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 B024T 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 B024T 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 diphasmatidglycerol, phosphatidyglycerol, phosphatidylcholine and four unknown phospholipids. The major cellular fatty acids were C18 : 1ω7c, C16 : 0, C18 : 1ω9c, 11-methyl C18 : 1ω7c and C19 : 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 B024T (=KCTC 22349T=CCCTCC AA 208033T).
oxidation, organic sulfur compound degradation, methyl-
-R. Arahal et al., 2005; Buchan et al., 2003; Martinez-

Strains YIM B024T 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 characteris-
tics, strains YIM B024T 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 incuba-
tion 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 (Gregersen, 1978). Degradation of
aesculin, casein, starch, Tween 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) accord-
ing to the manufacturer’s instructions. Antibiotic suscept-
bility 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 × 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 B024T 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 concentra-
tion 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 B024T 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 B024T 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 H5z was used as a standard.

The DNA G+C contents of strains YIM B024T 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 B024T and YIM B025 were C18:1ω7c,
C16:0ω9c, 11-methyl C18:1ω7c and C19:0 cyclo ω8c.
The presence of C18:1ω7c as the predominant fatty acid is a
feature that is characteristic of taxa within the class
Alphaproteobacteria. However, the cyclo-substituted fatty
acid (C19:0 cyclo ω8c) is not widely present in the Roseobacter
clad except for Sagittula stellata, Palleronia marisminoris and
Salipigus mucosus. The novel strains could be distinguished
from these bacteria on the basis of differences in the
proportions of several fatty acids, including C16:0ω9c,
C18:1ω9c, C19:0 3-OH, C12:1 3-OH and C19:0 cyclo ω8c (Table 1).
Table 1. Characteristics that distinguish strains YIM B024<sup>T</sup> and YIM B025 from related members of the *Roseobacter* clade

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

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*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 B024T (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 MEGAS (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 above tree 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 B024T 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 B024T and YIM B025 was 100 %, whereas the gene sequence similarity values between the two strains and Roseivivax halodurans JCM 10272T and Roseivivax halotolerans JCM 10271T 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 11314T, 93.4–94.3 %; Phaeobacter daeponensis TF-218T, 93.2–94.4 %; Yangia pacifica DX5-10T, 93.2–94.0 %; Roseovarius tolerans DSM 11457T, 92.9–93.8 %; Sagittula stellata ATCC 700073T, 92.7–93.7 %; Palleronia marismorinis B33T, 92.6–93.2 % and Salipiger mucosus CECT 5855T, 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.

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**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains YIM B024T 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 ± 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 B024T 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. *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 C₁₈:₁ω7c, C₁₆:₀, C₁₈:₁ω9c, 11-methyl C₁₈:₁ω7c and C₁₉:₂ cyclo ω8c. The polar lipids consist of diphasphatidylglycerol, 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, β-galactosidase, β-gluconidase, 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 B024T (=KCTC 22349T=CCTCC AA 208033T), 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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**References**


