Massilia yuzhufengensis sp. nov., isolated from an ice core

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A Gram-negative, rod-shaped, aerobic, motile bacterium, strain Y1243-1T, was isolated from an ice core drilled from Yuzhufeng Glacier, Tibetan Plateau, China. Cells had polar flagella. The novel strain shared 94.7–97.6 % 16S rRNA gene sequence similarity with the type strains of species of the genus Massilia. The novel isolate is thus classified in the genus Massilia. The major fatty acids of strain Y1243-1T were summed feature 3 (C16:1ω7c and/or iso-C15:0 2-0H) (43.98 %), C16:0 (27.86 %), C10:0 3-0H (7.10 %), C18:0 (6.95 %) and C18:1ω7c (5.01 %). The predominant isoprenoid quinone was Q-8. The DNA G+C content of strain Y1243-1T was 65.7 mol% (Tm). The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. A number of phenotypic characteristics distinguished the novel isolate from the type strains of recognized Massilia species. Furthermore, in DNA–DNA hybridization tests, strain Y1243-1T shared 45 % relatedness with its closest phylogenetic relative, Massilia consociata CCUG 58010T. From the genotypic and phenotypic data, it is evident that strain Y1243-1T represents a novel species of the genus Massilia, for which the name Massilia yuzhufengensis sp. nov. is proposed. The type strain is Y1243-1T (=KACC 16569T=CGMCC 1.12041T).

The genus Massilia was first proposed by La Scola et al. (1998) for an isolate from the blood of an immunocompromised patient with a cerebellar lesion. Members of the genus are characterized as aerobic, Gram-negative, motile, non-spore-forming rods, which contain several typical fatty acids, such as C12:0, C10:0 3-OH, iso-C15:0 2-OH and/or C16:1ω7c, C16:0 and C18:1ω7c. Q-8 is the predominant isoprenoid quinone. Phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol are the major polar lipids of these bacteria. The DNA G+C content ranges from 62.4 to 68.9 mol%. It was recently shown that four species of the genus Naxibacter were grouped together with the majority of species of the genus Massilia and no significant differences could be detected when comparing the chemotaxonomic makers described for species of the genera Naxibacter and Massilia. Therefore, Kämpfer et al. (2011) transferred all Naxibacter species to the genus Massilia. At the time of writing, a total of 18 Massilia species with validly published names have been described (La Scola et al., 1998; Gallego et al., 2006; Zhang et al., 2006; Zul et al., 2008; Weon et al., 2008, 2009, 2010; Kämpfer et al., 2011, 2012a; Wang et al., 2012).

Ice core samples were drilled from Yuzhufeng Glacier on the Qinghai–Tibetan Plateau of China (94° 14.77′ E 35° 39.64′ N). Temperatures on the glacier are extremely low. Thawed water from sections of the ice core with depth was used for cultivation. After incubation at 4 °C for 30 days with R2A agar medium (Reasoner & Geldreich, 1985), several bacterial colonies were recovered.

The genomic DNA of these strains was extracted according to the methods of Marmur (1961) from cells grown on R2A agar for 2 days at 28 °C. Purity was assessed using a NanoDrop spectrophotometer (2000c; Thermo). The 16S rRNA gene was amplified with universal primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GTACCTTGTAGACTCT3’) (Embley, 1991). Nearly full-length 16S rRNA gene sequences were compared with those in GenBank using the BLAST program (NCBI) and the EzTaxon server 2.1 (Chun et al., 2007) to determine their approximate phylogenetic affiliation. The 16S rRNA gene...
sequences were then analysed with the software package MEGA 5.05 (Tamura et al., 2011). A phylogenetic tree was constructed using the neighbour-joining and maximum-likelihood methods with bootstrap values based on 1000 replications (Fig. 1). One bacterial strain recovered from 119.6 m of the ice core, designated Y1243-1T, was shown to be phylogenetically related to members of the genus Massilia (97.6–94.7 % 16S rRNA gene sequence similarity) with the closest relative being Massilia consociata CCUG 58010T. We therefore considered that strain Y1243-1T may represent a novel species of the genus Massilia.

It was recently shown that Telluria species fell together with Massilia albidiflava, Massilia dura, Massilia lutea and Massilia plicata, and Duganella violaceinigra was reclassified within a novel genus as Pseudoduganella violaceinigra (Kämpfer et al., 2012b). The phylogenetic tree based on the neighbour-joining method in this study supports the reclassification of D. violaceinigra. Telluria species form the basal group in the tree. Furthermore, we found the topology of the maximum-likelihood tree and the neighbour-joining tree to be different for Telluria species (not shown). Therefore, further extensive taxonomic studies are required to determine whether to transfer members of the genus Telluria to the genus Massilia. Although the distinct phylogenetic positions and differences in chemotaxonomic markers such as polar lipids and fatty acids are usually used to distinguish bacteria at the genus level, assigning any novel isolate to Massilia–Duganella–Telluria species needs particular care because of the absence of

![Image](image_url)
**Table 1. Differential properties between strain Y1243-1\(^T\) and the type strains of recognized species of the genus *Massilia***

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Catalase/oxidase | +/- | ND/- | +/- | +/ | +/ | + | +/ | - | - | - | - | - | - | - | - | - | - | - | - |
| Isolation source | Ice core | Blood | Air | Air | Soil | Eye | Blood | Water | Air | Soil | Blood | Soil | Soil | Soil | Soil | Soil | Eye |
| Nitrate reduction | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Urease | + | + | - | + | + | - | ( + ) | - | + | + | + | + | + | + | + | + | + |
| Hydrolysis of: | | | | | | | | | | | | | | | | | | | |
| Aesculin | + | + | - | + | + | + | - | ( + ) | - | + | + | + | + | + | + | + | + |
| Gelatin | + | + | - | + | + | - | - | ND | ND | + | + | ND | + | ( + ) | + | + | + | ND |
| Assimilation of: | | | | | | | | | | | | | | | | | | | |
| d-Glucose | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| L-Arabinose | + | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Potassium gluconate | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | = | - | - |
| Adipic acid | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Trisodium citrate | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Enzyme activities | | | | | | | | | | | | | | | | | | | |
| Esterase lipase (C8) | + | + | + | - | + | + | + | ND | + | + | ND | + | - | + | + | + | + | ND | + | + |
| Cystine arylamidase | - | + | - | + | + | - | ( + ) | + | ND | - | + | ND | + | + | + | + | + | - | - | - | ND |
| β-Glucuronidase | + | - | - | + | - | ND | - | - | ND | - | - | - | - | - | - | - | - | - | - | - | ND |
| α-Glucosidase | - | - | - | + | - | + | + | ND | ( + ) | + | ND | - | - | - | - | - | - | - | - | ND |
| β-Glucosidase | - | - | - | + | - | + | + | ND | ( + ) | + | ND | - | - | - | - | - | - | - | - | ND |
| DNA G+C content (mol%) | 65.7 | 68.9 | 67.8 | 66.6 | 66.1 | 65.3 | 67.8 | ND | ND | 66.0 | 68.9 | 62.4 ± 0.3 | 64.0 | 68.7 | 65.3 | 63.3 | 65.1 | 65.9 | ND |

Data taken from: a, Kämpfer et al. (2011); b, Weon et al. (2009); c, Weon et al. (2010); d, Zul et al. (2008); e, Kämpfer et al. (2008); f, Gallego et al. (2006); g, Xu et al. (2005); h, Lindquist et al. (2003); i, Zhang et al. (2006); j, La Scola et al. (1998).
clear phylogenetic boundaries and genus-specific chemotaxonomic markers.

To further determine the taxonomic position of the novel isolate, a series of phenotypic and genotypic approaches were employed. Morphology was examined by transmission electron microscopy (JEM-1230; JEOL) (Fig. 2). Phenotypic characteristics such as Gram staining, catalase activity, and hydrolysis of casein, hypoxanthine, Tween 80 and starch were performed using the methods of Smibert & Krieg (1994). The pH range for growth was determined in R2A broth at 30°C. The pH (4–10) of the medium was adjusted with citrate phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994). Growth in the absence of NaCl and in the presence of 1, 2, 3, 4 and 5% (w/v) NaCl was also investigated in the same medium. Growth at various temperatures (0–40°C) was measured. Other physiological and biochemical properties were further determined with API 20NE, API 20E and API ZYM strips according to the manufacturer’s instructions (bioMérieux). Differences in physiological characterization between strain Y1243-1T and the type strains of recognized Massilia species are given in Table 1. Of particular note, strain Y1243-1T was able to grow at 2°C, whereas other species of this genus cannot.

Fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification System (MIDI, Version 6.0) with cells of strain Y1243-1T and all strains under comparison harvested from R2A agar.
after 48 h of growth at 28 °C. No significant differences in fatty acid profile were found between the novel isolate and closely related bacteria, but small quantitative differences were observed (Table 2). Strain Y1243-1T had a relatively higher proportion of C18:0 (6.95%) compared with recognized species of the genus Massilia.

For isoprenoid quinone and polar lipid analyses, cells were harvested after 48 h of growth at 28 °C. Isoprenoid quinones were analysed as described by Hiraishi et al. (1998), using a Waters Acquity Ultra Performance LC (UPLC)-Q-TOF-MS spectrometer by electrospray ionization (Romano et al., 2006). The predominant isoprenoid quinone of strain Y1243-1T was Q-8. Polar lipids were extracted and analysed by two-dimensional TLC (Altenburger et al., 1996; Tindall, 1990). The major polar lipids of strain Y1243-1T were similar to those of species of the genus Massilia (Fig. 3) (Kämpfer et al., 2008, 2011, 2012a; Weon et al., 2010).

Phospholipids PL1 and PL2 were also found in Massilia oculi (Kämpfer et al., 2012a), Massilia haematophila and Massilia varians (Kämpfer et al., 2008). One unknown aminolipid (AL), found in M. consociata, M. haematophila and M. varians, was also detected in strain Y1243-1T. The genomic DNA G+C content of the novel strain was estimated from the midpoint value (Tm) of the thermal denaturation profile (Mandel et al., 1970). The genomic DNA G+C content of strain Y1243-1T was 65.7 mol%. The predominant isoprenoid quinones, major polar lipids and DNA G+C content of strain Y1243-1T are consistent with the general characteristics of the genus Massilia.

The novel isolate shares a low 16S rRNA gene sequence similarity of 97.6% with M. consociata CCUG 58010T, and limited phenotypic variations can be observed between the two (Table 2). Therefore, we further compared the two bacteria at the genomic level by DNA–DNA hybridization experiments, which were carried out applying the optical renaturation method (De Ley et al., 1970; Huß et al., 1983; Jahne, 1992). The level of DNA–DNA relatedness between strain Y1243-1T and M. consociata CCUG 58010T was 45%.

Based on the genotypic and phenotypic data presented in this study, the psychrotolerant bacterium strain Y1243-1T represents a novel species of the genus Massilia, for which the name Massilia yuzhufengensis sp. nov. is proposed.

**Description of Massilia yuzhufengensis sp. nov.**

*Massilia yuzhufengensis* (yu.zhu.feng.en’sis. N.L. fem. adj. *yuzhufengensis*, of Yuzhufeng Glacier, Tibetan Plateau, China, where the type strain was isolated).

Cells are aerobic, Gram-negative rods (0.7–1 μm wide and 2.3–2.7 μm long) and have polar flagella. Yellow, round, smooth, convex and opaque colonies are produced on R2A agar after incubation at 28 °C for 2–3 days. Grows at 2–35 °C (optimally at 25 °C) on R2A agar, at pH 5–8 (optimally at pH 7) and with 0–3% NaCl (optimally at 2% NaCl). Positive for catalase, but negative for oxidase, nitrate reduction and urease (API 20NE test strips).

Degrades aesculin, gelatin and starch, but not casein or Tween 80. Assimilates L-arabinose, D-mannose, maltose and malic acid, but not D-glucose, D-mannitol, N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate or phenylacetic acid (API 20NE and API 20E test strips). In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucuronidase, but negative for lipase (C14), cystine arylamidase, x-chymotrypsin, x-galactosidase, β-galactosidase, x-glucosidase, β-glucosidase, N-acyl-β-glucosaminidase, x-mannosidase and x-fucosidase. The major fatty acids are summed feature 3 (C16:1 07c and/or iso-C15:0 2-OH) and C16:0. The major respiratory quinone is Q-8 and major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol.

The type strain Y1243-1T (=KACC 16569T=CGMCC 1.12041T) was isolated from a 119.6 m deep ice core section drilled from Yuzhufeng Glacier, Tibetan Plateau, China. The DNA G+C content of the type strain is 65.7 mol% (Tm).

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**References**


