Loktanella cinnabarina sp. nov., isolated from a deep subseafloor sediment, and emended description of the genus Loktanella

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A Gram-stain-negative, aerobic, heterotrophic and salt-tolerant bacterium, designated strain LL-001T, was isolated from a deep subseafloor sediment in Japanese waters. Cells were non-motile rods and colonies were smooth, convex, circular and vermilion. The conditions for growth were 15–35 °C, pH 5.5–7.5 and 1–8 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences confirmed that strain LL-001T belonged to the genus Loktanella within the family Rhodobacteraceae of the class Alphaproteobacteria. 16S rRNA gene sequence similarity between strain LL-001T and members of the genus Loktanella was 94.5–98.5 %; the highest sequence similarity was with Loktanella hongkongensis UST950701-009P7. DNA–DNA relatedness between strain LL-001T and Loktanella hongkongensis UST950701-009P7 was 41.5–43.6 %. The DNA G+C content of strain LL-001T was 69.3 mol%. On the basis of biochemical features and 16S rRNA gene sequence comparison, strain LL-001T is considered to represent a novel species of the genus Loktanella, for which the name Loktanella cinnabarina sp. nov. is proposed. The type strain is LL-001T (=JCM 18161T=CECT 8072T). The description of the genus Loktanella is also emended.

The genus Loktanella, which was described by Van Trappen et al. (2004), is the type species of the family Rhodobacteraceae, comprising three genera: Loktanella, Salsilacus and Fryxellensis. The type species, Loktanella salsilacus, was isolated from Antarctic lakes. Among these genera, Loktanella is the most species-rich, with eight described species, L. pyoseonensis, L. vestfoldensis, L. koreensis, L. rosea, L. tamlensis, L. litorea, L. atrilutea and L. agnita. Moreover, nine other species, L. pyoseonensis, L. vestfoldensis, L. koreensis, L. rosea, L. tamlensis, L. litorea, L. atrilutea and L. agnita, were described. Each species belonging to the genus Loktanella has been isolated from aqueous or marine environments such as microbial mats in Antarctic lakes, marine biofilms, sediment, sea sand and seawater.

A vermilion bacterium was isolated from a deep subseafloor sediment (108 m below the seafloor) off the Shimokita Peninsula of Japan in the north-western Pacific Ocean (site C9001: water depth 1180 m). Sediment subsamples (0.1 g) were suspended in 5 ml artificial seawater (Nihon Pharmaceutical). The suspension was incubated at 25 °C for 1 day and then spread on 1.5 % (w/v) agar containing basal seawater [BSW-5: 0.5 × artificial seawater, 0.5 % (w/v) marine broth 2216 (MB; Difco) and 0.25 % (w/v) NaCl]. Strain LL-001T was isolated after incubation at 25 °C for 5 days.

Genomic DNA of strain LL-001T was extracted and purified according to the method described by Wilson (1987). The sequencing and assembly of the 16S rRNA gene was performed as described by Lane (1991) with LA-Taq DNA polymerase (TaKaRa Bio). The resultant 16S rRNA gene sequence (1385 nt) of strain LL-001T was compared with those available in GenBank using ARB (Ludwig et al., 2004; Kumar et al., 2005, 2006), considering the secondary structure of the 16S rRNA to determine an approximate phylogenetic affiliation. Phylogenetic analyses were carried out using maximum likelihood (Felsenstein, 1981), neighbour joining (Saitou & Nei, 1987) with the substitution model of Jukes & Cantor (1969) and maximum parsimony (Fitch, 1971). A phylogenetic tree was constructed using the maximum-likelihood algorithm and evolutionary distances were calculated using the general time-reversible model (Tavaré, 1986; Zwickl & Holder, 2004) with
Hyphomonas polymorpha DSM 2665\textsuperscript{T} (AJ227813) as an outgroup taxon. A bootstrap analysis (Felsenstein, 1985) was performed with 1000 resampled datasets to estimate tree topology.

The maximum-likelihood tree based on 16S rRNA gene sequences (Fig. 1) indicated that strain LL-001\textsuperscript{T} belonged to the genus *Loktanella* and was closely related to *L. hongkongensis* UST950701-009P\textsuperscript{T}. This relationship was supported by a high bootstrap value (100\%). Essentially the same tree topologies were obtained with the neighbour-joining and maximum-parsimony algorithms (Fig. S1, available in IJSEM Online). 16S rRNA gene sequence similarities between strain LL-001\textsuperscript{T} and the other members of the genus *Loktanella* were as follows: *L. hongkongensis* UST950701-009P, 98.5\%; *L. pyoseonensis* JJM85\textsuperscript{T}, 95.9\%; *L. tamlensis* SSW-35\textsuperscript{T}, 95.9\%; *L. salsilacus* LMG 21507\textsuperscript{T}, 95.5\%; *L. vestfoldensis* LMG 22003\textsuperscript{T}, 95.3\%; *L. fryxellensis* LMG 22007\textsuperscript{T}, 95.1\%; *L. maricola* DSW-18\textsuperscript{T}, 95.0\%; *L. litorea* DPG-5\textsuperscript{T}, 95.0\%; *L. rosea* Fg36\textsuperscript{T}, 94.9\%; *L. atrilutea* IG8\textsuperscript{T}, 94.8\%; *L. koreensis* GA2-M3\textsuperscript{T}, 94.7\%; and *L. agnita* R10SW5\textsuperscript{T}, 94.5\%.

The G+C content of the genomic DNA of strain LL-001\textsuperscript{T} was determined by HPLC (Waters 600 series; Nihon Waters; Mesbah *et al.*, 1989) equipped with a Cosmosil 5C\textsubscript{18}-PAQ column (4.6 × 150 mm; NacalaiTesque) and was found to be 69.3 mol\%. DNA–DNA hybridization between strain LL-001\textsuperscript{T} and its closest phylogenetic neighbour was carried out with photobiotin-labelled

![Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences indicating the relationships between strain LL-001\textsuperscript{T} and members of the family Rhodobacteraceae. Bootstrap values (>50\%) based on 1000 resampled datasets are shown at branch nodes. Hyphomonas polymorpha DSM 2665\textsuperscript{T} (GenBank accession no. AJ227813) was used as an outgroup (not shown). Bar, 0.2 substitutions per nucleotide position.](http://ijs.sgmjournals.org)
probes in microplate wells as described by Ezaki et al. (1989) using a 1420 Multilabel Counter (Perkin Elmer) for fluorescence measurements. The hybridization temperature was 52 °C and reciprocal experiments were performed. DNA–DNA relatedness between strain LL-001T and L. hongkongensis UST950701-009P³ was 41.5–43.6 %, a difference that indicates these strains belong to different species (Wayne et al., 1987).

Cell morphology was observed under a light microscope equipped with phase-contrast optics at ×400 magnification (Olympus). Motility was assayed in semi-solid BSW-5 agar (0.3 % agar). Cells were inoculated by stabbing with a straight needle and the tube was incubated at 25 °C for 5 days. The external structure of strain LL-001T was checked with a JSM-6700F scanning electron microscope (Jeol). Gram-staining was carried out using a Gram-stain kit (Wako) according to the manufacturer's instructions. Growth at 4, 10, 20, 25, 30, 35, 37, 40 and 42 °C, at pH 5–9 (at intervals of 0.5 pH unit) and with 1–10 % (w/v) NaCl (at intervals of 1.0 % NaCl) and physiological saline was tested on BSW-5 agar with these conditions. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on BSW-5 agar and BSW-5 agar supplemented with nitrate, both prepared anaerobically using nitrogen. The decomposition of alginate was tested on medium supplemented with 1 % (w/v) alginate (Tang et al., 2009). Hydrolysis of casein, starch (Smibert & Feltham, 1993), Tween 80 (Barrow & Feltham, 1993), CM-cellulose (Rautela & Cowling, 1966), aesculin (Swan, 1954), xanthine and hypoxanthine (Barrow & Feltham, 1993) was tested on BSW-5 agar supplemented with nitrate, both prepared anaerobically using nitrogen. The decomposition of alginate was tested on medium supplemented with 1 % (w/v) alginate. Catalase activity was evaluated by observing oxygen bubble production in a 3 % (v/v) aqueous hydrogen peroxide solution. To assay enzyme activities, API ZYM (bioMérieux) tests were performed. Sensitivity to antibiotics was checked on BSW-5 agar using antibiotic discs (BD) containing (μg per disc unless otherwise stated): bacitracin (10 IU), polymyxin B (100 U), cephalothin (30), tetracycline (30), vancomycin (30), kanamycin (30), neomycin (30), lincomycin (15), streptomycin (10), novobiocin (30), gentamicin (10), rifampicin (5), chloramphenicol (30), erythromycin (15), ampicillin (10), carbenicillin (100) and penicillin G (10 U).

Colonies were vermillion, smooth, circular, convex and 0.5–2.0 mm in diameter on BSW-5 agar (Fig. S2b). Cells of strain LL-001T were approximately 1.2–5.3 μm in length and 0.9–1.0 μm in width, Gram-stain-negative and smooth rods (Fig. S2c and d). Strain LL-001T, similarly to other species of the genus Loktanella except for L. atrilutea, L. pyoseonensis and L. tamensis, was non-motile. Growth occurred at 15–35 °C, at pH 5.5–7.5 and with 1–8 % NaCl on BSW-5 agar. In anaerobic conditions, growth was not observed after 2 weeks of cultivation on BSW-5 agar at 25 °C. No growth of colonies was observed on nutrient agar or trypticase soy agar. The differences in cultural, biochemical and biophysical assays between strain LL-001T and L. hongkongensis UST950701-009P³, L. pyoseonensis JMM5T and L. salisalicus LMG 21507T are given in Fig. S2a, Table 1 and the species description.

The chemotaxonomic characteristics of strains LL-001T and the reference strains were determined using cells cultured in BSW-5 liquid medium to the exponential phase of growth at 25 °C. Isoprenoid quinones, phospholipids and cellular fatty acids were analysed according to the methods described by Minnikin et al. (1977, 1984), Kroppenstedt (1985) and Nishijima et al. (1997) and compared with results obtained for the reference strains. Isoprenoid quinones were determined by HPLC. Phospholipids were separated by twodimensional TLC with an aluminium-backed silica gel plate (no. 5514; Merck) in the solvent systems described by Ivanova et al. (2005). The phospholipid composition was determined in comparison to that of L. hongkongensis UST950701-009P³ with an authentic sample. For detection, iodine (Merck) for total lipids, Dittmer-Lester reagent (Merck) for phosphorus, ninhydrin reagent (Merck) for amino groups, Dragendorff reagent (Merck) for choline phospholipids, p-anisaldehyde reagent (Wako) for glycolipids and periodic acid-Schiff's reagent for glycerolipids were used. For analysis of cellular fatty acids, cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification system (version 4.5; MIDI) and the fatty acid methyl esters were determined by GC with reference to the TSBA40 MIS standard library.

Ubiquinone Q-10 was the predominant isoprenoid quinone. Phosphatidylcholine, diphosphatidylglycerol and phosphatidylglycerol were the major phospholipids (28.5, 21.1 and 20.3 % of the total, respectively). The cellular fatty acid compositions of strain LL-001T and L. hongkongensis UST950701-009P³ consisted of straight-chain saturated and unsaturated fatty acids, with small amounts of hydroxy fatty acids, which is typical for the genus Loktanella (Van Trappen et al., 2004; Ivanova et al., 2005; Weon et al., 2006; Hosoya & Yokota, 2007; Yoon et al., 2007, 2013; Moon et al., 2010; Lee, 2012). The dominant cellular fatty acid was C₁₈:₁ω7c, which was in similar proportions in both strains. However, the strains differed in the abundance of minor components: C₁₆:₀ω7 and C₁₄:₁ω6 unsaturated fatty acids, C₁₆:₀ and C₁₆:ω₉ω6 saturated fatty acids C₁₂:₀ 3-OH and C₁₀:₀ 3-OH. The fatty acid compositions of strain LL-001T and L. hongkongensis UST950701-009P³ are given in Table S1.

On the basis of the phenotypic features and phylogenetic evidence presented here, strain LL-001T represents a novel
species of the genus *Loktanella*, for which the name *Loktanella cinnabarina* sp. nov. is proposed. The description of the genus *Loktanella* is also emended.

**Emended description of the genus Loktanella Van Trappen et al. 2004 emend. Lee 2012**

The description is as given by Lee (2012) with the following amendment. The DNA G+C content is 55.0–69.3 mol%.

**Description of Loktanella cinnabarina sp. nov.**

*Loktanella cinnabarina* (cin.na.ba’ri.na. L. n. *cinnabar* -aris cinnabar; L. suff. -inis -a -um suffix used with the sense of belonging to; N.L. fem. adj. *cinnabarina* belonging to cinnabar, referring to the vermillion colour of the cells).

Cells are Gram-stain-negative, aerobic, heterotrophic, salt-tolerant, non-motile rods, approximately 1.2–5.3 μm in length and 0.9–1.0 μm in width. Colonies on BSW-5 agar after incubation for 5 days are round and brilliant vermillion. Cytochrome oxidase- and catalase-positive. Growth occurs at 15–35 °C (optimum, 20–30 °C) but not at 37 °C. Growth occurs at pH 5.5–7.5 (optimum, pH 6.0–7.0). Growth occurs on BSW-5 agar with 1–8 % (w/v) NaCl (optimum, 1–5 % NaCl). Hydrolysis of aesculin and degradation of L-tyrosine are observed, but production of H₂S, decomposition of alginate and hydrolysis of casein, starch, Tween 80, CM-cellulose, xanthine and hypoxanthine are not observed. No identifiable growth is observed on carbohydrates (API 20 NE), and acids are not produced from any substrate (GN2 MicroPlates). With API ZYM, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, α-galactosidase and α-glucosidase, weakly positive for esterase (C4), trypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase and β-glucosidase, but negative for lipase (C4), cystine arylamidase, α-chymotrypsin, acid phosphatase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. With API 20 NE assay, weakly positive for hydrolysis of aesculin and β-glucosidase, but negative for nitrate/nitrite reduction, indole production, glucose fermentation, arginine hydrolyase and urease. Sensitive to bacitracin, polymyxin B, tetracycline, vancomycin, kanamycin, neomycin, streptomycin and carbenicillin; slightly sensitive to novobiocin, gentamicin, rifampicin, chloramphenicol, erythromycin and cephalothin; resistant to ampicillin, penicillin G and lincomycin. Other physiological and biochemical properties are given in Table 1. The major phospholipids

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Table 1. Differential phenotypic characteristics of strain LL-001T, its closest phylogenetic neighbours and the type strain of the type species of the genus *Loktanella*

Strains: 1, LL-001T; 2, *L. hongkongensis* UST950701-009PT; 3, *L. pyoseonensis* JIM857; 4, *L. salsilacus* LMG 21507T. All data were taken from this study. All strains were positive for leucine arylamidase and catalase activities, and negative for hydrolysis of urea and gelatin. +, Positive; w, weakly positive; v, variable; −, negative; ND, no data available.
are phosphatidylcholine, diphostathidylglycerol and phosphatidylglycerol. Q-10 is the predominant ubiquinone. The predominant cellular fatty acid is C_{18:1}ω7c.

The type strain, LL-001^T (=JCM 18161^T=CECT 8072^T), was isolated from a deep subseaflower sediment (108 m below the seafloor, water depth 1180 m) off the Shimokita Peninsula of Japan. The DNA G+C content of the type strain is 69.3 mol%.

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References


