Sediminimonas qiahouensis 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 diphasphatidylglycerol, phosphatidylglycerol, 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 qiahouensis gen. nov., sp. nov. is proposed. The type strain of the type species is YIM B024T (=KCTC 22349T=CCTCC AA 208033T).

The Roseobacter clade (also known as the Roseobacter–Sulfotobacter–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).

†These authors equally contributed to this work.

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

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM B024T and YIM B025 are EU878003 and EU878004, respectively.

A supplementary figure showing the polar lipid profile of strain YIM B024T 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 (Martinez-Canoas et al., 2004), Roseisalminus (Labrenz et al., 2005), Thalassobacter (Macian et al., 2005), Palleronia (Martinez-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), Wexvinia (Ying et al., 2007), Thalassosoccus (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
Y.-X. Wang and others

oxidation, organic sulfur compound degradation, methyl-

otrophy, gas vacuoles, poly-β-hydroxybutyrate (PHB)

granules, rosette formation and production of exopolysac-

charide) (Arahah et al., 2005; Buchan et al., 2003; Martinez-

Checa et al., 2005).

Strains YIM B024 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 (Grgersen, 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) accor-
ding to the manufacturer’s instructions. Antibiotic suscepti-
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

chmetatnomic 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. (1999), 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,

C16:0, C18:1ω9c, 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 clade except for Sagittula stellata, Palleronia marismorinis and

Salipiger mucosus. The novel strains could be distinguished from these bacteria on the basis of differences in the

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

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<td>C&lt;sub&gt;19:0&lt;/sub&gt; cyclo</td>
<td></td>
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<td>12.8</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>63.0</td>
<td>64.1</td>
<td>66</td>
<td>64.9</td>
<td>63.3</td>
<td>63.3–63.4</td>
<td>65</td>
<td>64.4</td>
<td>59.7</td>
<td>64.2</td>
<td>64.5</td>
</tr>
</tbody>
</table>

*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-completed 16S rRNA gene sequences of strains YIM B024<sup>T</sup> (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 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 (Klug & Farris, 1969) trees (not shown) were generated using the treeing algorithms contained in the PHYLIPI 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<sup>T</sup> 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<sup>T</sup> and YIM B025 was 100%, whereas the gene sequence similarity values between the two strains and *Roseivivax halodurans* JCM 10272<sup>T</sup> and *Roseivivax halotolerans* JCM 10271<sup>T</sup> 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 lacuscoraeilensis* DSM 11314<sup>T</sup>, 93.4–94.3%; *Phaeobacter daeponensis* TF-218<sup>T</sup>, 93.2–94.4%; *Yangia pacifica* DX5-10<sup>T</sup>, 93.2–94.0%; *Roseovarius tolerans* DSM 11457<sup>T</sup>, 92.9–93.8%; *Sagittula stellata* ATCC 700073<sup>T</sup>, 92.7–93.7%; *Palleronia marisminoris* B33<sup>T</sup>, 92.6–93.2% and *Salipiger mucosus* CECT 5855<sup>T</sup>, 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 B024<sup>T</sup> 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 trees generated with the maximum-likelihood 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. *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. Chemoherotrophic 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 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 C19:0 cyclo ω8c. The polar lipids consist of phosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol 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 aerocellulases 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-hydroxybutyrylacetic 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, χ-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), cefalothin (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.

**Acknowledgements**

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**References**


