

# *Loktanella salsilacus* gen. nov., sp. nov., *Loktanella fryxellensis* sp. nov. and *Loktanella vestfoldensis* sp. nov., new members of the *Rhodobacter* group, isolated from microbial mats in Antarctic lakes

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A taxonomic study was performed on 26 strains isolated from microbial mats in Antarctic lakes of the Vestfold Hills and the McMurdo Dry Valleys. Phylogenetic analysis based on 16S rRNA gene sequences placed these strains within the *Rhodobacter* group of the  $\alpha$ -subclass of the *Proteobacteria*. Sequence similarity values for the strains with their nearest phylogenetic neighbours (*Jannaschia*, *Octadecabacter* and *Ketogulonicigenium*) ranged between 94.0 and 95.8%. DNA–DNA hybridizations and comparison of repetitive extragenic palindromic DNA–PCR (rep-PCR) fingerprinting patterns revealed that these strains are members of three distinct species. The isolates are Gram-negative, chemoheterotrophic, non-motile rods and their DNA G + C contents range from 59.4 to 66.4 mol%. Whole-cell fatty acid profiles are similar and the primary fatty acid in all the strains is 18:1  $\omega$ 7c (74.1–87.7% of total). Genotypic results together with phenotypic characteristics allowed the differentiation of these species from related recognized species of the  $\alpha$ -*Proteobacteria* and the strains are assigned to a new genus, *Loktanella* gen. nov., with three novel species: *Loktanella salsilacus* sp. nov. (type species), consisting of ten strains with LMG 21507<sup>T</sup> (= CIP 108322<sup>T</sup>) as type strain; *Loktanella fryxellensis* sp. nov., consisting of 12 strains with LMG 22007<sup>T</sup> (= CIP 108323<sup>T</sup>) as type strain; and *Loktanella vestfoldensis* sp. nov., consisting of four strains with LMG 22003<sup>T</sup> (= CIP 108321<sup>T</sup>) as type strain.

During the last few years, there has been an increase in the isolation and description of novel marine and freshwater bacteria and several of these novel isolates represent members of the  $\alpha$ -subclass of the *Proteobacteria*, in which they are phylogenetically related to the genus *Rhodobacter*. The abundance of some members from the *Rhodobacter* group in these aquatic environments has been correlated with the presence of algal blooms and it has been suggested that they play an important role in sulfur cycling (González *et al.*, 1999, 2000).

Several of these novel members originate from Antarctic habitats: *Antarctobacter heliothermus* (Labrenz *et al.*, 1998),

*Roseovarius tolerans* (Labrenz *et al.*, 1999), *Staleyella guttiformis* and *Sulfitobacter brevis* (Labrenz *et al.*, 2000) from Ekho Lake, and *Octadecabacter arcticus* and *Octadecabacter antarcticus* (Gosink *et al.*, 1997) from polar sea ice and sea water. Recently, two new genera have been added to this *Rhodobacter* group: *Ketogulonicigenium* (Urbance *et al.*, 2001), isolated from soil and which oxidizes L-sorbose to 2-keto-L-gulonic acid, and *Jannaschia helgolandensis* (Wagner-Döbler *et al.*, 2003), isolated from the North Sea.

During the MICROMAT project (November 1998 to February 2001), 746 heterotrophic bacterial strains were isolated from microbial mat samples, collected from ten Antarctic lakes (Van Trappen *et al.*, 2002). Numerical analysis of the fatty acid composition of the isolates revealed 41 clusters, and 16S rRNA gene sequence analysis, performed on representative strains, showed that they belong to the  $\alpha$ -,  $\beta$ - and  $\gamma$ -subclasses of the *Proteobacteria*, the Gram-positives and the *Bacteroidetes* (Van Trappen *et al.*, 2002). Fatty acid analysis and 16S rRNA gene sequence analysis showed that diversity of heterotrophic bacteria in microbial mats from Antarctic lakes is very high. Moreover, many fatty acid clusters were shown to contain multiple taxa when tested by repetitive extragenic palindromic DNA–PCR

Published online ahead of print on 23 January 2004 as DOI 10.1099/ijs.0.3006-0.

Abbreviation: rep-PCR, repetitive extragenic palindromic DNA–PCR.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 22007<sup>T</sup>, LMG 22003<sup>T</sup>, LMG 21507<sup>T</sup>, LMG 22000, LMG 22002 and LMG 22006 are AJ582225, AJ582226, AJ440997, AJ582228, AJ582229 and AJ582227, respectively.

Normalized rep-PCR profiles and a dendrogram are available as supplementary material in IJSEM Online.

(rep-PCR) fingerprinting, a technique used to investigate the genomic diversity of each fatty acid cluster in greater detail, especially those belonging to the *Bacteroidetes* group (Van Trappen *et al.*, 2003, 2004).

In the present work we studied the relationships of 26 strains from fatty acid cluster 41 (Van Trappen *et al.*, 2002, belonging to the  $\alpha$ -*Proteobacteria*), using polyphasic taxonomic characterization.

The investigated isolates, their origin and genomic profile grouping are given in Table 1. Strains were routinely cultivated on marine agar 2216 (Difco) at 25 °C for 48 h, except when indicated otherwise.

DNA was prepared according to the method of Pitcher *et al.* (1989). rep-PCR fingerprinting (based on primers targeting the repetitive extragenic palindromic sequence) was performed on all strains of fatty acid cluster 41 (59 strains) isolated by Van Trappen *et al.* (2002), using the primers GTG<sub>5</sub> and REP1R-I and REP2-I (Versalovic *et al.*, 1991), as described by Rademaker & de Bruijn (1997) and Rademaker *et al.* (2000). Numerical analysis was carried out using the BIONUMERICS software package (Applied Maths). Twenty-six of these strains, listed in Table 1, could be divided into three different clusters according to their combined profile type (available as supplementary material in IJSEM Online) and these clusters were delineated by numerical analysis at a Pearson correlation coefficient level of 50 %. They are hereafter referred to as rep-PCR profile type I (comprising 12 strains), type II (four strains) and type III (ten strains). It is now well established that similar rep-PCR profiles are correlated to high total genomic DNA–DNA hybridization values (Versalovic *et al.*, 1994; Rademaker & De Bruijn, 1997; Rademaker *et al.*, 2000; Van Trappen *et al.*, 2003, 2004).

The almost-complete 16S rRNA gene sequences (1404–1449 nt) of strains LMG 22003<sup>T</sup>, LMG 22006, LMG 22007<sup>T</sup>, LMG 21507<sup>T</sup>, LMG 22000 and LMG 22002 were

determined as described previously (Van Trappen *et al.*, 2004). The closest related sequences were found using the FASTA program and the sequences from reference strains were aligned, with editing of the alignment and reformatting performed with the BIOEDIT program (Hall, 1999) and FORCON (Raes & Van de Peer, 1999). Evolutionary distances were calculated using the evolutionary model of Jukes and Cantor and a phylogenetic tree (shown in Fig. 1) was constructed using the neighbour-joining method (Saitou & Nei, 1987) with the TRECON program (Van de Peer & De Wachter, 1994). Dendrograms obtained by maximum-parsimony and maximum-likelihood analyses showed essentially the same topography (data not shown).

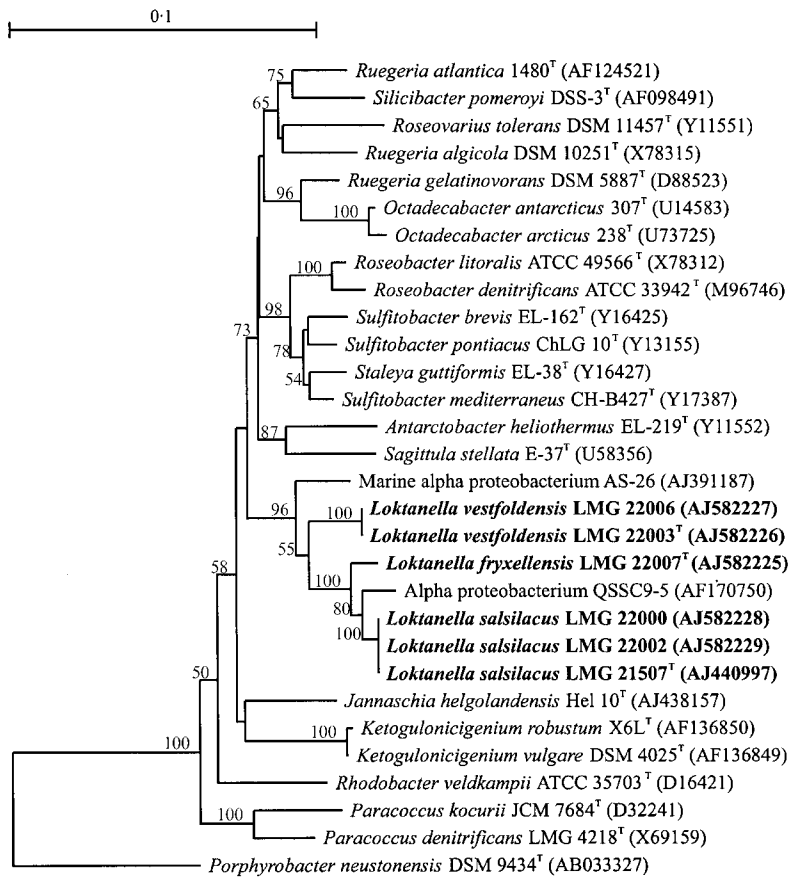
The novel Antarctic strains form a distinct evolutionary clade, supported by high bootstrap values, within the  $\alpha$ -*Proteobacteria* and are associated with the *Rhodobacter* group. A phylogenetic tree showing the position of the strains to their closest neighbours is shown in Fig. 1.

The 16S rRNA gene sequence of strain LMG 22007<sup>T</sup> (representative for the strains of rep-PCR profile type I) revealed 98.6 % similarity to that of strain LMG 21507<sup>T</sup> (identical to that of LMG 22000 and LMG 22002, and representative for rep-PCR profile type III) and 95.4 % to that of strain LMG 22003<sup>T</sup> (identical to that of strain LMG 22006, and representative for rep-PCR profile type II). The strains with nearest related sequences to that of strain LMG 22007<sup>T</sup> (rep-PCR profile I) were *J. helgolandensis* Hel 10<sup>T</sup> (95.8 %), *O. antarcticus* 307<sup>T</sup> (94.5 %) and the currently unclassified marine alpha proteobacterium strain QSSC9-5 (97.3 %). The 16S rRNA gene sequence of strain LMG 22003<sup>T</sup> (rep-PCR profile type II) showed 95.4 % sequence similarity to *J. helgolandensis* Hel 10<sup>T</sup>, 94.2 % to *Ketogulonicigenium vulgare* DSM 4025<sup>T</sup>, 94.3 % to *Ruegeria algicola* DSM 10251<sup>T</sup> and 96.2 % to the currently unclassified strain AS-26. The 16S rRNA gene sequence of strain LMG 21507<sup>T</sup> (rep-PCR profile type III) showed 95.7 % similarity to *J. helgolandensis* Hel 10<sup>T</sup>, 94.2 % to

**Table 1.** Strains investigated, source of isolation and rep-PCR profile type

LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, Gent, Belgium; numbers prefixed 'R-' refer to the strain numbers as preserved in the research collection of the Laboratorium voor Microbiologie, Universiteit Gent, Belgium and as used by Van Trappen *et al.* (2002).

Species (rep-PCR cluster)	Strain designations	Isolation site
<i>Loktanella fryxellensis</i> (I)	LMG 22007 <sup>T</sup> (=R-7670 <sup>T</sup> ), LMG 22008 (=R-7672), LMG 22009 (=R-7726), LMG 22010 (=R-7728), R-7601, R-7605, R-7671, R-7729, R-7732, R-7735, R-8013, R-8014	Lake Fryxell, Dry Valleys
<i>Loktanella vestfoldensis</i> (II)	LMG 22003 <sup>T</sup> (=R-9477 <sup>T</sup> ), LMG 22006 (=R-9184)	Ace Lake, Vestfold Hills
	LMG 22004 (=R-9054), LMG 22005 (=R-9057)	Pendant Lake, Vestfold Hills
<i>Loktanella salsilacus</i> (III)	LMG 21507 <sup>T</sup> (=R-8904 <sup>T</sup> ), LMG 21999 (=R-8968), R-8884, R-8901, R-9036	Ace Lake, Vestfold Hills
	LMG 22000 (=R-9030), LMG 22001 (=R-9066), LMG 22002 (=R-9068), R-9064, R-9186	Organic Lake, Vestfold Hills



**Fig. 1.** Neighbour-joining dendrogram showing the estimated phylogenetic relationships of *Loktanella salsilacus*, *Loktanella fryxellensis*, *Loktanella vestfoldensis* and other related genera of the  $\alpha$ -Proteobacteria on the basis of 16S rRNA gene sequences. *Porphyrobacter neustonensis* was chosen as outgroup. Bootstrap values are shown as percentages of 500 replicates, when more than 50%. Bar, 1 nt substitution per 100 nt. The GenBank accession number for each reference strain is given in parentheses.

*O. antarcticus* 307<sup>T</sup>, 94.2% to *K. vulgare* DSM 4025<sup>T</sup> and 98.4% to strain QSSC9. The low level of sequence similarities of the novel strains with recognized bacteria belonging to the *Rhodobacter* group of the  $\alpha$ -Proteobacteria (91.0–95.8%) clearly demonstrates that they represent a new genus.

Genomic relatedness between the novel Antarctic strains, representing the three different rep-PCR profile types, was determined by DNA–DNA hybridizations. DNA was prepared according to the method of Marmur (1961) and DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader (Perkin Elmer) for fluorescence measurements. Hybridization temperature was 45 °C and reciprocal experiments were performed for every pair of strains. The mean hybridization level between strains LMG 22007<sup>T</sup> (rep-PCR profile type I), LMG 22003<sup>T</sup> (rep-PCR profile type II) and LMG 21507<sup>T</sup> (rep-PCR profile type III) ranged between 10.5 and 17.6%, indicating that the strains represent three different species (Wayne *et al.*, 1987). Differences between reciprocal experiments were less than 10%. The rep-PCR profiles within each of the clusters I and II were almost identical (see Table 1 and supplementary data in IJSEM Online), indicating that within each of these clusters strains represent a single species (Versalovic *et al.*, 1994). Indeed, the 16S rRNA gene sequences of two strains from rep-PCR

group II are identical. Hybridization values of the three representative strains (LMG 21507<sup>T</sup>, LMG 22000 and LMG 22002) of rep-PCR profile type III were between 78.2 and 85.5%, indicating that these strains represent a single new species, as would be expected from their identical 16S rRNA gene sequences.

The G+C content of the DNA from the Antarctic strains was determined using an HPLC method, as described by Van Trappen *et al.* (2003). G+C values of strains LMG 22007<sup>T</sup>, LMG 22008, LMG 22009 and LMG 22010 from rep-PCR cluster I are 65.7, 66.2, 66.4 and 66.3 mol%, respectively; values for strains LMG 22003<sup>T</sup>, LMG 22004, LMG 22005 and LMG 22006 from rep-PCR cluster II are 62.1, 62.6, 62.3 and 63.1 mol%, respectively; and values for strains LMG 21507<sup>T</sup>, LMG 21999, LMG 22000, LMG 22001 and LMG 22002 of rep-PCR cluster III are 60.4, 60.3, 59.7, 60.1 and 59.4 mol%, respectively. These values are consistent with the G+C content of the *Rhodobacter* group, which ranges between 52.1 and 65 mol% (Labrenz *et al.*, 2000; Urbance *et al.*, 2001; Wagner-Döbler *et al.*, 2003; González *et al.*, 2003).

Cellular fatty acid patterns of the Antarctic strains are based on the data generated by Van Trappen *et al.* (2002). The strains showed similar fatty acid profiles (see Table 2), with the most abundant fatty acid being 18:1  $\omega$ 7c, accounting for 74.1–87.7% of the total fatty acids. This profile is

**Table 2.** Fatty acid composition of the three species of the genus *Loktanella*

The mean percentages of total fatty acids with the corresponding standard deviations are given. ND, Not detected; Tr, trace amount (<1% of total). Summed feature 2 comprises any combination of 12:0 aldehyde, unknown 10.928, 16:1 iso I and 14:0 3OH. Summed feature 3 comprises 15:0 iso 2OH or 16:1  $\omega$ 7c or both. Summed feature 7 comprises any combination of unknown 18.846, 19:1  $\omega$ 6c and 19:0 cyclo  $\omega$ 10c. Unknown fatty acids are designated by their equivalent chain lengths, relative to the chain lengths of known straight-chain saturated fatty acids.

Fatty acid	<i>L. salsilacus</i> (10 strains)	<i>L. fryxellensis</i> (12 strains)	<i>L. vestfoldensis</i> (4 strains)
10:0 3OH	2.4 ± 0.7	3.7 ± 1.1	6.1 ± 1.5
12:1 3OH	ND	ND	5.6 ± 1.4
16:0	2.9 ± 0.9	2.7 ± 1.1	2.9 ± 0.7
18:0	1.4 ± 0.8	1.6 ± 0.9	1.8 ± 0.3
18:1 $\omega$ 7c	87.7 ± 1.9	84.9 ± 3.7	74.1 ± 3.1
18:1 $\omega$ 7c 11 Methyl	Tr	ND	1.9 ± 0.8
Summed feature 2	Tr	1.7 ± 0.7	ND
Summed feature 3	2.8 ± 0.9	ND	ND
Summed feature 7	1.2 ± 1.0	4.7 ± 2.0	4.7 ± 0.7
Unknown 11.799	ND	ND	2.3 ± 1.2

characteristic for several major phylogenetic groups of the  $\alpha$ -*Proteobacteria*. Other fatty acids, in lower proportions, are 10:0 3OH, 16:0, 18:0 and summed feature 7 (comprising the unknown fatty acid 18.846, 19:1  $\omega$ 6c and 19:0 cyclo  $\omega$ 10c). The Antarctic strains can be differentiated from phylogenetic neighbour *J. helgolandensis* by the relative amount of 18:1  $\omega$ 7c (45–52%) and 19:0 cyclo (20–25%) and from *Ketogulonicigenium* species by the relative amount of 16:0 (32–39%) and 18:1  $\omega$ 7c (41–55%). The strains belonging to the different rep-PCR clusters can be differentiated from each other by the presence or absence of, for example, summed feature 2 (comprising any combination of 12:0 aldehyde, unknown 10.928, 16:1 iso I and 14:0 3OH), 18:1  $\omega$ 7c 11 methyl and the unknown fatty acid 11.799.

The following morphological, physiological and biochemical tests were performed. The strains were aerobic and chemoheterotrophic, and there was no growth under anaerobic conditions. Growth at different temperatures (5–45 °C) was tested on marine agar, and salt tolerance on R2A agar (composition per litre: 0.5 g yeast extract, 0.5 g proteose peptone No.3, 0.5 g Casamino acids, 0.5 g glucose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g dipotassium phosphate, 0.05 g magnesium sulfate, 15.0 g agar), supplemented with 1–20% NaCl at 25 °C. The strains of rep-PCR cluster III and rep-PCR cluster I were able to grow at 5–30 °C and 5–25 °C, respectively, whereas the strains of rep-PCR cluster II tolerated temperatures up to 37 °C. No growth occurred at 40 °C. Growth appeared on R2A agar with up to 10% NaCl for the strains of rep-PCR cluster III and rep-PCR cluster II, whereas the strains of rep-PCR cluster I only grew with up to 5% NaCl.

Colony morphology was determined on marine agar after

7 days. In addition, growth and adherence of colonies on R2A, nutrient and trypticase/soy agars were tested. Cells were tested for their reaction to the Gram stain and for catalase and oxidase activity. Tests in the commercial systems API ZYM, API 20NE and API 20E (bioMérieux) were generally performed according to the manufacturer's instructions. The API ZYM tests were read after 4 h incubation at 25 °C, the other API tests after 48 h at 25 °C. Degradation of casein (Reichenbach & Dworkin, 1981), DNA (using DNA agar from Difco, supplemented with 0.01% toluidine blue from Merck), starch, Tween 80 and L-tyrosine (Barrow & Feltham, 1993) was tested and reactions were read after 5 days.

The strains show the typical morphological characteristics of the *Rhodobacter* group (Labrenz *et al.*, 2000; Urbance *et al.*, 2001; Wagner-Döbler *et al.*, 2003; González *et al.*, 2003) and their physiological and biochemical characteristics are given in the descriptions below. The strains of rep-PCR clusters I, II and III can be differentiated from each other and related genera by several phenotypic characteristics (see Table 3 and Table 4).

On the basis of these results, a new genus with the name *Loktanella* gen. nov. is proposed with three species, *Loktanella salsilacus* sp. nov. (rep-PCR cluster III, type species), *Loktanella fryxellensis* sp. nov. (rep-PCR cluster I) and *Loktanella vestfoldensis* sp. nov. (rep-PCR cluster II).

### Description of *Loktanella* gen. nov.

*Loktanella* (Lok.tan.el'la. N.L. fem. n. *Loktanella* named after Tjhing-Lok Tan from the Alfred Wegener Institute in Bremerhaven, who contributed to our understanding of marine and polar bacteriology and ecology).

**Table 3.** Phenotypic characteristics that differentiate the three species of the genus *Loktanella*

–, Negative; +, positive; (+), weakly positive.

Characteristic	<i>L. salsilacus</i>	<i>L. fryxellensis</i>	<i>L. vestfoldensis</i>
Pigmentation	Beige	Pink–beige	Pink
Growth on:			
Trypticase/soy agar	–	–	(+)
Nutrient agar	–	–	(+)
Salinity range (% NaCl)	0–10	0–5	0–10
Temperature range (°C)	5–30	5–25	5–37
Hydrolysis of urea	–	–	+
Production of:			
Trypsin	–	–	+
$\alpha$ -Galactosidase	+	–	–
G + C content (mol%)	59.4–60.4	65.7–66.4	62.1–63.1

Gram-negative, rod-shaped cells that are strictly aerobic, moderately halotolerant and chemoheterotrophic. They do not form spores and the optimal growth temperature is 25 °C. Motility is not observed. Cytochrome oxidase-, catalase- and  $\beta$ -galactosidase-positive. The dominant fatty acid is 18:1  $\omega$ 7c and other characteristic fatty acids are 10:0 3OH, 16:0, 18:0 and summed feature 7 (which comprises the unknown fatty acid 18.846, 19:1  $\omega$ 6c and 19:0 cyclo  $\omega$ 10c). The DNA G+C content ranges from 59.4 to 66.4 mol%. As determined by 16S rRNA gene sequence analysis, the genus *Loktanella* belongs to the *Rhodobacter* group within the  $\alpha$ -*Proteobacteria*.

The type species is *Loktanella salsilacus*.

#### Description of *Loktanella salsilacus* sp. nov.

*Loktanella salsilacus* (sal.si.la'cus. L. adj. *salsus* salt, salty; L. gen. n. *lacus* of a lake; N.L. gen. n. *salsilacus* of a salt lake, referring to the isolation source, Ace Lake and Organic Lake, Vestfold Hills, Antarctica).

Cells are Gram-negative, short rods (<1  $\mu$ m by 3–4  $\mu$ m), often forming pairs or short chains. Strains grow at 5–30 °C; weak growth is observed at 37 °C and no growth occurs at 45 °C. Beige, convex, translucent colonies with a diameter

**Table 4.** Phenotypic characteristics that differentiate *Loktanella* from other related members of the '*Rhodobacteraceae*'

Genus: 1, *Loktanella*; 2, *Ketogulonicigenium*; 3, *Jannaschia*; 4, *Octadecabacter*; 5, *Antarctobacter*; 6, *Sulfitobacter*; 7, *Roseobacter*. Data for *Ketogulonicigenium*, *Jannaschia*, *Octadecabacter*, *Antarctobacter*, *Sulfitobacter* and *Roseobacter* are from the literature (Urbance *et al.*, 2001; Wagner-Döbler *et al.*, 2003; Gosink *et al.*, 1997; Labrenz *et al.*, 1998, 1999, 2000; Pukall *et al.*, 1999; Shiba, 1991; Lafay *et al.*, 1995; Ruiz-Ponte *et al.*, 1998). –, Negative; +, positive; v, variable results; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Rosettes formed	–	–	–	–	+	+	v
Colony colour	Pink–beige	Brown	White	White	Brown–yellow	v	v
Motility	–	+	–	–	+/–	+	+
Optimum temperature (°C)	25	25–31	25–30	4–15	16–26	17–28	20–30
Oxidase	+	+	(+)	–	+	+	+
Carbon utilization	–	+	+	+	+	+	+
Hydrolysis of:							
Aesculin	+	ND	–	ND	ND	ND	+
Gelatin	–	ND	–	–	+	–	+
DNA	–	ND	ND	ND	+	–	ND
Tween 80	+	ND	–	ND	–	+	ND
Nitrate reduction	–	ND	–	–	+	–	v
G + C content (mol%)	59–66	52–54	63	56–57	62	57–63	56–60

of 1–2 mm, with entire margins are formed on marine agar plates. Growth also occurs on R2A, but no growth is observed on trypticase/soy agar and nutrient agar. Colonies do not adhere to the agar. Degrades aesculin, Tween 80 and citrate. Growth on carbohydrates (API 20NE) is not observed and acids from carbohydrates are not produced (API 20E). Agar, casein, DNA, gelatin, starch, tyrosine and urea are not degraded. Tests for indole production, nitrate reduction, the Voges–Proskauer reaction and hydrogen sulfide production are negative. None of the strains shows activity for the enzymes arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase (API 20E) and lipase ( $C_{14}$ ), valine arylamidase, cystine arylamidase,  $\alpha$ -chymotrypsin, trypsin,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase (API ZYM). Weak enzymic activity is observed for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase,  $\alpha$ -glucosidase and  $\beta$ -glucosidase, medium activity for esterase ( $C_4$ ), esterase lipase ( $C_8$ ) and leucine arylamidase, and strong activity for  $\alpha$ -galactosidase (API ZYM). Growth occurs in 0–5 % NaCl, with weak growth in 10 % NaCl. The G + C content of the strains is 59.4–60.4 mol%. Isolated from microbial mats from lakes Ace and Organic in the Vestfold Hills, Antarctica.

The type strain is LMG 21507<sup>T</sup> (= CIP 108322<sup>T</sup>).

#### Description of *Loktanella fryxellensis* sp. nov.

*Loktanella fryxellensis* (fry.xell.en'sis N.L. fem. adj. *fryxellensis* referring to the isolation source, Lake Fryxell, Antarctica).

Cells are Gram-negative, short rods (<1  $\mu$ m by 2–3  $\mu$ m), often forming pairs or short chains. Strains grow at 5–25 °C; optimal growth temperature is 25 °C but weak growth occurs at 30 °C. Pale-pink to beige, convex, translucent colonies with a diameter of 1 mm, with entire margins formed on marine agar plates after 6 days incubation. Growth also occurs on R2A agar, but the strains do not grow on nutrient agar or trypticase/soy agar, and colonies do not adhere to the agar. Degrades aesculin, Tween 80 and citrate (weak reaction). No growth is observed (API 20NE) on carbohydrates and acids are not produced from carbohydrates (API 20E). Agar, casein, DNA, gelatin, tyrosine and urea are not degraded. Tests for indole production, nitrate reduction, the Voges–Proskauer reaction and hydrogen sulfide production are negative. None of the strains shows activity for the enzymes arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase (API 20E) and lipase ( $C_{14}$ ), cystine arylamidase,  $\alpha$ -chymotrypsin, trypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase (API ZYM). Weak enzymic activity is observed for valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and  $\alpha$ -glucosidase, medium activity for alkaline phosphatase, esterase ( $C_4$ ), esterase lipase ( $C_8$ ),  $\beta$ -galactosidase and leucine arylamidase, and strong activity for  $\beta$ -glucosidase (API ZYM). Growth

occurs in 0–5 % NaCl but not in 10 % NaCl. The G + C content of the strains is 65.7–66.4 mol%. Isolated from microbial mats from Lake Fryxell, in the McMurdo Dry Valleys, Antarctica.

The type strain is LMG 22007<sup>T</sup> (= CIP 108323<sup>T</sup>).

#### Description of *Loktanella vestfoldensis* sp. nov.

*Loktanella vestfoldensis* (vest.fold.en'sis. N.L. fem. adj. *vestfoldensis* referring to the isolation source, lakes Ace and Pendant, Vestfold Hills, Antarctica).

Cells are Gram-negative, short rods (<1  $\mu$ m by 3–4  $\mu$ m), often forming pairs or short chains. Strains grow at 5–37 °C, but no growth is observed at 45 °C. Pale-pink, convex, translucent colonies with a diameter of <1 mm, with entire margins formed on marine agar plates. Growth also occurs on trypticase/soy agar (weak growth), nutrient agar (weak growth) and R2A agar. Colonies do not adhere to the agar. Degrades aesculin, Tween 80, citrate and urea. No growth is observed (API 20NE) on carbohydrates and acids are not produced from carbohydrates (API 20E). Agar, casein, DNA, gelatin, tyrosine and starch are not degraded. Tests for indole production, nitrate reduction, hydrogen sulfide production and the Voges–Proskauer reaction are negative. None of the strains shows activity for the enzymes arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase (API 20E) and lipase ( $C_{14}$ ), valine arylamidase, cystine arylamidase,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase (API ZYM). Weak enzymic activity is observed for alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase and  $\beta$ -glucosidase, medium activity for esterase ( $C_4$ ), esterase lipase ( $C_8$ ) and acid phosphatase, and strong activity for trypsin (API ZYM). Growth occurs in 0–5 % NaCl and weak growth in 10 % NaCl. The G + C content of the strains is 62.1–63.1 mol%. Isolated from microbial mats from lakes Ace and Pendant in the Vestfold Hills, Antarctica.

The type strain is LMG 22003<sup>T</sup> (= CIP 108321<sup>T</sup>).

#### Acknowledgements

This work was funded by the Bijzonder Onderzoeksfonds (BOF), Universiteit Gent, Belgium. Part of this work was conducted in the framework of the MICROMAT project 'Biodiversity of microbial mats in Antarctica' (project no. BIO4980040), funded by the European Commission under the Biotech Programme. J.S. acknowledges the Fund for Scientific Research FWO (Belgium). We are grateful to Dr J. P. Euzéby for his help with nomenclature.

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