Salinarimonas rosea gen. nov., sp. nov., a new member of the χ-2 subgroup of the Proteobacteria

Ji-Hui Liu,† Yong-Xia Wang,† Xiao-Xia Zhang, Zhi-Gang Wang, Yi-Guang Chen, Meng-Liang Wen, Li-Hua Xu, Qian Peng and Xiao-Long Cui

The order Rhizobiales is a phenotypically heterogeneous assemblage within the χ-2 subgroup of the phylum Proteobacteria (Woese et al., 1984). The cluster includes members with a variety of morphological, physiological and biological features, which may impede their taxonomic definition (Lee et al., 2005). Garrity et al. (2004) proposed that the order Rhizobiales comprised 11 families based on 16S rRNA gene sequence analysis: Rhizobiaceae, ‘Aurantimonadaceae’, Bartonellaceae, Brucellaceae, Phyllobacteriaceae, Methylocystaceae, Beijerinckiaceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Methylobacteriaceae and Rhodobacteriaceae. In addition to these families, a new family, Xanthobacteriaceae (Lee et al., 2005), was described subsequently as a result of phylogenetic analysis of 16S rRNA gene sequences. Recently, another new family, Cohaesibacteriaceae (type genus Cohaesibacter), within the order Rhizobiales was identified by Hwang & Cho (2008) using a polyphasic approach. In this paper, characterization and classification of a novel genus-level strain within the order Rhizobiales is reported.

Strain YIM YD3T was isolated from a deposit sample collected from a salt mine in Moxie, Yunnan Province, south-west China. The novel strain was isolated by the standard dilution-plating technique on marine agar 2216 (MA; Difco) cultivated in an anaerobic chamber (GasPak Anaerobic systems; BBL) at 28 °C for 14 days. Subsequently, the isolate was purified by cultivation on MA at 28 °C for 4 times and then cultivated under aerobic or anaerobic conditions. The isolate was able to grow well under aerobic conditions. It was maintained on MA slants at 4 °C and stored as 20% glycerol suspensions at −80 °C.

Morphological, physiological and biochemical characteristics of strain YIM YD3T were investigated using routine cultivation on MA. Gram staining was carried out using the standard Gram reaction combined with the KOH lysis...
test method (Gregersen, 1978). Cellular morphology and the presence of flagella were determined using light microscopy (BH-2; Olympus) after staining. Motility was determined by the hanging drop method (Smibert & Krieg, 1994). Bacteriochlorophyll a production was analysed spectrophotometrically using the procedure of Cohen-Bazire et al. (1957) following the recommendations of Allgaier et al. (2003). The presence of poly-β-hydroxybutyrate was determined by the Sudan Black B staining method (Smibert & Krieg, 1994) under a light microscope. Thioulate oxidation was tested as described by Takeda et al. (2004). Growth was also tested on trypticase soy agar (TSA; BBL), nutrient agar and MY medium (Quesada et al., 1993). The temperature range (5, 10, 15, 20, 28, 30, 35, 37 and 40 °C) and pH range (pH 4.0–10.0 at unit intervals) for growth were tested on the basis of colony formation on MA plates. To test NaCl tolerance, TSA containing various concentrations of NaCl (0, 0.5, 1, 2, 3, 5, 7, 10, 15, 20 and 25 %, w/v) was used. 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). Degradation of ascuscin, casein, starch, cellulose, Tweens 20, 40 and 60, xanthine and hypoxanthine was determined as described by Cowan & Steel (1965). In addition, nitrate reduction, production of indole and H2S, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and gelatinase activities and the Voges–Proskauer test were assayed using API 20E and API 20NE kits (bioMérieux), respectively, with 3 % (w/v) NaCl solution except that cell suspensions were supplemented with 3 % (w/v) NaCl. Other enzyme activities and acid production were assayed using the API ZYM and API 50CH kits (bioMérieux), respectively, with 3 % (w/v) NaCl solution as the suspension medium. Carbon utilization was tested using artificial seawater medium (Cho & Giovannoni, 2006) as the basal medium containing each carbon source at a final concentration of 0.5 % (w/v or v/v). Antibiotic sensitivities were determined using the disk-diffusion methodology (Bauer et al., 1966).

Polar lipids were extracted as described by Minnikin et al. (1979) and identified by two-dimensional TLC followed by spraying with specific reagents (Collins & Jones, 1980). Respiratory quinones were extracted according to 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 strain YIM YD3T was obtained from sediment of Guanting Reservoir (Qu et al., 2008). Sequence comparisons with representative bacteria with validly published names indicated that strain YIM YD3T belonged to the class Alphaproteobacteria in the order Rhizobiales (Fig. 1; a global tree is available as Supplementary Fig. S1 in IJSEM Online). Strain YIM YD3T formed a distinct phylogenetic lineage within a clade containing the genera Balneimonas, Bosea, Chelatococcus, Microvirga and Methylobacterium in the order Rhizobiales and was most closely related to the type strains of Balneimonas flocculans (93.5 % 16S rRNA gene sequence similarity), Chelatococcus daeguensis (93.0 %), Bosea massiliensis (92.6 %), Bosea minititanlanis (92.5 %), Bosea enea (92.3 %), Bosea vestrisii (92.3 %), Bosea thiooxidans (92.3 %), Chelatococcus ascharavorans (92.2 %) and Microvirga subterranea (92.0 %). The 16S rRNA gene sequence similarity levels to the type strains of other type species in the order Rhizobiales were below 92.0 %. Therefore, the low sequence similarities between strain YIM YD3T and its phylogenetic neighbours strongly indicate that strain YIM YD3T is a member of a new genus in the order Rhizobiales.

Morphological, physiological and biochemical characteristics of strain YIM YD3T are given in the genus and species descriptions and in Table 1. Major fatty acids of strain YIM YD3T were C18:1ω7c (39.80 %), C18:1ω9c (13.27 %), C16:0 (13.15 %), C19:0 cyclo ω8c (12.07 %), C18:0 (7.79 %), summed feature 3 (iso-C15:0 2-OH and/or C16:0ω7c 2.44 %), summed feature 5 (C18:2ω6ω9c and/or anteiso-C18:1ω7c 2.36 %), C17:0 cyclo (1.80 %) and C17:0 (1.06 %). Other minor fatty acids (<1 %) were C14:0, C20:1ω9c, C18:0 3-OH, summed feature 2, C20:1ω7c, C15:0, C17:0ω8c, anteiso-C15:0 and anteiso-C17:0. Strain YIM YD3T was Gram-negative and catalase- and oxidase-positive; these phenotypic properties were common to Balneimonas flocculans, C. daeguensis, Bosea massiliensis, Bosea mina-
tiltanensis, Bosea eneae, Bosea vestrisii and C. asaccharovorans. However, numerous other properties of strain YIM YD3T differed from those of these close phylogenetic relatives (Table 1). Strain YIM YD3T could be differentiated from the type strain of Balneimonas flocculans based on the presence of C19 : 0cyclo[8 as the major fatty acid (Table 1). These two strains could also be distinguished by their differing abilities to reduce nitrate, produce urease, oxidize thiosulfate and produce acid from glucose, their different optimal growth temperatures (i.e. 28–30 °C for strain YIM YD3T and 40–45 °C for members of the genus Chelatococcus) and utilization of maltose and sucrose (Table 1; Yoon et al., 2008). A comparison of polar lipids in strain YIM YD3T and members of the genus Chelatococcus revealed an additional diphosphatidylglycerol in strain YIM YD3T, although phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and unknown phospholipids are commonly found in members of the genus Chelatococcus (Yoon et al., 2008). Another major difference in the polar lipid composition was the absence in strain YIM YD3T of two aminolipids that are commonly found in members of the genus Chelatococcus (Yoon et al., 2008). Strain YIM YD3T produced oxidase and urease and oxidized thiosulfate, but did not produce gelatinase, thus enabling it to be differentiated from Microvirga subterranea (Table 1; Kanso & Patel, 2003); other differences included optimal growth temperature (i.e. 28–30 °C for strain YIM YD3T and 41 °C for Microvirga subterranea) and utilization of glucose, sucrose, citrate and glycerol (Table 1; Kanso & Patel, 2003). In addition,
Table 1. Differential characteristics of strain YIM YD3<sup>T</sup> and related strains

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>Shape</td>
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<td>Size (μm)</td>
<td>0.4–1.0 × 1.20–1.45</td>
<td>0.5–0.7 × 1.5–3.5</td>
<td>0.3–0.6 × 1.0–4.0</td>
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<td>0.5 × 1.5–2.0</td>
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<td>ND</td>
<td>0.83 × 1.4–1.6</td>
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<td>Flagella</td>
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<td>Single polar</td>
<td>Absent</td>
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<td>Optimum growth pH</td>
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<td>7.0–7.5</td>
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<td>D-Xylose</td>
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<td>+</td>
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<td>ND</td>
<td>ND</td>
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<td>Fructose</td>
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<td>Sucrose</td>
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<td>Citrate</td>
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<td>Glycerol</td>
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<td>Arabinose</td>
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<td>Glucose</td>
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<td>Thiosulphate oxidation</td>
<td>+</td>
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<td>ND</td>
<td>−</td>
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<td>+</td>
<td>−</td>
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<td>Major fatty acids (≥10% of total fatty acids)</td>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;ω7c, C&lt;sub&gt;16:0&lt;/sub&gt;, C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;ω7c, C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω8c</td>
<td>ND</td>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;ω7c, C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω8c</td>
<td>ND</td>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;ω7c</td>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;ω7c, C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω8c</td>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;ω7c, C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω8c</td>
<td>ND</td>
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<td>DNA G+C content (mol%)</td>
<td>71.8</td>
<td>64.0</td>
<td>68.3</td>
<td>63</td>
<td>66.7</td>
<td>68.5 ± 0.4</td>
<td>69.0</td>
<td>65.0</td>
<td>68.2</td>
<td>63.5 ± 0.5</td>
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combinations of phenotypic and chemotaxonomic characteristics could be used to differentiate strain YIM YD3\(^T\) from members of related genera in the order *Rhizobiales*. The results of the present study indicate that strain YIM YD3\(^T\) represents a novel species in a new genus, for which the name *Salinarimonas rosea* gen. nov., sp. nov. is proposed.

**Description of *Salinarimonas* gen. nov.**

*Salinarimonas* (Sa.li.na.ri.mo’nas. L. fem. pl. n. salinae - arum salt works; L. fem. n. monas a monad, unit; N.L. fem. n. Salinarimonas a monad from salt works).

Facultatively anaerobic, rod-shaped cells. Gram-negative, motile and oxidase- and catalase-positive. Produces pink-pigmented colonies. The predominant fatty acids are C\(_{18:1}\) v, C\(_{18:1\omega 9c}\), C\(_{16:0}\) and C\(_{19:0}\) cyclo \(\omega 8c\). The predominant respiratory ubiquinone is Q-10. The major polar lipids are diphasatidglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylcholine and two unknown phospholipids. The genus is a member of the order *Rhizobiales*. The type species is *Salinarimonas rosea*.

**Description of *Salinarimonas rosea* sp. nov.**

*Salinarimonas rosea* (ro’se.a. L. fem. adj. rosea rose-coloured, pink).

In addition to the characteristics reported for the genus, cells are halotolerant, motile by means of a single polar flagellum and approximately 0.4–1.0 \(\mu\)m wide by 1.20–1.45 \(\mu\)m long. Growth occurs at 15–37 °C (optimum, 28–30 °C), at pH 6.0–9.0 (optimum, pH 7.0–8.0) and in NaCl concentrations of 0–5 % (w/v) (optimum, 3 %). Growth occurs on TSA, nutrient agar and MY medium. On MA or MY medium supplemented with 3 % (w/v) NaCl, colonies are circular, entire, convex and pink in colour. After incubation for 5 days under optimal growth conditions, colonies are approximately 2.5 mm in diameter. Aesculin, xanthine and hypoxanthine are decomposed. Poly-\(\beta\)-hydroxybutyrate and bacteriochlorophyll \(a\) are not detected. Positive for nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease, but negative for hydrolysis of gelatin and starch, and for production of indole and H\(_2\)S. With the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, \(\alpha\)-chymotrypsin, acid phosphatase and naphthol-AS-Bl-phosphohydrolase are present, but \(\alpha\) - and \(\beta\)-glucosidase, \(\alpha\) - and \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\gamma\)-acetyl-\(\beta\)-glucosaminidase, \(\alpha\)-mannosidase and \(\alpha\)-fucosidase are absent. Acid is produced from glycerol, D-glucose, L-arabinose, D-ribose, DL-xylene, D-galactose, D-fructose, D-mannose, maltose, sucrose, trehalose, raffinose, turanose, D-fucose and potassium 2-ketogluconate. Utilizes acetate, citrate, D-fructose, D-glucose, D-mannose, ribose, D-xylene, sorbitol, p-glycerol, glycerol, inositol, inulin, L-arginine, L-asparagine, L-lysine, L-ornithine, pyruvate, sodium succinate, sucrose, Casamino acids, L-proline, peptone and tryptone as sole carbon sources, but does not utilize acetamide, benzoate, cellobiose, D-galactose, raffinose, salicin, trehalose, L-xylene, ethanol, L-rhamnose, maleic acid or tartrate. Cells are sensitive to (\(\mu\)g per disc) amikacin (30), norfloxacin (10), ampicillin (10), cephalexin (30), benzylpenicillin (10), ciprofloxacin (5), carbenicillin (100), erythromycin (15) and chloramphenicol (30), but resistant to gentamicin (10). Major fatty acids are C\(_{18:1\omega 7c}\), C\(_{18:1\omega 9c}\), C\(_{16:0}\) cyclo \(\omega 8c\), C\(_{18:0}\) summed feature 3 (iso-C\(_{15:0}\) 2-OH and/or C\(_{16:1\omega 7c}\)), summed feature 5 (C\(_{18:1\omega 6}\)c9c and/or anteiso-C\(_{18:0}\)), C\(_{17:0}\) cyclo and C\(_{17:0}\) minor fatty acids are C\(_{14:0}\) \(\omega 2\), C\(_{20:1\omega 9c}\), C\(_{18:0}\) 3-OH, summed feature 2, C\(_{20:1\omega 7c}\), C\(_{15:0}\) \(\omega 10c\), anteiso-C\(_{15:0}\) and anteiso-C\(_{17:0}\).

The type strain, YIM YD3\(^T\) (=KCTC 22346\(^T\)=CCTCC AA208038\(^T\)), was isolated from a deposit from a salt mine in Yunnan, China. The DNA G+C content of the type strain is 71.8 mol%.

**Acknowledgements**

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**References**


