Aliifodinibius roseus gen. nov., sp. nov., and *Aliifodinibius sediminis* sp. nov., two moderately halophilic bacteria isolated from salt mine samples

Yong-Xia Wang,[1]† Ji-Hui Liu,[1,2]† Wei Xiao,[1] Xiao-Liang Ma,[1] Yong-Hong Lai,[1] Zhi-Ying Li,[1] Kai-Yan Ji,[1] Meng-Liang Wen[1] and Xiao-Long Cui[1]

Correspondence
Xiao-Long Cui
xlcuiynu@aliyun.com *or*
xlcui@ynu.edu.cn
Meng-Liang Wen
mlwen@ynu.edu.cn

[1]The Key Laboratory for Microbial Resources of the Ministry of Education, and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China

[2]The Technology Research Center, HongyunHonghe Tobacco (Group), Co., Ltd, Kunming, Yunnan 650202, PR China

Two rod-shaped, non-motile bacteria were isolated from two separate salt mines in Yunnan, south-western China. These strains, designated YIM D15^T and YIM J21^T, were Gram-negative and moderately halophilic. The two strains required 6–10 % NaCl (w/v; optimal) for growth. The DNA G+C contents of strains YIM D15^T and YIM J21^T were 49.0 mol% and 48.4 mol%, respectively. The predominant isoprenoid quinone was MK-7. The polar lipid profiles of strains YIM D15^T and YIM J21^T were composed predominantly of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, three unknown polar lipids and one glycolipid. Minor amounts of other lipids were also detectable. The predominant cellular fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:1}\omega9c$/10 methyl-$C_{16:0}$ and $C_{16:1}\omega7c$/$C_{16:1}\omega6c$. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that the two isolates formed a distinct clade with the genus *Fodinibius* (in the phylum *Bacteroidetes*) and were related to the species *Fodinibius salinus*, with sequence similarities of 91.9–92.4 %. Analyses of 16S rRNA gene sequences revealed that strains YIM D15^T and YIM J21^T were related to each other (97.3 % sequence similarity). The DNA–DNA hybridization relatedness between the two isolates was 34 %. On the basis of the phylogenetic analysis, DNA–DNA hybridization relatedness, phenotypic and chemotaxonomic characteristics, strains YIM D15^T and YIM J21^T should be classified as members of a novel genus and as two novel species, for which the names *Aliifodinibius roseus* gen. nov., sp. nov. (type strain YIM D15^T=ACCC 10715^T=KCTC 23442^T) and *Aliifodinibius sediminis* sp. nov. (type strain YIM J21^T=ACCC 10714^T=DSM 21194^T) are proposed.

The taxonomy of the large group of bacteria formerly classified as the *Cytophaga–Flavobacterium–Bacteroides* (Olsen *et al.*, 1994) is in need of taxonomic clarification. Stoecker *et al.* (2006), for example, presented molecular evidence that indicated that the genus *Crenothrix* belonged to the order *Methylococcales* of the class *Gammaproteobacteria*. Consequently, other genera originally assigned to the family *Crenotrichaceae* within the order *Sphingobacteriales* are currently considered *Sphingobacteriales genera incertae sedis*. Such genera are *Rhodothermus* (Alfredsson *et al.*, 1988; Sako *et al.*, 1996) and *Salinibacter* (Antón *et al.*, 2002; Makhdoumi-Kakhki *et al.*, 2012), both of which include species that show extremophilic characteristics. *Rhodothermus* strains are thermophilic whereas strains of the genus *Salinibacter* are extreme halophiles. Recently three genera that are phylogenetically related to *Rhodothermus* and *Salinibacter* have been described and named *Balneola* (Urios *et al.*, 2006), *Gracilimonas* (Choi *et al.*, 2009) and *Fodinibius* (Wang *et al.*, 2012). The former two genera, which were both isolated from seawater, do not exhibit any of the extreme features found in the species of the genera *Rhodothermus* and *Salinibacter*. The last genus, which was isolated from a salt mine, exhibits the moderately halophilic feature. In this paper, characterization

†These authors contributed equally to this work.

Abbreviations: AL, aminolipid; DPG, diphosphatidylglycerol; GL, glycolipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Aliifodinibius roseus* YIM D15^T and *Aliifodinibius sediminis* YIM J21^T are JQ923475 and JQ923476, respectively.

Two supplementary figures are available with the online version of this paper.

and classification of two strains (from the phylum *Bacteroidetes*) that appear taxonomically novel at the genus level are reported.

During an investigation of the microbial diversity of the hypersaline environments in Yunnan, south-western China, strain YIM D15^T was isolated from a sediment sample (with 49.3 %, w/v, NaCl) collected from the Fenggang salt mine (23° 28′ N 100° 43′ E) and strain YIM J21^T was isolated from the another sediment sample (with 12.9 %, w/v, NaCl) collected from the Mengye salt mine (22° 71′ N 101° 63′ E). These strains were isolated at 28 °C, by the standard dilution-plating technique, on marine agar 2216 (MA; Difco) supplemented with 10 % (w/v) NaCl (modified MA). They were subsequently maintained on modified MA slants and preserved at −80 °C as a suspension in marine broth 2216 (MB; Difco) supplemented with 10 % (w/v) NaCl (modified MB) containing 20 % (v/v) glycerol.

Gram staining was carried out using the standard Gram reaction combined with KOH lysis (Gregersen, 1978). Cell morphology and flagella were examined by transmission electron microscopy and light microscopy (BH-2; Olympus) after Leifson staining (Murray *et al.*, 1994). Growth at various temperatures (4, 10, 15, 20, 25, 28, 37, 42, 45, 50, 55 and 60 °C) and pH (4.0–11.0, in increments of 0.5 pH units, at 28 °C) was assessed in modified MB. To test NaCl tolerance, growth on nutrient agar (Difco) and tryptone soy agar (TSA; BD), each containing 0, 1, 2, 3, 4, 5, 7, 8, 10, 12, 15, 16, 18, 20, 21, 22, 23, 25 and 30 % (w/v) NaCl, was investigated at 28 °C. Growth under anaerobic conditions was explored by incubation on modified MA in an anaerobic jar (GasPak anaerobic systems; BBL). Accumulation of poly-β-hydroxybutyrate was observed by Sudan black staining (Smibert & Krieg, 1994) under a light microscope. Bacteriochlorophyll *a* was analysed spectrophotometrically by using the method of Cohen-Bazire *et al.* (1957) following the recommendations of Allgaier *et al.* (2003). Catalase and oxidase activities were determined using 3 % (v/v) H_2O_2 and Kovacs' reagent (Kovacs, 1956), respectively. Glucose fermentation, arginine dihydrolase, indole production, β-galactosidase and urease activities, hydrolysis of aesculin, casein, DNA, gelatin, starch, Tween 40 and Tween 80 were examined as described by Hansen & Sørheim (1991). Nitrate reduction was tested in modified MB medium containing 0.2 % KNO_3. Nitrite was monitored with naphthylamine/sulphanilic acid reagents and residual nitrate with Zn powder; gas production was detected in Durham tubes. H_2S production was tested in modified MB medium supplemented with 0.01 % (w/v) L-cysteine; the indicator was a strip of paper impregnated with lead acetate placed in the neck of the tube. Other enzyme activities were assayed by using the API ZYM kit (bioMérieux) according to the manufacturer's instructions, and cell suspension in autoclaved saline containing 10 % (w/v) NaCl. To explore carbon utilization, a potential carbon source, at a final concentration of 0.5 % (w/v or v/v), was added to a basal medium of artificial seawater (Cho & Giovannoni, 2006) (without NH_4Cl if an amino acid was being tested) supplemented with 10 % (w/v) NaCl. Acid production from carbohydrates was tested as described by Hansen & Sørheim (1991), the basal medium was supplemented with 10 % (w/v) NaCl for the two isolates. Antibiotic sensitivity was explored by placing commercial antibiotic discs (Himedia) on the modified MA plates that had been spread with the isolates and then incubated at 28 °C for 5 days.

Polar lipids of strains YIM D15^T and YIM J21^T were extracted (Minnikin *et al.*, 1979) and identified by two-dimensional TLC (Collins & Jones, 1980). Isoprenoid quinones of the two isolates were also extracted (Collins *et al.*, 1977) and analysed by HPLC (Tamaoka *et al.*, 1983). Biomass for the quantitative analysis of fatty acids was obtained by scraping cells from the (same) third quadrant of cultures of strains YIM D15^T and YIM J21^T (grown on modified MA for 5 days at 28 °C). Analysis of the cellular fatty acid pattern followed the method described by Sasser (1990) but used version 6.0 of the Sherlock Microbial Identification System (MIDI).

Genomic DNA extraction, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out as described previously (Cui *et al.*, 2001). The sequenced lengths of the 16S rRNA genes of strains YIM D15^T and YIM J21^T were 1466 bp and 1421 bp, respectively. The 16S rRNA gene sequences of the closely related taxa were retrieved from the GenBank database using BLAST (Altschul *et al.*, 1997). All the sequences were aligned and the levels of similarity were calculated using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods, in MEGA version 4 (Tamura *et al.*, 2007), while a maximum-likelihood (Felsenstein, 1981) tree was generated using the treeing algorithm contained in the PHYLIP package (Felsenstein, 1993). Evolutionary distances for the neighbour-joining tree were calculated by using the Kimura two-parameter model (Kimura, 1980). Bootstrap analysis with 1000 resamplings (Felsenstein, 1985) was used to evaluate tree topology.

The cells of strains YIM D15^T and YIM J21^T were Gram-reaction-negative, catalase- and oxidase-positive, rod-shaped, 0.3 μm wide and 0.4–0.7 μm long (Fig. S1, available in IJSEM Online). Neither flagella nor endospores were observed. Growth was observed for strain YIM D15^T under anaerobic conditions on modified MA. Growth of strain YIM D15^T occurred on nutrient agar and TSA supplemented with 4–20 % (w/v) NaCl. After growth on modified MA or TSA or nutrient agar supplemented with 10 % (w/v) NaCl, at 28 °C for 5 days, colonies of strain YIM D15^T were rose-red, circular, convex and opaque with regular margins. The temperature range of strain YIM D15^T for growth was 20–42 °C while the pH range for growth at 28 °C was pH 6.5–8.0. While, for strain YIM J21^T, growth was not observed under anaerobic conditions

on modified MA. Growth occurred on nutrient agar and TSA supplemented with 4–16 % (w/v) NaCl. After growth on modified MA or TSA or nutrient agar supplemented with 10 % (w/v) NaCl, at 28 °C for 5 days, colonies were salmon pink, circular, convex and opaque with regular margins. The temperature range for growth was 25–45 °C while the pH range for growth at 28 °C was pH 6.5–8.5. The two isolates were sensitive to ampicillin (10 μg), erythromycin (15 μg) and chloramphenicol (30 μg) and tolerant of gentamicin (10 μg), carbenicillin (100 μg) and ciprofloxacin (5 μg). Phenotypically the two isolates differed from each other as well as differing from the species with validly published names to which they were most closely related (*Fodinibius salinus*, *Gracilimonas tropica*, *Balneola vulgaris* and *Balneola alkaliphila*) in several features (Table 1). Other phenotypic characteristics of strains YIM D15^T and YIM J21^T are given in the species description.

The G+C contents of the DNA of strains YIM D15^T and YIM J21^T, extracted according to the method of Cui *et al.* (2001), were determined to be 49.0 mol% and 48.4 mol%, respectively, by HPLC (Mesbah *et al.*, 1989), with the DNA of *Escherichia coli* DH5α used as a standard.

Strains YIM D15^T and YIM J21^T shared similar polar lipid profiles. Although the two isolates and *F. salinus* YIM D17^T and *G. tropica* DSM 19535^T shared some major polar lipids, differences in their lipid profiles enabled the two novel isolates to be distinguished from *F. salinus* YIM D17^T and *G. tropica* DSM 19535^T (Table 1 and Fig. S2). Major polar lipid components of strain YIM D15^T were diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), one unknown phospholipid (PL2), three unknown polar lipids (L1–L3) and one unknown glycolipid (GL) detectable with molybdatophosphoric acid. Minor to trace amounts of phosphatidylglycerol (PG) and an unknown phospholipid (PL1) and phosphatidylglycerol (PG) were also detected. Major polar lipid components of strain YIM J21^T were DPG, PC, PE, PL2, three unknown polar lipids (L1–L3) and GL. Minor to trace amounts of PG and unknown phospholipids (PL1, PL3 and PL4) and PG were also detected. *F. salinus* YIM D17^T had DPG, PE, PC, PG, PL5, GL and unknown aminolipid (AL) as major lipids. Minor to trace amounts of two unknown phospholipids (PL2 and PL4) and three polar lipids (L2, L5 and L6) were also detected. *G. tropica* DSM 19535^T had DPG, PE, PC, PG, GL and one unknown phospholipid (PL5) as major lipids and minor amounts of two unknown phospholipids (PL1 and PL2) and three polar lipids (L3, L4 and L7).

The major difference between the two novel isolates and *F. salinus* YIM D17^T was that the two novel isolates had no detectable AL and PL5. Unlike *G. tropica* DSM 19535^T, strains YIM D15^T and YIM J21^T did not contain PL5 as a major polar lipid. In addition, strains YIM D15^T and YIM J21^T could be distinguished from *F. salinus* YIM D17^T and *G. tropica* DSM 19535^T (Table 2) by very high levels of anteiso-$C_{15:0}$ (18.4–21.0 %).

In terms of polar lipid profiles, strains YIM D15^T and YIM J21^T appeared more similar to the two strains of species of the genus *Balneola* investigated (*B. vulgaris* DSM 17893^T and *B. alkaliphila* DSM 19538^T) than to *F. salinus* YIM D17^T and *G. tropica* DSM 19535^T, unlike either type strain of the species of the genus *Balneola*, strains YIM D15^T and YIM J21^T had PC as their major polar lipid. The major polar lipids of *B. vulgaris* DSM 17893^T and *B. alkaliphila* DSM 19538^T (Wang *et al.*, 2012) are DPG, phosphatidylglycerol (PG), PE, GL and an unknown polar lipid (L). The fatty acid profiles also allowed the two strains of species of the genus *Balneola* to be distinguished from strains YIM D15^T and YIM J21^T (Table 2). The major respiratory quinone of strains YIM D15^T and YIM J21^T was menaquinone-7 (MK-7). The dominant fatty acids of strains YIM D15^T and YIM J21^T were iso-$C_{15:0}$ (19.2 % and 16.0 % respectively), anteiso-$C_{15:0}$ (21.0 % and 18.4 %), iso-$C_{17:1}\omega9c$/10-methyl $C_{16:0}$ (10.2 % and 15.5 %) and $C_{16:1}\omega7c$/$C_{16:1}\omega6c$ (14.3 % and 12.9 %). The cellular fatty acid profiles of strains YIM D15^T and YIM J21^T were similar to those of the type strains of *F. salinus*, *G. tropica*, *B. vulgaris* and *B. alkaliphila*, but clear differences could also be observed (Table 2).

Phylogenetic analysis of their almost-complete 16S rRNA gene sequences revealed that strains YIM D15^T and YIM J21^T occupied a distinct lineage with the species of the genus *Fodinibius*, within the phylum *Bacteroidetes* (Fig. 1). The topologies of the phylogenetic tree reconstructed using the neighbour-joining algorithm (Fig. 1) were similar to those of the trees reconstructed using the maximum-likelihood and maximum-parsimony algorithms (not shown). Sequence analysis revealed that strains YIM D15^T and YIM J21^T were related to each other, with 97.3 % sequence similarity. The two novel strains were closely related to *F. salinus* YIM D17^T (91.9–92.4 % 16S rRNA gene sequence similarity), *G. tropica* CL-CB462^T (88.4–88.9 %), *B. vulgaris* 131X/A01/164^T (85.0–85.1 %) and *B. alkaliphila* CM41_14b^T (85.6–85.7 %). The two novel strains exhibited less than 80.0 % 16S rRNA gene sequence similarity to other established members of the phylum *Bacteroidetes*. The low sequence similarity values between strains YIM D15^T and YIM J21^T and the most closely related members of the phylum *Bacteroidetes* demonstrated that the two strains represent a distinct genus in the phylum *Bacteroidetes*.

DNA–DNA hybridization was carried out using the photobiotin-labelling method (Ezaki *et al.*, 1989), with a multiwell plate reader (CytoFluor; PerSeptive Biosystems). The DNA–DNA hybridization relatedness between strains YIM D15^T and YIM J21^T was 34 %. When the recommendation of a threshold value of 70 % DNA–DNA similarity for the definition of bacterial species (Wayne *et al.*, 1987) is considered, strains YIM D15^T and YIM J21^T should be considered to represent two separate species.

From the results of the phenotypic, chemotaxonomic, DNA–DNA hybridization relatedness and phylogenetic

Table 1. Characteristics that distinguish strains YIM D15^T and YIM J21^T from closely related species with validly published names

Strains: 1, YIM D15^T (this study); 2, YIM J21^T (this study); 3, *Fodinibius salinus* YIM D17^T (Wang *et al.*, 2012); 4, *Gracilimonas tropica* DSM 19535^T (Wang *et al.*, 2012); 5, *Balneola vulgaris* DSM 17893^T (Wang *et al.*, 2012); 6, *Balneola alkaliphila* DSM 19538^T (Wang *et al.*, 2012). +, Positive; −, negative.

Characteristic	1	2	3	4	5	6
Colony colour	Rose red	Salmon pink	Pink	Pale pink	Orange	Pale yellow
Cell shape	Rods	Rods	Rods/slightly curved	Long irregular rods	Rods	Rods
Motility	−	−	−	−	+	−
Salinity range for growth (percentage NaCl)	4–20	4–16	5–20	1–20	1–8	1–7
Temperature range for growth (°C)	20–42	25–45	25–45	20–37	10–45	15–37
pH range for growth	6.5–8.0	6.5–8.5	6.0–9.0	6.0–9.0	6.0–10.0	7.0–9.0
Nitrate reduced to nitrite	−	+	+	−	−	−
Glucose fermentation	+	−	−	+	−	−
Hydrolysis of:						
Aesculin	+	+	+	+	−	−
Gelatin	+	+	+	+	−	−
Starch	−	−	−	+	−	+
Tween 40	−	+	+	+	−	+
Tween 80	+	+	+	+	−	−
Utilization of:						
Acetate	+	+	−	−	+	+
Adenine	−	−	−	−	+	−
D-Glucose	+	+	−	+	+	−
Hypoxanthine	−	−	−	−	+	+
myo-Inositol	−	+	−	+	+	+
Maltose	+	+	−	+	−	−
D-Mannitol	−	+	−	+	+	+
D-Sorbitol	+	−	−	−	+	−
Sucrose	+	+	−	−	+	+
Enzymic activities (API ZYM)						
Esterase (C4)	+	+	−	+	+	+
Trypsin	−	+	−	−	+	−
α-Chymotrypsin	−	+	−	+	+	+
Acid phosphatase	+	+	−	−	+	+
N-Acetyl-β-glucosaminidase	−	+	+	+	−	−
Major polar lipids	DPG, PC, PE, GL, L, PL	DPG, PC, PE, GL, L, PL	DPG, PE, PC, PG, GL, AL, PL, L	DPG, PE, PG, PC, GL, PL, L	DPG, PE, PG, GL, L	DPG, PE, PG, GL, AL, L
DNA G+C content (mol%)	49.0	48.4	43.0	42.3	41.7	39.5

Table 2. Cellular fatty acid composition of strains YIM D15^T and YIM J21^T and the type strains of phylogenetically related species

Strains: 1, YIM D15^T (this study); 2, YIM J21^T (this study); 3, *Fodinibius salinus* YIM D17^T (Wang *et al.*, 2012); 4, *Gracilimonas tropica* DSM 19535^T (Wang *et al.*, 2012); 5, *Balneola vulgaris* DSM 17893^T (Wang *et al.*, 2012); 6, *Balneola alkaliphila* DSM 19538^T (Wang *et al.*, 2012). Results are presented as percentages of the total fatty acids; the major fatty acids (>10 %) are highlighted in bold type; fatty acids that represent <1 % in all strains are omitted. –, Not detected.

Fatty acid	1	2	3	4	5	6
$C_{14:0}$	0.3	0.8	0.7	0.6	1.6	1.1
$C_{16:0}$	2.6	2.1	3.7	1.0	2.5	0.8
$C_{18:0}$	0.4	0.7	1.4	–	0.1	–
iso-$C_{13:0}$	0.2	0.2	0.4	1.1	**10.2**	**16.0**
iso-$C_{14:0}$	0.9	0.5	1.7	1.1	**15.0**	4.1
iso-$C_{15:0}$	**19.2**	**16.0**	**23.6**	**42.4**	**25.6**	**36.3**
anteiso-$C_{15:0}$	**21.0**	**18.4**	8.1	5.6	1.8	1.6
iso-$C_{15:1}$ F	1.1	1.2	2.4	0.6	1.0	4.0
iso-$C_{16:1}$ H	0.5	0.7	–	–	–	–
iso-$C_{16:0}$	9.0	7.9	3.8	2.6	6.8	1.8
iso-$C_{17:0}$	1.6	2.3	0.3	3.4	1.7	0.6
anteiso-$C_{17:0}$	3.9	6.8	0.4	0.5	0.1	–
$C_{15:1}\omega6c$	1.9	0.5	0.6	6.4	3.9	4.6
$C_{15:1}\omega8c$	0.5	–	–	0.9	0.7	1.6
$C_{16:1}\omega5c$	2.3	2.9	1.5	1.5	1.0	2.3
$C_{17:1}\omega6c$	1.8	0.5	0.6	4.3	0.7	0.4
$C_{17:1}\omega8c$	1.7	0.3	0.5	5.4	2.8	1.2
$C_{18:1}\omega7c$	0.3	2.9	–	–	–	–
$C_{18:1}\omega9c$	0.3	0.5	4.5	–	–	–
anteiso-$C_{17:1}\omega9c$	2.8	3.4	1.4	0.8	–	–
10-Methyl $C_{18:0}$	–	–	3.1	–	–	–
iso-$C_{17:0}$ 3-OH	1.5	–	0.3	–	–	–
$C_{16:1}\omega7c/C_{16:1}\omega6c$	**14.3**	**12.9**	**13.8**	8.5	**17.2**	**14.7**
iso-$C_{17:1}\omega9c$/10-methyl $C_{16:0}$	**10.2**	**15.5**	**24.0**	**12.0**	5.1	6.2

analyses, strains YIM D15^T and YIM J21^T could not be assigned to any known taxa. Some phenotypic features (Table 1), their fatty acids (Table 2) and polar lipids profiles (Fig S2) and 16S rRNA gene sequences clearly differentiate the two isolates from the phylogenetically related, recognized taxa and indicate that the two isolates represent a novel genus and two novel species, for which the names *Aliifodinibius roseus* gen. nov., sp. nov. and *Aliifodinibius sediminis* sp. nov. are proposed.

Description of *Aliifodinibius* gen. nov.

Aliifodinibius (A.li.i.fo.di.ni.bi′us. L. pronoun. *alius* other, another; N.L. masc. n. *Fodinibius* a name of a bacterial genus; N.L. masc. n. *Aliifodinibius* the other *Fodinibius*).

Cells are Gram-negative, non-motile, moderately halophilic, non-endospore forming rods. Catalase- and oxidase-positive. The major respiratory quinone is MK-7. The major polar lipids are DPG, PE, PC, one phospholipid, three unknown polar lipids and one glycolipid. The dominant fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:1}\omega9c$/10-methyl $C_{16:0}$ and $C_{16:1}\omega7c/C_{16:1}\omega6c$. The DNA G+C content of members of the genus ranges between 48.4 and 49.0 mol%. The genus *Aliifodinibius* is a member of the phylum *Bacteroidetes*. The type species is *Aliifodinibius roseus*.

Description of *Aliifodinibius roseus* sp. nov.

Aliifodinibius roseus (ro′se.us. L. masc. adj. *roseus* rose-coloured, pink).

Shows the following characteristics in addition to the description given above for the genus. Colonies are rose-red, circular, convex and opaque, with regular margins. Cells are facultatively anaerobic, 0.5–0.6 μm in length and 0.3 μm in width. Does not accumulate poly-β-hydroxybutyrate or produce bacteriochlorophyll *a*. Temperature range for growth is 20–42 °C (optimum 28 °C) and pH range for growth at 28 °C is 6.5–8.0 (optimum 7.0). Growth at 28 °C occurs at NaCl concentrations of 4–20 % (w/v) (optimum 6–10 %). Hydrolyses aesculin, gelatin and Tween 80, but not DNA, casein, starch or Tween 40. Nitrate is not reduced. Arginine dihydrolase, β-galactosidase, glucose fermentation and urease are present. Indole production and H_2S production are absent. Utilizes acetate, aesculin, aspartate, D-galactose, glycerol, D-glucose, maltose, D-mannose, D-sorbitol and sucrose, but does not utilize adenine, citrate, ethanol, glycine, *myo*-inositol, α-lactose, D-mannitol, methanol, xanthine, D-xylose or hypoxanthine. Acid is produced from D-fructose, D-glucose, α-lactose, maltose, D-mannose and sucrose, but not from glycerol, *myo*-inositol, D-mannitol, D-sorbitol or D-ribose. Alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucosidase and α-mannosidase activities are present, but trypsin, α-chymotrypsin, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase activities are absent. Sensitive to ampicillin (10 μg), erythromycin (15 μg), penicillin G (20 U), streptomycin (10 μg), norfloxacin (10 μg) and chloramphenicol (30 μg). Tolerant of gentamicin (10 μg), carbenicillin (100 μg) and ciprofloxacin (5 μg).

The type strain, YIM D15^T (=ACCC 10715^T=KCTC 23442^T), was isolated from a salt sediment collected from the Fenggang salt mine (23° 28′ N 100° 43′ E) in Yunnan, south-western China. The genomic DNA G+C content of the type strain is 49.0 mol%.

Description of *Aliifodinibius sediminis* sp. nov.

Aliifodinibius sediminis (se.di′mi.nis, L. gen. n. *sediminis* of sediment).

Exhibits the following properties in addition to those given in the genus description. Colonies are salmon pink,

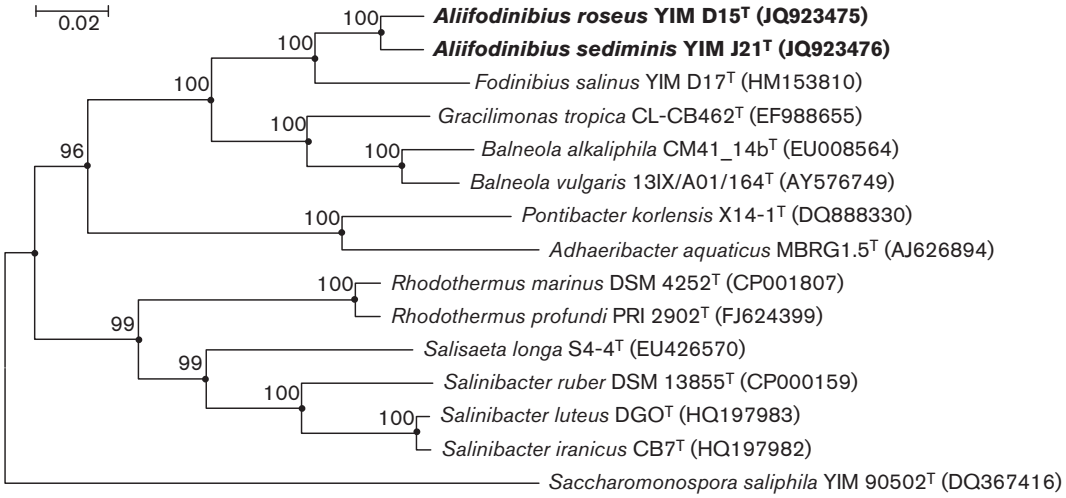

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships between strains YIM D15^T and YIM J21^T and type strains of species of related genera. Bootstrap percentages (based on 1000 replications) >70 % are shown at branching points. *Saccharomonospora saliphila* YIM 90502^T was used as an outgroup. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood method. Bar, 0.02 substitutions per nucleotide position.

circular, convex and opaque with regular margins. Cells are aerobic, 0.4–0.7 μm in length and 0.3 μm in width. Does not accumulate poly-β-hydroxybutyrate or produce bacteriochlorophyll *a*. Temperature range for growth is 25–45 °C (optimum 28 °C) and pH range for growth at 28 °C is 6.5–8.5 (optimum 7.0). Growth at 28 °C occurs at NaCl concentrations of 4–16 % (w/v) (optimum 6–10 %). Hydrolyses aesculin, gelatin, Tween 40 and Tween 80, but not DNA, casein or starch. Nitrate is reduced. Arginine dihydrolase, β-Galactosidase and urease are present. Glucose fermentation, indole production and H_2S production are absent. Utilizes acetate, aspartate, D-glucose, *myo*-inositol, α-lactose, maltose, D-mannitol and sucrose, but does not utilize aesculin, adenine, citrate, ethanol, D-galactose, glycerol, glycine, D-mannose, methanol, D-sorbitol, xanthine, D-xylose or hypoxanthine. Acid is produced from D-fructose, D-glucose, *myo*-inositol, α-lactose, maltose, D-mannose and sucrose, but not from glycerol, D-mannitol, D-sorbitol or D-ribose. Alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase activities are present, but β-glucuronidase and α-fucosidase activities are absent. Sensitive to ampicillin (10 μg), erythromycin (15 μg) and chloramphenicol (30 μg). Tolerant of gentamicin (10 μg), carbenicillin (100 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), penicillin G (20 U) and streptomycin (10 μg).

The type strain, YIM J21^T (= ACCC 10714^T=DSM 21194^T), was isolated from a salt sediment collected from the Mengye salt mine (22° 71′ N 101° 63′ E) in Yunnan, south-western China. The genomic DNA G+C content of the type strain is 48.4 mol%.

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References

Alfredsson, G. A., Kristjánsson, J. K., Hjörleifsdottir, S. & Stetter, K. O. (1988). *Rhodothermus marinus* gen. nov., sp. nov., a thermophilic, halophilic bacterium from submarine hot springs in Iceland. *J Gen Microbiol* **134**, 299–306.

Allgaier, M., Uphoff, H., Felske, A. & Wagner-Döbler, I. (2003). Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microbiol* **69**, 5051–5059.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Antón, J., Oren, A., Benlloch, S., Rodríguez-Valera, F., Amann, R. & Rosselló-Mora, R. (2002). *Salinibacter ruber* gen. nov., sp. nov., a novel, extremely halophilic member of the *Bacteria* from saltern crystallizer ponds. *Int J Syst Evol Microbiol* **52**, 485–491.

Cho, J. C. & Giovannoni, S. J. (2006). *Pelagibaca bermudensis* gen. nov., sp. nov., a novel marine bacterium within the *Roseobacter* clade in the order *Rhodobacterales*. *Int J Syst Evol Microbiol* **56**, 855–859.

Choi, D. H., Zhang, G. I., Noh, J. H., Kim, W. S. & Cho, B. C. (2009). *Gracilimonas tropica* gen. nov., sp. nov., isolated from a *Synechococcus* culture. *Int J Syst Evol Microbiol* **59**, 1167–1172.

Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by non-sulfur purple bacteria. *J Cell Physiol* **49**, 25–68.

Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Cui, X. L., Mao, P. H., Zeng, M., Li, W. J., Zhang, L. P., Xu, L. H. & Jiang, C. L. (2001). *Streptimonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. *Int J Syst Evol Microbiol* **51**, 357–363.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.

Hansen, G. H. & Sørheim, R. (1991). Improved method for phenotypical characterization of marine bacteria. *J Microbiol Methods* **13**, 231–241.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703–704.

Makhdoumi-Kakhki, A., Amoozegar, M. A. & Ventosa, A. (2012). *Salinibacter iranicus* sp. nov. and *Salinibacter luteus* sp. nov., isolated from a salt lake, and emended descriptions of the genus *Salinibacter* and of *Salinibacter ruber*. *Int J Syst Evol Microbiol* **62**, 1521–1527.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.

Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy.. In *Methods for General and Molecular Bacteriology*. pp. 21–41. Edited by P. Gephardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Olsen, G. J., Woese, C. R. & Overbeek, R. (1994). The winds of (evolutionary) change: breathing new life into microbiology. *J Bacteriol* **176**, 1–6.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sako, Y., Takai, K., Ishida, Y., Uchida, A. & Katayama, Y. (1996). *Rhodothermus obamensis* sp. nov., a modern lineage of extremely thermophilic marine bacteria. *Int J Syst Bacteriol* **46**, 1099–1104.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Microbiol*, pp. 611–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Stoecker, K., Bendinger, B., Schöning, B., Nielsen, P. H., Nielsen, J. L., Baranyi, C., Toenshoff, E. R., Daims, H. & Wagner, M. (2006). Cohn's *Crenothrix* is a filamentous methane oxidizer with an unusual methane monooxygenase. *Proc Natl Acad Sci U S A* **103**, 2363–2367.

Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Urios, L., Agogué, H., Lesongeur, F., Stackebrandt, E. & Lebaron, P. (2006). *Balneola vulgaris* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* from the north-western Mediterranean Sea. *Int J Syst Evol Microbiol* **56**, 1883–1887.

Wang, Y.-X., Liu, J. H., Xiao, W., Zhang, X. X., Li, Y. Q., Lai, Y. H., Ji, K. Y., Wen, M. L. & Cui, X. L. (2012). *Fodinibius salinus* gen. nov., sp. nov., a moderately halophilic bacterium isolated from a salt mine. *Int J Syst Evol Microbiol* **62**, 390–396.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.