**Tangfeifania diversioriginum** gen. nov., sp. nov., a representative of the family *Draconibacteriaceae*

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A novel Gram-stain-negative, facultatively anaerobic, catalase- and oxidase-positive, non-motile and pink-pigmented bacterium, designated G22ᵀ, was isolated from Gahai, a saltwater lake in Qinghai province, China. Optimal growth occurred at 33–35°C, pH 7.0–7.5, and in the presence of 2–4% (w/v) NaCl. The DNA G+C content was 40.0 mol%. The major polar lipids were phosphatidylethanolamine and three unknown lipids. The predominant cellular fatty acids were iso-C₁₅:₀, anteiso-C₁₅:₀, iso-C₁₇:₀ 3-OH and iso-C₁₅:₀ 3-OH, and MK-7 was the main respiratory quinone. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain G22ᵀ fell within the class *Bacteroidia*. Its closest phylogenetic neighbour was the recently described species *Draconibacterium orientale*, the sole member of the family *Draconibacteriaceae*, with merely 90.04% sequence similarity. On the basis of phenotypic, chemotaxonomic and phylogenetic evidence observed, a novel species in a new genus, *Tangfeifania diversioriginum* gen. nov., sp. nov., is proposed within the family *Draconibacteriaceae*. The type strain is G22ᵀ (=CICC 10587ᵀ =DSM 27063ᵀ).

At the time of writing, the class *Bacteroidia* (phylum *Bacteroidetes*) comprises seven families: *Bacteroidaceae*, *Marinilabiliaceae*, *Porphyromonadaceae*, *Prevotellaceae*, *Rikenellaceae*, *Prolixibacteraceae* and *Draconibacteriaceae*. During the last decade, many novel taxa belonging to this class have been described, of which the phylogenetic placement for many is still unresolved, such as the genera *Marinilimum* and *Phocaeicola* (Al Masalma et al., 2009; Na et al., 2009; Ruvira et al., 2013). By contrast, the classification of certain taxa has been re-evaluated, resulting in a clarification of many outstanding problems. For instance, the misclassified [*Cytophaga* *fermentans* has recently been reclassified as *Saccharicrinis fermentans* within the family *Marinilabiliaceae* (Yang et al., 2014). Likewise, the genera *Prolixibacter* and *Sunxiqinia* (Holmes et al., 2007; Qu et al., 2011; Takai et al., 2013), which represent deep lineages in the phylum that were unable to be classified systematically for many years, were re-evaluated and transferred to the family *Prolixibacteraceae* (Huang et al., 2014). Recently, another novel family, *Draconibacteriaceae*, was created to accommodate the newly described genus *Draconibacterium* (Du et al., 2014). In this paper, we report the characterization of a novel species in a new genus within the family *Draconibacteriaceae*, for which we propose the name *Tangfeifania diversioriginum* gen. nov., sp. nov.

Strain G22ᵀ was isolated from a sediment sample collected from Gahai, a saltwater lake (35 g Na⁺ 1⁻¹, 100° 32′ 33″ E 36° 59′ 50″ N) located in Qinghai Province, China. The sediment sample was treated with an enrichment culture technique described by Du et al. (2014), except that the plates were incubated at 28°C for 2 weeks. Subsequently, a pink colony was isolated and purified through repeated streaking on marine agar 2216 (MA; Difco) at 33°C. Pure cultures were stored at −80°C in 20% (v/v) glycerol or maintained as lyophilized pellets in vacuum-sealed glass vials stored at 4°C.

Cell morphology was observed using light microscopy (E600; Nikon). Catalase activity was determined by observing bubble production in 3% (v/v) H₂O₂ solution and oxidase activity was determined using an oxidase reagent (bioMérieux). Gliding motility was examined by using oil-immersion phase-contrast microscopy (AX70; Olympus) as described by Bowman (2000). The optimal growth temperature and pH and tolerance of NaCl were studied as

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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain G22ᵀ is J0683777.

Three supplementary figures are available with the online Supplementary Material.
described previously (Du et al., 2014). Gram staining, reduction of nitrate, the oxidation–fermentation test and tests for degradation of agar, starch, CM-cellulose, alginate and Tween 80 were performed and evaluated by using MA medium as described by Dong & Cai (2001). Additional biochemical tests were carried out by using API 20E, API ZYM strips and API 50 CHB fermentation kits (bioMérieux) and Biolog GEN III microplates according to the manufacturers’ instructions, except that the NaCl concentration was adjusted in all tests to 3%. Antimicrobial susceptibility testing was performed by the disc diffusion method, as described by Jorgensen et al. (1999), using antibiotic-impregnated discs (Tianhe) on MA incubated at 33 °C for 7 days. Results of these phenotypic tests are given in the species description and in Table 1.

Fatty acid compositions were determined as described by Sasser (1990) by using the Microbial Identification System (Microbial ID). Cultures for fatty acid analysis were incubated in MB at 33 °C for 7 days. Isoprenoid quinones were extracted, purified and analysed by HPLC, as described by Hiraiishi et al. (1996). Polar lipids analysis was carried out by the Identification Service, Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

The predominant cellular fatty acids (>5%) of strain G22T were iso-C15:0 (32.6%), anteiso-C15:0 (15.4%), iso-C17:0 3-OH (14.6%) and iso-C15:0 3-OH (5.4%), which was similar to those seen in Draconibacterium orientale except that G22T possessed more iso-C15:0 3-OH and less iso-C17:0 2-OH.

The major polar lipids were phosphatidylethanolamine and three unknown lipids. In addition, moderate to minor amounts of glycolipid, aminolipid, two unknown phospholipids and another unknown lipid were detected (Fig. S1, available in the online Supplementary Material). The main respiratory quinone was MK-7. By comparison, D. orientale also contains phosphatidylethanolamine and an unknown lipid as the major polar lipids, and MK-7 as the main respiratory quinone.

DNA was extracted and purified using a genomic DNA extraction kit (Tiangen) following the manufacturer’s protocol. The G+C content of DNA was determined by HPLC (Mesbah et al., 1989) and found to be 40.0 mol%. The 16S rRNA gene was amplified by PCR with universal primers 27f and 1492r (Qu et al., 2011). The amplified gene was cloned into pGM-T vector (Tiangen) and recombinant plasmids were reproduced in Escherichia coli DH5α cells. Sequencing reactions were carried out using an ABI BigDye 3.1 Sequencing kit (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). The resulting 16S rRNA gene sequence was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm; in addition, the EzTaxon-e database was queried in order to identify the most similar 16S rRNA gene sequences from verified type strains (Kim et al., 2012). The sequence was aligned via CLUSTAL X (version 1.81) (Thompson et al., 1997) with related sequences retrieved from the databases, and the alignments were edited manually. A phylogenetic tree was reconstructed using the neighbor-joining (NJ) method implemented in MEGA version 6 (Tamura et al., 2013). The maximum-likelihood (ML) method, as implemented in MEGA 6, and the maximum-parsimony (MP) method, as implemented in PAUP (Swofford, 2002), were also used to reconstruct phylogenetic trees in order to determine the phylogenetic placement of the novel isolate and reference species on the tree. The resultant tree topologies generated from all three methods were evaluated by bootstrap analysis based on 1000 replicates.

The nearly complete 16S rRNA gene sequence (1446 nt) of strain G22T was obtained. From the results of initial 16S rRNA gene sequence analysis by BLAST, some uncultured bacterial clones were found showing 93–99% sequence similarity to G22T. However, those studies (Alain et al., 2006; Liu et al., 2009; Chang et al., 2012; Harris et al., 2013;)}

Table 1. Differential characteristics of strain G22T and related genera of the class Bacteroidia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Saltwater lake</td>
<td>Marine</td>
<td>Marine</td>
<td>Marine</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Pink</td>
<td>Light pink to tawny</td>
<td>Ivory or brownish ivory/ unpigmented</td>
<td>White</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indole production</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth at:</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>4 °C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>15 °C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>pH 5.5</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of starch</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Respiratory quinones</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7, MK-7(H6)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>40.0</td>
<td>42.0</td>
<td>36</td>
<td>44.9</td>
</tr>
</tbody>
</table>
Zhang et al., 2013) merely discovered the existence of the novel 16S rRNA gene sequences with probable distribution in different environments. To our knowledge, no bacterial organisms belonging to a monophyletic lineage with strain G22T have been isolated previously. The EzTaxon-e analysis revealed that strain G22T was most closely related to D. orientale, with 90.04% sequence similarity, and more distantly related to the representative members within the families Prolixibacteraceae and Marinilabiliaceae and the incertae sedis genus Marinifilum, with sequence similarities of 84.58–88.05%. Such low levels of 16S rRNA gene sequence similarity between strain G22T and its nearest neighbours indicate that the isolate represents a novel genus. As shown in the NJ tree (Fig. 1), strain G22T is a member of the class Bacteroidia and forms a distinct line of descent within the family Draconibacteriaceae. The ML and MP tree topologies were similar to the NJ tree topology (Figs S2 and S3).

In addition to phylogenetic distance, strain G22T can be distinguished from its closest relatives by a number of chemotaxonomic and phenotypic features (Table 1). It is pertinent to note that strain G22T can be readily distinguished from members of the genera Draconibacterium and Marinifilum by the ability of members of the latter two genera to produce indole, whereas strain G22T is unable to do so. Members of the genus Draconibacterium are also able to grow at pH 5.5 whereas strain G22T cannot. In addition, G22T shows catalase activity, whereas members of the genus Marinifilum do not.

Strain G22T also displays a number of differences from members of the genera Sunxiuqinia, Prolixibacter and Mangrovibacterium. Major distinctive properties of the genus Sunxiuqinia include its inability to grow at 15°C and at pH 5.5 as well as its ability to hydrolyse starch and gelatin. The genus Prolixibacter contains MK7 and MK-7(H6) as predominant menaquinones, whereas G22T displays only MK-7. Although the genus Mangrovibacterium contains MK-7 as a component of respiratory quinone, it also has MK-7(H2), MK-7(H6) and MK-9, which is distinct from strain G22T. In addition, the members of the family Marinilabiliaceae display gliding motility, whereas this is not observed for strain G22T.

In view of the results of our polyphasic chemotaxonomic analysis, we propose that strain G22T be assigned to a novel species of a new genus within the family Draconibacteriaceae, for which the name Tangfeifania diversioriginum gen. nov., sp. nov. is proposed. A detailed description of the morphological, physiological, biochemical and chemotaxonomic characteristics is given in the genus and species descriptions.
Description of Tangfeifania gen. nov.

Tangfeifania (Tang. fei.fan’i.a. N. L. fem. n. Tangfeifania named in honour of Tang Feifan (1897–1958), a Chinese microbiologist who was the first scientist to successfully isolate and culture Chlamydia trachomatis).

Cells are Gram-stain-negative, straight or slightly curved rods, non-endospore-forming, facultatively anaerobic and non-motile. The major polar lipids are phosphatidylethanolamine and three unknown lipids. The predominant cellular fatty acids are iso-C₁₅:₀ anteiso-C₁₅:₀ and iso-C₁₇:₀ 3-OH. MK-7 is the main respiratory quinone. The type species is Tangfeifania diversioriginum.

Description of Tangfeifania diversioriginum sp. nov.

Tangfeifania diversioriginum (di. ver.si.o.rí.gi.num. L. adj. diversus diverse, different; orí.go -inis origin; N. L. gen. pl. n. diversioriginum of different origins).

Displays the following properties in addition to those described for the genus. Cells are approximately 0.25–0.3 μm wide and 2.3–4.3 μm long. Surface colonies on MA are pink, circular, convex, entire, smooth, opaque and about 0.5 mm in diameter after 7 days of incubation at 33 °C. Catalase- and oxidase-positive. Growth occurs at 25–40 °C, pH 6.5–8.5 and in the presence of 1–8% (w/v) NaCl, with optimum growth between 33 and 35 °C, at pH 7.0–7.5 and with 2–4% (w/v) NaCl. No growth occurs in the absence of NaCl. Nitrate is not reduced. Oxidation–fermentation test is weakly positive. Starch and Tween 80 are hydrolysed, but agar, alginate and CM-cellulose are not. Results of API 20E assays are positive for tryptophan deaminase, gelatinase and Simmons’ citrate utilization, but negative for ONPG, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, H₂S production, indole production and Voges–Proskauer reaction.

D-Fructose 6-phosphate, D-alanine and glucuronamide are oxidized in Biolog GEN III assays. Acid is produced from D-arabinose, L-arabinose, D-ribose, D-xylene, L-xylene, D-fructose, L-sorbos, asesin, turanose, D-lyxose, D-tagatose and potassium 5-ketogluconate, but not from glycerol, erythritol, D-adonitol, methyl β-D-xlyopyranoside, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, sucrose, trehalose, inulin, melezitose, starch, glycerogen, xylitol, gentiobiose, D-fucose, D-arabitol, L-arabitol, potassium gluconate or potassium 2-ketogluconate. Acid production from D-galactose, D-glucose, D-mannose, N-acetylglucosamine, cellobiose, maltose, lactose, melibiose, raffinose and L-fucose is weakly positive in API 50CH assays. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase and arabinosidase are absent. Cells are resistant to cefalotin, oxacillin, gentamicin, ciprofloxacin, kanamycin, norfloxacin, neomycin, amikacin, polymyxin B and trimethoprim, but sensitive to spectinomycin, erythromycin, midecamycin, acetylsypramycin, clindamy- cin, lincomycin and carbencillin. The predominant cellular fatty acids are iso-C₁₅:₀ anteiso-C₁₅:₀ iso-C₁₇:₀ 3-0H and iso-C₁₅:₀ 3-OH. In addition to the major polar lipids phosphatidylethanolamine and three unknown lipids, moderate to minor amounts of glycolipid, aminolipid, two unknown phospholipids and another unknown lipid are present in the polar lipid profile.

The type strain, G22T (=CICC 10587T =DSM 27063T), was isolated from Ghahai, a saltwater lake located in Qinghai Province, China. The DNA G+C content of the type strain is 40.0 mol%.

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References


