Frateuria terrea sp. nov., isolated from forest soil, and emended description of the genus *Frateuria*

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A bacterial strain, designated VA24^T, was isolated from forest soil of the Changbai Mountains, Heilongjiang province, China. Cells of strain VA24^T were Gram-reaction-negative, aerobic, non-spore-forming, long rods, 0.3–0.5×2.0–3.0 µm in size and were motile by means of a subpolar flagellum. Strain VA24^T was oxidase-positive and catalase-negative. Growth occurred at 21–36 °C, pH 5–10 and in 0–2% (w/v) NaCl but did not occur at 37 °C. The predominant respiratory quinone was Q-8, the major polar lipids were phosphatidylethanolamine and phosphatidylmonomethylethanolamine and the major cellular fatty acids were iso-C_{15:0} (14.9%), iso-C_{17:1ω9c} (14.1%), iso-C_{17:0} (10.8%) and iso-C_{16:0} (10.3%). The DNA G + C content was 67.4 mol% (*T_m*). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain VA24^T was closely related to *Frateuria aurantia* IFO 13300^T with 96.9% sequence similarity. DNA–DNA relatedness of strain VA24^T to *F. aurantia* DSM 6220^T was 15.8%. Based on its phenotypic and genotypic features, strain VA24^T represents a novel species of the genus *Frateuria*, for which the name *Frateuria terrea* sp. nov. is proposed. The type strain is VA24^T (=CGMCC 1.7053^T =NBRC 104236^T).

At the time of writing, the genus *Frateuria* contains only one species with a validly published name, *Frateuria aurantia*, which was isolated from the lily plant *Lilium auratum* and was previously designated ‘*Acetobacter aurantium*’ (Kondo & Ameyama, 1958). Based on comprehensive phenotypic, chemotaxonomic and DNA–DNA hybridization studies, ‘*A. aurantium*’ was reclassified as the sole representative of a newly established genus, *Frateuria*, and was assigned as the type species of that genus (Swings *et al.*, 1980, 1984).

In an attempt to study the diversity and cultivability of microbes present in forest soil of the Changbai Mountains, Heilongjiang province, PR China, bacterial strains were isolated by using a serial dilution method (Sait *et al.*, 2002). A bacterial strain, designated VA24^T, was isolated on VA medium that was modified from VL55 medium (Sait *et al.*, 2002) and inoculated with the 10^{−7} dilution by repeated

streaking as described by Zhang *et al.* (2009). After 1 week, each plate displayed several small yellow colonies.

The nearly complete 16S rRNA gene (1531 bp) of strain VA24^T was amplified and sequenced as described by Zhang *et al.* (2003). DNA BLAST searches on the NCBI database (<http://blast.ncbi.nlm.nih.gov>) showed that strain VA24^T was related to members of the *Frateuria–Dyella–Rhodanobacter* group within the family *Xanthomonadaceae*. Based on 16S rRNA gene sequence similarities, the closest relatives to strain VA24^T were *F. aurantia* IFO 13330^T (96.9%) and *Dyella ginsengisoli* Gsoil 3046^T (96.9%), both of which are members of the family *Xanthomonadaceae*. Alignments of 16S rRNA gene sequences of members from this family were performed with the CLUSTAL_X program version 1.64b (Thompson *et al.*, 1997) and alignment positions with insertions or deletions were excluded from the analysis. Phylogenetic trees, based on 16S rRNA gene sequence analyses, were reconstructed by the neighbour-joining (NJ) and maximum-parsimony (MP) methods using Kimura’s two-parameter calculation model in MEGA version 3.1 software (Kumar *et al.*, 2004). The phylogenetic trees clearly showed that strain VA24^T, together with *F. aurantia* IFO 13330^T, formed a distinct phylogenetic lineage within the *Frateuria–Dyella–Rhodanobacter* cluster of genera within the family *Xanthomonadaceae* (Fig. 1). A close

Abbreviations: MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain VA24^T is EU682683.

Three supplementary figures and one supplementary table are available with the online version of this paper.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain VA24^T in the family Xanthomonadaceae. GenBank accession numbers are given in parentheses. Numbers represent bootstrap support (>50% are shown) based on 1000 replicates. *Xanthomonas campestris* LMG 568^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position (K_{nuc}).

relationship between strain VA24^T and *F. aurantia* IFO 13330^T was evident in the tree topology, which was also supported when trees were reconstructed using other tools such as the MP method (see Supplementary Fig. S1, available in IJSEM Online).

The morphological, physiological and biochemical characteristics of strain VA24^T were investigated using routine cultivation at 30 °C on diluted glucose and yeast-extract medium (D-GY; 10 g glucose and 2 g yeast extract l⁻¹). Growth was assessed on various media, including tryptic soy broth (TSB; 30 g Bacto tryptic soy broth l⁻¹), 1/10 strength TSB broth, Luria–Bertani (LB) broth, 1/10 strength LB broth, R2A medium (Reasoner & Geldreich, 1985), GY medium (50 g glucose and 10 g yeast extract l⁻¹) and 1/2, 1/5 and 1/10 strength GY medium. Gram reactions were performed as described by Gerhardt *et al.* (1994) using exponential-growth-phase cells grown on D-GY medium. Endospore formation was detected by malachite green staining. Cell motility was determined by transmission electron microscopy (H-600; Hitachi) after negative staining with 1% (w/v) phosphotungstic acid. Gliding motility and growth under anaerobic conditions were examined according to the methods of Dong & Cai (2001). Growth was determined at 4, 11, 13.5, 14, 15, 17.5, 19, 20, 21, 22.5, 23.5, 25, 27, 29, 30, 31, 33, 36, 37, 41, 45.5, 47, 50, 57 and 60 °C using a TN3F temperature-gradient incubator (Advantec). Growth was tested at pH 2.1, 3.1, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.1, 8.5, 9.0, 10.0 and 11.0 in D-GY broth adjusted by the addition of 5 M NaOH or HCl solutions and verified after autoclaving. Tolerance to NaCl was examined in D-GY broth supplemented with 0, 0.5, 1, 2 and 5% (w/v) NaCl. Tests for catalase and oxidase activities, hydrolysis of casein, starch and Tweens 20 and 80 were performed according to the methods of Dong & Cai (2001). Formation of hydrogen sulfide from thiosulfate was detected using lead acetate paper with the GPY medium as described by Xie & Yokota (2005). Strain VA24^T and *F. aurantia* DSM 6220^T were characterized biochemically by using API 20 NE, API 50 CH and API ZYM identification systems (bioMérieux) at 30 °C.

Strain VA24^T was strictly aerobic. Cells of strain VA24^T were oxidase-positive and catalase-negative and motile by means of a subpolar flagellum (Supplementary Fig. S2). Detailed physiological and biochemical characteristics of strain VA24^T are provided in the species description and differential properties of its closest relatives, *F. aurantia* DSM 6220^T, *D. ginsengisoli* Gsoil 3046^T, *Dyella japonica* DSM 16301^T and *Rhodanobacter spathiphylli* B39^T, are listed in Table 1.

For the analysis of cellular fatty acids, cells of strain VA24^T and *F. aurantia* DSM 6220^T were grown on D-GY agar at 30 °C for 2 days. The fatty acids were extracted, methylated and analysed by the Sherlock Microbial Identification System (MIDI) following the manufacturer's instructions. Isoprenoid quinones were extracted and purified according to the method of Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989). Polar lipids of strain VA24^T and *F. aurantia* DSM 6220^T were extracted by following the modified method of Kamekura (1993). Cells were harvested by centrifugation, washed and resuspended in water. The polar lipids were extracted by mixing the cell suspension with methanol/chloroform (2:1). After shaking (at 200 r.p.m.) for 4–5 h, the mixture was filtered and the filtrate was transferred to separatory funnels with a mixture of water and chloroform (1:1) at 4 °C. The chloroform fraction was harvested and dried and the residue was dissolved in 0.5 ml chloroform and separated by two-dimensional TLC on Merck Kieselgel 60-HPTLC plates as described by Ross *et al.* (1985). Phospholipids were detected with Zinzadze reagent (Dittmer & Lester, 1964) immediately without heating.

The predominant isoprenoid quinone of strain VA24^T was Q-8, which was similar to that of *F. aurantia* DSM 6220^T, the type species of the genus *Frateuria*. The major phospholipids of strain VA24^T were phosphatidylethanolamine and phosphatidylmonomethylethanolamine and the minor polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol (Supplementary Fig. S3a). The phospholipid profile of strain VA24^T was the same as that of *F. aurantia* DSM 6220^T (Supplementary Fig. S3b). The

Table 1. Phenotypic characteristics that differentiate strain VA24^T from *F. aurantia* IFO 3245^T and other related taxa

Taxa: 1, strain VA24^T; 2, *F. aurantia* DSM 6220^T; 3, *Dyella ginsengisoli* Gsoil 3046^T (Jung *et al.*, 2009); 4, *Dyella japonica* DSM 16301^T (Xie & Yokota, 2005; Kim *et al.*, 2006); 5, *Rhodanobacter spathiphylli* B39^T (De Clercq *et al.*, 2006). Strain VA24^T and *F. aurantia* DSM 6220^T were tested in parallel with API 20 NE, API 50 CH and API ZYM systems in this study; all other data from Swings *et al.* (1980, 1984). All strains are positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-B1-phosphohydrolase and acid phosphatase activities. All strains are negative for indole production, arginine dihydrolase, lipase (C14), trypsin, α -chymotrypsin and β -glucuronidase activities, assimilation of D-malate, L-arabinose, caprate, D-mannitol, adipate, citrate and phenylacetate and production of acid from glycerol, m-erythritol, D-arabinose, L-xylose, D-adonitol, methyl β -D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, lactose, melibiose, sucrose, inulin, melezitose, raffinose, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, L-fucose, D- and L-arabitol, gluconate and 2-ketogluconate. +, Positive; –, negative; (+), weakly positive; ND, no data; Y, yellow; BY, brown–yellow.

Characteristic	1	2	3	4	5
Colony colour	Y	BY	Y	Y	Y
Flagella	+	+	+	+	–
Optimum temperature for growth (°C)	33	30	30	25–30	35
Oxidase activity	+	–	+	–	+
Catalase activity	–	+	+	+	+
Biochemical characteristic					
(API 20 NE)					
Nitrate reduction	–	–	–	+	–
Urease activity	–	+	–	–	–
Aesculin hydrolysis	+	–	+	–	+
Gelatin hydrolysis	–	–	+	–	+
Assimilation of (API 20 NE):					
D-Glucose	+	+	+	+	–
D-Mannose	+	–	+	+	–
N-Acetyl-D-glucosamine	+	–	+	+	–
Maltose	–	–	+	+	–
Gluconate	+	–	–	–	–
Enzyme activities (API ZYM)					
Esterase (C4)	–	+	–	+	+
Cystine arylamidase	–	–	+	–	+
α -Galactosidase	–	–	+	–	–
β -Galactosidase	–	–	+	–	–
α -Glucosidase	–	–	+	+	–
β -Glucosidase	–	–	+	+	+
N-Acetyl- β -glucosaminidase	+	–	+	–	–
α -Mannosidase	–	–	+	–	–
α -Fucosidase	–	–	+	–	–
Acid production from					
(API 50 CH):					
L-Arabinose	+	+	–	+	–
D-Galactose	+	+	–	+	–
D-Glucose	+	+	+	+	–
Maltose	(+)	–	–	+	(+)
Trehalose	(+)	–	–	+	–
Aesculin	+	–	+	+	(+)
D-Fucose	+	+	+	+	–
D-Ribose	–	+	–	–	–
D-Mannose	–	+	–	+	–
D-Xylose	–	+	–	–	–
D-Fructose	–	–	–	+	–
myo-Inositol	–	+	–	–	–
Arbutin	–	–	+	–	–
Salicin	–	–	–	–	(+)
Cellobiose	–	–	–	+	–
Starch	–	–	–	+	(+)
5-Ketogluconate	–	–	–	–	+
DNA G + C content (mol%)	67.4	63.5	66.6	63.4–64.0	67.7

major cellular fatty acids of strain VA24^T were iso-C_{15:0} (14.9%), iso-C_{17:1ω9c} (14.1%), iso-C_{17:0} (10.8%) and iso-C_{16:0} (10.3%). The fatty acids iso-C_{15:0}, iso-C_{17:1ω9c} and iso-C_{17:0} were also predominant in *F. aurantia* DSM 6220^T when grown under the same conditions but were present in different proportions. The minor fatty acids of strain VA24^T, however, were different from those of *F. aurantia* DSM 6220^T and included some hydroxyl fatty acids. Detailed cellular fatty acid compositions of strain VA24^T and *F. aurantia* DSM 6220^T are provided in Supplementary Table S1.

The DNA G + C contents of strain VA24^T and *F. aurantia* DSM 6220^T, determined by thermal denaturation (Marmur & Doty, 1962) using *Escherichia coli* K-12^T as reference, were 67.4 mol% and 63.5 mol%, respectively.

Strain VA24^T showed phenotypic and chemotaxonomic properties typical of those of the genus *Frateuria*; it contained Q-8 as major isoprenoid quinone, showed similar phospholipid and cellular fatty acid profiles to the type species *F. aurantia* and produced hydrogen sulfide. Despite the fact that strain VA24^T shared 96.9% 16S rRNA gene sequence similarity to both *F. aurantia* IFO 13330^T and *D. ginsengisoli* Gsoil 3046^T, the phylogenetic trees, based on 16S rRNA gene sequence analysis, showed that strain VA24^T and *F. aurantia* IFO 13330^T formed a phylogenetic lineage distinct from *D. japonica* XD53^T and other type species of the genus *Dyella*. Furthermore, species of the genus *Dyella* did not produce hydrogen sulfide. This evidence suggested that strain VA24^T was a member of the genus *Frateuria* and not the genus *Dyella*. It was also clear, however, that a range of phenotypic characteristics differentiated strain VA24^T from *F. aurantia* DSM 6220^T (Table 1), currently the only described species of the genus *Frateuria*, such as the colony colour, the minor fatty acid composition and the ability to produce urease and hydrolyse casein and aesculin. To further distinguish strain VA24^T from *F. aurantia* DSM 6220^T, DNA–DNA hybridization was performed using the thermal denaturation and renaturation method of De Ley *et al.* (1970) modified by Huß *et al.* (1983). Results showed that the DNA–DNA relatedness of strain VA24^T to *F. aurantia* DSM 6220^T was 15.8 ± 2.3% (SD of three replicates), which was significantly lower than 70%, the recommended threshold value for the delineation of genomic species (Wayne *et al.*, 1987). On the basis of its phylogenetic and phenotypic properties, strain VA24^T represents a novel species of the genus *Frateuria*, for which the name *Frateuria terrea* sp. nov. is proposed.

Emended description of the genus *Frateuria* Swings *et al.* 1980

Characteristics of the genus *Frateuria* are as described by Swings *et al.* (1980, 1984), with the following modifications. The oxidase activity is positive or negative. Cells are motile by means of a polar or subpolar flagellum. The predominant fatty acids (>10% of total fatty acids) are iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1ω9c}. The phospholipid pattern contains phosphatidylethanolamine and phosphatidylmonomethyl-

ethanolamine and may contain diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The G + C content of the DNA is 62–67.4 mol%.

Description of *Frateuria terrea* sp. nov.

Frateuria terrea (ter're.a. L. fem. adj. *terrea* of earth).

Cells are strictly aerobic, Gram-reaction-negative, heterotrophic, oxidase-positive and catalase-negative, rod-shaped, 0.3–0.5 × 2.0–4.0 µm and motile by means of a subpolar flagellum. Colonies on diluted glucose-yeast extract agar are circular, entire and yellow. No growth is detected on TSB agar, 1/10 strength TSB agar, LB agar, 1/10 strength LB agar, R2A agar, GY agar or 1/2 strength GY agar, but growth does occur on 1/5 and 1/10 strength GY agar. Growth occurs at 19–36 °C (optimum 33 °C), pH 5.0–9.0 (optimum pH 6.5–8.0) and in the presence of 0–1% NaCl (optimum 0%) and is inhibited by 3% NaCl. Nitrate is not reduced. Casein and aesculin are hydrolysed but gelatin, starch and Tweens 20 and 80 are not. Cells produce hydrogen sulfide weakly. Urease and arginine dihydrolase activities are absent. Indole is not produced. Assimilates D-glucose, D-mannose, gluconate and N-acetyl-D-glucosamine but not L-arabinose, D-mannitol, maltose, caprate, adipate, malate, citrate or phenylacetate. In the API 50 CH test system, acids are produced from D-glucose, D-galactose, L-arabinose, maltose, trehalose and D-fucose; all the other API 50 CH test results are negative. In the API ZYM system, tests are positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, N-acetyl-β-glucosaminidase and naphthol-AS-B1-phosphohydrolase activities but are negative for esterase (C4), lipase (C14), trypsin, α-chymotrypsin, cystine arylamidase, α-galactosidase, α-glucosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities. The major phospholipids are phosphatidylethanolamine and phosphatidylmonomethylethanolamine and the minor phospholipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The major cellular fatty acids are iso-C_{15:0}, iso-C_{17:1ω9c}, iso-C_{17:0} and iso-C_{16:0}. The predominant isoprenoid quinone is Q-8. The DNA G + C content of the type strain is 67.4 mol%.

The type strain, VA24^T (=CGMCC 1.7053^T =NBRC 104236^T) was isolated from forest soil of the Changbai mountains in north-east China.

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