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

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A Gram-negative, rod-shaped, facultatively anaerobic, halotolerant bacterial strain, designated YIM YD3^T, was isolated from a salt mine in Yunnan, south-west China. The taxonomy of strain YIM YD3^T was investigated by a polyphasic approach. Strain YIM YD3^T was motile, formed pink colonies and was positive for catalase and oxidase activities. Q-10 was the predominant respiratory ubiquinone. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylcholine and two unknown phospholipids. The major fatty acids (>10% of total fatty acids) were C_{18:1} ω 7c, C_{18:1} ω 9c, C_{16:0} and C_{19:0} cyclo ω 8c. The DNA G+C content was 71.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that the isolate formed a distinct line within a clade containing the genera *Balneimonas*, *Bosea*, *Chelatococcus* and *Microvirga* in the order *Rhizobiales*, with highest levels of 16S rRNA gene sequence similarity to the type strain of *Balneimonas flocculans* (93.5%). On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain YIM YD3^T represents a novel species in a new genus, for which the name *Salinarimonas rosea* gen. nov., sp. nov. is proposed, with strain YIM YD3^T (=KCTC 22346^T=CCTCC AA208038^T) as the type strain.

The order *Rhizobiales* is a phenotypically heterogeneous assemblage within the α -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 *Rhodobiaceae*. In addition to these families, a new family, *Xanthobacteraceae* (Lee *et al.*, 2005), was described subsequently as a result of phylogenetic analysis of 16S

rRNA gene sequences. Recently, another new family, *Cohaesibacteraceae* (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 YD3^T 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 four 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 YD3^T were investigated using routine cultivation on MA. Gram staining was carried out using the standard Gram reaction combined with the KOH lysis

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM YD3^T is EU878006.

An extended neighbour-joining phylogenetic tree showing the relationships between strain YIM YD3^T and most species in the order *Rhizobiales* with validly published names, is available with the online version of this paper.

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. Thiosulfate 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 aesculin, 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) according to the manufacturer's instructions, 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 YD3^T was obtained by scraping cells from MA that had been incubated for 5 days at 28 °C. Analysis of the whole-cell fatty acid pattern followed the method of Sasser (1990) using the Microbial Identification system (MIDI). The genomic DNA G+C content was determined by HPLC according to Mesbah *et al.* (1989) after DNA extraction according to the method of Cui *et al.* (2001). Genomic DNA of *Escherichia coli* H5 α was used as a standard.

Genomic DNA extraction, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products

were carried out as described previously (Cui *et al.*, 2001). The almost-complete 16S rRNA gene sequence of strain YIM YD3^T (1412 bp) was obtained and compared with sequences available in GenBank/EMBL/DDBJ using BLAST searches (Altschul *et al.*, 1990). The sequence of strain YIM YD3^T was aligned with those of 190 species with validly published names belonging to related families in the order *Rhizobiales* obtained from GenBank using the program CLUSTAL_X (Thompson *et al.*, 1997). 16S rRNA gene sequence similarities were calculated using the program CLUSTAL_X. Phylogenetic trees were obtained by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to Kimura's two-parameter model (Kimura, 1980). The robustness of tree topologies was assessed by bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods.

Preliminary BLAST network searches in GenBank showed that the closest relative of strain YIM YD3^T was clone 69-29 (98.1 % sequence similarity), which was obtained from sediment of Guanting Reservoir (Qu *et al.*, 2008). Sequence comparisons with representative bacteria with validly published names indicated that strain YIM YD3^T 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 YD3^T 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 minatitlanensis* (92.5 %), *Bosea eneae* (92.3 %), *Bosea vestrisii* (92.3 %), *Bosea thiooxidans* (92.3 %), *Chelatococcus asaccharovorans* (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 YD3^T and its phylogenetic neighbours strongly indicate that strain YIM YD3^T is a member of a new genus in the order *Rhizobiales*.

Morphological, physiological and biochemical characteristics of strain YIM YD3^T are given in the genus and species descriptions and in Table 1. Major fatty acids of strain YIM YD3^T were C_{18:1} ω 7c (39.80 %), C_{18:1} ω 9c (13.27 %), C_{16:0} (13.15 %), C_{19:0} cyclo ω 8c (12.07 %), C_{18:0} (7.79 %), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c; 2.44 %), summed feature 5 (C_{18:2} ω 6,9c and/or anteiso-C_{18:0}; 2.36 %), C_{17:0} cyclo (1.80 %) and C_{17:0} (1.06 %). Other minor fatty acids (<1 %) were C_{14:0}, C_{20:1} ω 9c, C_{18:0} 3-OH, summed feature 2, C_{20:1} ω 7c, C_{15:0}, C_{17:1} ω 8c, anteiso-C_{15:0} and anteiso-C_{17:0}. Strain YIM YD3^T was Gram-negative and catalase- and oxidase-positive; these phenotypic properties were common to *Balneimonas flocculans*, *C. daeguensis*, *Bosea massiliensis*, *Bosea minatitlanensis*, *Bosea eneae*, *Bosea vestrisii*, *Bosea thiooxidans*, *Chelatococcus asaccharovorans*, *Chelatococcus daeguensis*, *Chelatococcus massiliensis*, *Chelatococcus minatitlanensis*, *Chelatococcus subterranea*, *Microvirga subterranea*, *Methylobacterium* and *Methylobacterium*.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain YIM YD3^T and some related taxa in the order *Rhizobiales*. *Bradyrhizobium betae* PL7HG1^T was used as the outgroup. Filled circles indicate that the corresponding nodes were also found in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bootstrap percentages (based on 1000 replications) >50% are shown at branch points. Bar. 1 substitution per 100 nt positions.

tiltanensis, *Bosea eneae*, *Bosea vestrisii* and *C. asaccharovorans*. However, numerous other properties of strain YIM YD3^T differed from those of these close phylogenetic relatives (Table 1). Strain YIM YD3^T could be differentiated from the type strain of *Balneimonas flocculans* based on the presence of *C*_{19:0} cyclo ω 8c 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 YD3^T and 40–45 °C for *Balneimonas flocculans*) and utilization of glucose, maltose, D-xylose, fructose, sucrose, citrate and glycerol (Table 1; Takeda *et al.*, 2004). In addition, the DNA G+C content of strain YIM YD3^T (71.8 mol%) was very different from that of *Balneimonas flocculans* (64.0 mol%). Strain YIM YD3^T could be distinguished from members of the genus *Chelatococcus* based on motility, optimal growth temperature (i.e. 28–30 °C for strain YIM YD3^T and 30–37 °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 YD3^T and members of the genus *Chelatococcus* revealed an additional diphosphatidylglycerol in strain YIM YD3^T, 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 YD3^T of two aminolipids that are commonly found in members of the genus *Chelatococcus* (Yoon *et al.*, 2008). Strain YIM YD3^T 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 YD3^T 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^T and related strains

Strains: 1, YIM YD3^T; 2, *Balneimonas flocculans* TFB^T (Takeda *et al.*, 2004); 3, *Chelatococcus daeguensis* K106^T (Yoon *et al.*, 2008); 4, *Chelatococcus asaccharovorans* TE2^T (Auling *et al.*, 1993; Yoon *et al.*, 2008); 5, *Bosea massiliensis* 63287^T (La Scola *et al.*, 2003); 6, *Bosea minatitlanensis* AMX 51^T (Ouattara *et al.*, 2003); 7, *Bosea eneeae* 34614^T (La Scola *et al.*, 2003); 8, *Bosea vestrisii* 34635^T (La Scola *et al.*, 2003); 9, *Bosea thiooxidans* DSM 9653^T (Das *et al.*, 1996; La Scola *et al.*, 2003); 10, *Microvirga subterranea* Fail4^T (Kanso & Patel, 2003). All strains are Gram-negative and catalase-positive. +, Positive; –, negative; w, weakly positive; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colony pigmentation	Pink	Absent	Yellow	White	Cream	Absent	Cream	Cream	Cream	Pink
Cell morphology:										
Shape	Rods	Rods	Rods	Diplococci	Rods	Rods	Rods	Rods	Rods	Rods
Size (µm)	0.4–1.0 × 1.20–1.45	0.5–0.7 × 1.5–3.5	0.3–0.6 × 1.0–4.0	1.5–2.0 × 1.2–1.5	ND	0.5 × 1.5–2.0	ND	ND	0.83 × 1.4–1.6	1.0 × 1.5–4.0
Motility	+	+	–	–	+	+	+	+	+	+
Flagella	Single polar	Single polar	Absent	Absent	Single polar	Single polar	Single polar	Single polar	Single polar	Single polar
Optimum growth temperature (°C)	28–30	40–45	30–37	36	25–37	37	25–37	25–37	30–32	41
Optimum growth pH	7.0–8.0	7.0	7.0–7.5	7.0–8.0	ND	6.0	ND	ND	7.5–8.0	7.0
Urease activity	+	–	–	+	+	+	+	+	–	–
Nitrate reduction	+	–	+	+	+	–	–	–	+	+
Growth on:										
Glucose	+	–	+	+	–	–	–	+	+	–
Maltose	+	–	–	–	+	–	–	+	+	ND
D-Xylose	+	–	+	+	ND	–	ND	ND	+	+
Fructose	+	–	+	–	ND	–	ND	ND	+	–
Sucrose	+	–	–	–	ND	–	ND	ND	ND	–
Citrate	+	–	+	–	–	–	–	+	+	–
Glycerol	+	–	+	–	ND	ND	ND	ND	–	–
Acid production from:										
Arabinose	–	–	+	–	–	–	–	–	–	w
Glucose	+	–	+	–	–	–	–	–	–	w
Thiosulfate oxidation	+	–	ND	ND	–	+	–	–	+	–
Major fatty acids	C _{18:1} ω7c	C _{16:0} , C _{18:0} , C _{18:1}	C _{18:1} ω7c, C _{19:0}	C _{18:1} ω7c, C _{19:0}	C _{18:1} ω7c	C _{18:1} ω7c	C _{18:1} ω7c, C _{19:0}	C _{18:1} ω7c, C _{16:1} ω7c	C _{18:1} ω7c	ND
(>10 % of total fatty acids)	C _{18:1} ω9c, C _{16:0} , C _{19:0} cyclo ω8c		cyclo ω8c	cyclo ω8c		C _{19:0} cyclo ω8c	cyclo ω8c, C _{16:1} ω5c			
DNA G + C content (mol%)	71.8	64.0	68.3	63	66.7	68.5 ± 0.4	69.0	65.0	68.2	63.5 ± 0.5

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}ω7c, C_{18:1}ω9c, C_{16:0} and C_{19:0} cyclo ω8c. The predominant respiratory ubiquinone is Q-10. The major polar lipids are diphosphatidylglycerol, 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 μm wide by 1.20–1.45 μm 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-β-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 H₂S. With the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Acid is produced from glycerol, D-glucose, L-arabinose, D-ribose, DL-xylose, 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-xylose, sorbitol, glycerol,

glycogen, 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-xylose, ethanol, L-rhamnose, maleic acid or tartrate. Cells are sensitive to (μg per disc) amikacin (30), norfloxacin (10), ampicillin (10), cephalothin (30), benzylpenicillin (10), ciprofloxacin (5), carbenicillin (100), erythromycin (15) and chloramphenicol (30), but resistant to gentamicin (10). Major fatty acids are C_{18:1}ω7c, C_{18:1}ω9c, C_{16:0}, C_{19:0} cyclo ω8c, C_{18:0}, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), summed feature 5 (C_{18:2}ω6,9c and/or anteiso-C_{18:0}), C_{17:0} cyclo and C_{17:0}; minor fatty acids are C_{14:0}, C_{20:1}ω9c, C_{18:0} 3-OH, summed feature 2, C_{20:1}ω7c, C_{15:0}, C_{17:1}ω8c, 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%.

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