manca *et al.* (1996). Accumulation of PHB was determined by the Sudan Black staining method (Smibert & Krieg, 1994) under a light microscope. Bacteriochlorophyll *a* was analysed spectrophotometrically using the procedure of Cohen-Bazire *et al.* (1957) following the recommendations of Allgaier *et al.* (2003). Gram staining was performed using the standard Gram reaction combined with the KOH lysis test method (Gegersen, 1978). Degradation of aesculin, casein, starch, Tweens 20, 40 and 60, xanthine and hypoxanthine were determined according to the protocols described by Cowan & Steel (1965). Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using a 1 % (w/v) solution of tetramethyl-*p*-phenylenediamine (Kovacs, 1956). The ability of the novel strains to utilize 95 carbon or energy sources was determined using the Biolog GN2 microplate system and enzyme activity tests were performed using API ZYM test kits (bioMérieux) according to the manufacturer's instructions. Antibiotic susceptibility was determined as described by Groth *et al.* (2004) using antibiotic discs (Himedia). Other biochemical tests were carried out with API 20NE and API 20E kits (bioMérieux). For all of these tests, cell suspensions were supplemented with 3 % NaCl (w/v).

The two novel strains were very similar in terms of morphological characteristics. Cells of both strains were Gram-negative, catalase- and oxidase-positive, non-motile and non-spore-forming irregular rods. The size of the cells ranged from 0.35–0.5 μm $\times$ 1.5–3.5 μm . Colonies were faint brown–yellow, 0.5–1.75 mm in diameter, uniformly circular and convex and opaque after growth on MA or MY agar medium at 28 °C for 5 days. Neither of the novel isolates grew on trypticase soy agar nor on nutrient agar. No growth was found under strict anaerobic conditions, even with prolonged incubations of 30 days. However, strains YIM B024^T and YIM B025 were able to sustain their growth activity under microaerobic conditions, but growth was poor. The temperature range for growth was 15–37 °C (optimum 28–30 °C). The pH range for growth was pH 6.5–9.0 (optimum 7.0–8.5) and the NaCl concentration for growth was 0.25–20 % (w/v) (optimum 1.5–10 %). The physiological characteristics of the novel strains are given in Table 1 and in the genus and species descriptions. The two novel strains were very similar in terms of carbon source assimilation and enzyme content.

Strains YIM B024^T and YIM B025 were cultivated for 5 days in MB at 28 °C to obtain the cell mass required for chemotaxonomic analysis. Polar lipids were extracted as described by Minnikin *et al.* (1979) and identified by two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Respiratory quinones were extracted by using the method of Collins *et al.* (1977) and analysed by HPLC as described by Tamaoka *et al.* (1983). Biomass for quantitative fatty acid analysis of strains YIM B024^T and YIM B025 was prepared by scraping growth from MA plates that had been incubated for 5 days at 28 °C. Analysis of the whole-cell fatty acid pattern followed the methods described by Sasser (1990) using the Microbial Identification System (MIDI). The G+C content of the genomic DNA was determined by HPLC according to Mesbah *et al.* (1989), after DNA extraction by the method of Cui *et al.* (2001). The genomic DNA of *Escherichia coli* H5 α was used as a standard.

The DNA G+C contents of strains YIM B024^T and YIM B025 were 63.0 mol% and 64.1 mol%, respectively. The polar lipid compositions of the two isolates were very similar and contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and four unknown phospholipids (see Supplementary Fig. S1, available in IJSEM Online). The sole respiratory quinone was Q-10. The major cellular fatty acids (>5 %) in strains YIM B024^T and YIM B025 were C_{18:1} ω 7c, C_{16:0}, C_{18:1} ω 9c, 11-methyl C_{18:1} ω 7c and C_{19:0} cyclo ω 8c. The presence of C_{18:1} ω 7c as the predominant fatty acid is a feature that is characteristic of taxa within the class *Alphaproteobacteria*. However, the cyclo-substituted fatty acid (C_{19:0} cyclo ω 8c) is not widely present in the *Roseobacter* clade except for *Sagittula stellata*, *Palleronia marisminoris* and *Salipiger mucosus*. The novel strains could be distinguished from these bacteria on the basis of differences in the proportions of several fatty acids, including C_{16:0}, C_{18:1} ω 9c, C_{10:0} 3-OH, C_{12:1} 3-OH and C_{19:0} cyclo ω 8c (Table 1).

Table 1. Characteristics that distinguish strains YIM B024^T and YIM B025 from related members of the *Roseobacter* clade

Strains/species: 1, YIM B024^T; 2, YIM B025; 3, *Ruegeria lacuscaerulensis* DSM 11314^T (Petursdottir & Kristjansson, 1997; Yi *et al.*, 2007); 4, *Phaeobacter daeponensis* TF-218^T (Yoon *et al.*, 2007); 5, *Yangia pacifica* DX5-10^T (Dai *et al.*, 2006); 6, *Roseovarius tolerans* EL-172^T (Labrenz *et al.*, 1999); 7, *Sagittula stellata* ATCC 700073^T (González *et al.*, 1997); 8, *Roseivivax halodurans* JCM 10272^T; 9, *Roseivivax halotolerans* JCM 10271^T (Suzuki *et al.*, 1999; Nishimura *et al.*, 1994); 10, *Palleronia marisminoris* B33^T (Martínez-Checa *et al.*, 2005); 11, *Salipiger mucosus* CECT 5855^T (Martínez-Cánovas *et al.*, 2004). +, Positive; –, negative; w, weakly positive; ND, no data available; NQ, not quantified.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Pigment	Faint brown–yellow	Light brown–yellow	Tan	Yellowish white	Faint yellow	Red	Cream	Pink	Pink	Pink	–
Cell shape	Rods	Rods	Rods	Oval	Rods	Rods	Rods	Rods	Rods	Short rods	Rods
Motile	–	–	–	+	+	+	+	+	+	–	–
Oxidase	+	+	+	+	w	+	+	+	+	–	+
PHB	–	–	–	ND	+	+	+	ND	ND	+	+
Bacteriochlorophyll <i>a</i>	–	–	–	–	–	+	–	+	+	–	–
Na ⁺ requirement	+	+	+	+	+	+	+	–	+	+	+
NaCl optimum (%)	1.5–10	1.5–10	3.5	ND	5	1.0–8.0	ND	ND	ND	ND	9–10
Temperature optimum (°C)	28–30	28–30	45	ND	37	8.5–33.5	30	27–30	27–30	ND	20–40
Nitrate to nitrite	+	+	+	+	–	+	–	+	–	–	–
Acid from glucose	+	+	–	–	–	ND	–	+	+	–	–
Indole	–	–	–	–	–	–	ND	+	+	–	–
β-Galactosidase	–	–	+	–	ND	ND	ND	+	+	+	–
Major fatty acids (%)*											
C _{10:0}			2.7								
C _{12:0}			2.3	1.2							
C _{14:0}	1.47	1.06									
C _{14:0} 2-OH						2.4					
C _{15:0}	2.99	2.38									
C _{16:0}	14.98	13.90	2.1	8.6	8.95	6.2	8.6			4.3	12.4
C _{16:0} 2-OH				5.6							
C _{16:1} ω7c					3.93						1.3
C _{17:0}	2.76	2.62			1.44						
C _{17:1} ω8c					3.93						
C _{18:0}	3.42	3.10	2.1	2.4			6.8			3.4	2.0
C _{18:1}						72.0		NQ	NQ		
C _{18:1} ω7c	21.31	29.76	72.9	57.7			NQ			68.9	78.0
C _{18:1} ω7c/ω9c/ω12t					81.67						
C _{18:1} ω9c	10.51	7.07									
C _{18:2}						10.6					
3-OH fatty acids											
C _{10:0}	1.51	1.96	1.6	1.7						5.0	
C _{12:0}			4.0	2.6	3.2						
C _{12:1}	2.59	3.07				3.6	3.6				2.3
Methyl fatty acids											
11 methyl C _{18:1} ω7c	8.31	6.76	7.3	16.6							1.9
Cyclo-substituted fatty acids											
C _{19:0} cyclo ω8c	16.29	17.60					1.8			12.8	2.3
C _{19:0} cyclo			2.1								
DNA G + C content (mol%)	63.0	64.1	66	64.9	63.3	63.3–63.4	65	64.4	59.7	64.2	64.5

*Only percentages >1 % are shown.

Genomic DNA extraction, PCR amplification of 16S rRNA gene and sequencing of the purified PCR products were performed as described previously (Cui *et al.*, 2001). The almost-complete 16S rRNA gene sequences of strains YIM B024^T (1368 bp) and YIM B025 (1357 bp) were obtained and compared with those available in GenBank using BLAST (Altschul *et al.*, 1990). Alignments and similarities were obtained with the CLUSTAL_X program (Thompson *et al.*, 1997). Phylogenetic analyses were performed using MEGA3 (Kumar *et al.*, 2004). Distances (corrected by Kimura's two-parameter model; Kimura, 1980) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees (not shown) were generated using the treeing algorithms contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1000 resamplings (Felsenstein, 1985). Fluorometric DNA–DNA hybridization experiments were performed with photobiotin-labelled probes as described by Ezaki *et al.* (1989).

Preliminary BLAST searches showed that the two novel isolates belonged to the *Roseobacter* clade in the order *Rhodobacterales*. To clarify the phylogenetic position of

strains YIM B024^T and YIM B025, the phylogenetic tree was constructed using the neighbour-joining algorithm (Fig. 1). The two strains formed a monophyletic clade loosely associated with the genus *Roseivivax*. This relationship was maintained in the trees constructed using the maximum-likelihood and maximum-parsimony algorithms. The 16S rRNA gene sequence similarity value between strains YIM B024^T and YIM B025 was 100%, whereas the gene sequence similarity values between the two strains and *Roseivivax halodurans* JCM 10272^T and *Roseivivax halotolerans* JCM 10271^T ranged from 92.8 to 93.9% and from 92.4 to 93.5%, respectively. The gene sequence similarity values for the two novel isolates and other closely related phylogenetic neighbours of the *Roseobacter* clade were as follows: *Ruegeria lacuscaerulensis* DSM 11314^T, 93.4–94.3%; *Phaeobacter daeponensis* TF-218^T, 93.2–94.4%; *Yangia pacifica* DX5-10^T, 93.2–94.0%; *Roseovarius tolerans* DSM 11457^T, 92.9–93.8%; *Sagittula stellata* ATCC 700073^T, 92.7–93.7%; *Palleronia marisminoris* B33^T, 92.6–93.2% and *Salipiger mucosus* CECT 5855^T, 92.4–93.2%. These values indicated that the novel strains could represent a new taxon of genus rank.

To confirm that the two novel strains belonged to a novel species, DNA–DNA hybridization studies were performed.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains YIM B024^T and YIM B025 and representatives of the *Roseobacter* clade. Bootstrap percentages (based on 1000 replications) >50% are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-parsimony and maximum-likelihood methods. Open circles indicate that the corresponding nodes were found only in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position.

The level of DNA–DNA relatedness between the two novel strains was $90 \pm 0.4\%$. When the recommendation of a threshold value of 70 % DNA–DNA similarity for the delineation of bacterial species (Wayne *et al.*, 1987) is considered, this result strongly suggests that the new isolates belong to the same separate novel species.

The evidence collected using a polyphasic approach, including fatty acid profiles, quinone determination, DNA–DNA hybridization values, 16S rRNA gene sequence analyses, differences in phenotypic characteristics and the inability of the novel strains to synthesize bacteriochlorophyll *a* or to accumulate PHB, demonstrated conclusively (Table 1) that strains YIM B024^T and YIM B025 should be recognized as representing a new species of a novel genus within the order *Rhodobacterales*. The name *Sediminimonas* gen. nov. is proposed for this novel genus and the name *Sediminimonas qiaohouensis* sp. nov. is proposed for the type species.

Description of *Sediminimonas* gen. nov.

Sediminimonas (se.di.mi.ni.mo'nas. N.L. n. *sedimen* sediment; Gr. fem. n. *monas* monad unit; N.L. fem. n. *Sediminimonas* monad isolated from sediment).

Cells are Gram-negative, non-motile, aerobic, short rods (1.5–3.5 µm long, 0.35–0.5 µm wide). Bacteriochlorophyll *a* is not found. Do not produce PHB or exopolysaccharides. Nitrate and nitrite are reduced. Chemoheterotrophic and slightly halophilic, requiring NaCl for growth. Produce acids from glucose (API 20NE) and utilize a variety of carbon compounds as sole carbon sources. The major fatty acids are C_{18:1}ω7c, C_{16:0}, C_{18:1}ω9c, 11-methyl C_{18:1}ω7c and C_{19:0} cyclo ω8c. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and four unknown phospholipids. The sole respiratory ubiquinone is Q-10. The DNA G+C content is 63.0–64.1 mol%. The genus is affiliated to the *Roseobacter* clade in the order *Rhodobacterales* and currently contains only one species, the type species, *Sediminimonas qiaohouensis*.

Description of *Sediminimonas qiaohouensis* sp. nov.

Sediminimonas qiaohouensis (qi.ao.hou.en'sis. N.L. fem. adj. *qiaohouensis* from the Qiaohou salt mine, where the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Colonies on MA and MY media are circular, convex, faint brown–yellow and 0.5–1.75 mm in diameter. Grows at 15–37 °C, optimally at 28–30 °C. Growth occurs at pH 6.5–9.0 and 0.25–20 % (w/v) NaCl; optimum growth occurs at pH 7.0–8.5 and at 1.5–10 % NaCl. Catalase- and oxidase-positive. Tween 20 and aesculin are hydrolysed. In tests with Biolog GN2 microplates, the following substrates are utilized: dextrin, *N*-acetyl-D-glucosamine, D-fructose, D-galactose, α-D-glucose, D-gluconic acid, maltose, D-maltotriose, D-mannitol,

D-mannose, D-psicose, sucrose, trehalose, turanose, γ-hydroxyphenylacetic acid, D- and L-alanine, L-glutamic acid, L-serine, glycerol, thymidine and uridine. With the API ZYM system (bioMérieux), positive reactions are obtained for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, β-glucosidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Negative reactions are obtained for cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. With API 20NE kits, β-glucosidase activity is present, but gelatin is not hydrolysed. With API 20E kits, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease formation are positive, however, indole production, H₂S production and the Voges–Proskauer test are negative. Resistant to gentamicin (10 µg), amikacin (30 µg) and norfloxacin (10 µg), but susceptible to ampicillin (10 µg), cephalothin (30 µg), benzylpenicillin (10 µg), ciprofloxacin (5 µg), carbenicillin (100 µg), erythromycin (15 µg) and chloramphenicol (30 µg).

The type strain, YIM B024^T (=KCTC 22349^T=CCTCC AA 208033^T), and reference strain YIM B025 (=KCTC 22350=CCTCC AA 208034), were isolated from ancient salt sediment collected from the Qiaohou salt mine in Yunnan, south-west China.

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