Draconibacterium orientale gen. nov., sp. nov., isolated from two distinct marine environments, and proposal of Draconibacteriaceae fam. nov.

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The taxonomic characteristics of two bacterial strains, FH5T and SS4, isolated from enrichment cultures obtained from two distinct marine environments, were determined. These bacteria were Gram-stain-negative, facultatively anaerobic rods. Growth occurred at 20–40 °C (optimum, 28–32 °C), pH 5.5–9.0 (optimum, pH 7.0–7.5) and in the presence of 1–7 % NaCl (optimum, 2–4 %). The major cellular fatty acids were anteiso-C15:0 and iso-C15:0. Menaquinone 7 (MK-7) was the sole respiratory quinone. The major polar lipids were phosphatidylethanolamine, an unknown phospholipid and an unknown lipid. The DNA G+C contents of strains FH5T and SS4 were both determined to be 42.0 mol%. The results of DNA–DNA hybridization studies indicated that the FH5T and SS4 genomes share greater than 95 % relatedness. The strains formed a distinct phylogenetic line within the class Bacteroidia, with less than 89.4 % sequence similarity to their closest relatives with validly published names. On the basis of physiological and biochemical characteristics, 16S rRNA gene sequences and chemical properties, a novel genus and species, Draconibacterium orientale gen. nov., sp. nov., within the class Bacteroidia, are proposed, with strain FH5T (=DSM 25947T=CICC 10585T) as the type strain. In addition, a new family, Draconibacteriaceae fam. nov., is proposed to accommodate Draconibacterium gen. nov.

The class Bacteroidia was proposed by Krieg (2011) to accommodate Gram-stain-negative, non-spore-forming rods and cocccobacilli that were non-motile or capable of gliding motility. Although most of the members identified in this class are anaerobic, several aerobic and facultatively anaerobic strains have been reported recently. Sunxiuqinia fæciciva and Sunxiuqinia elliptica were isolated from deep sub-seafloor sediment and sediment in a sea cucumber farm, respectively (Qu et al., 2011; Takai et al., 2013). Prolíxisbacter bellarívorsans was found in a marine-sediment fuel cell (Holmes et al., 2007), and Marinífilum fragile and Marinífilum flexuosum were recovered from tidal flat sediment and surface seawater, respectively (Na et al., 2009; Ruvira et al. 2013). Recently, a novel family, ‘Prolíxisbacteracé fam. nov., was proposed to accommodate the genera Sunxiuqinia, Prolíxisbacter and a newly described genus ‘Mariátobacterium’ (Huang et al. 2013). In this study, we report the characterization of two deep-branching strains isolated from a marine sediment sample and a shark gill sample. The novel isolates represent a deep-rooting lineage belonging to the class Bacteroidia which, at present, constitutes a single order, the Bacteroidales. The novel isolate showed closest 16S rRNA gene sequence similarity with members of the established genera Marinífilum and Prolíxisbacter. On the basis of the distinct genotypic and phenotypic properties when compared with members of the most closely related taxon, the novel strain is proposed as representing a novel genus and species for which the name Draconibacterium orientale gen. nov., sp. nov. is proposed.

Strains FH5T and SS4 were isolated from a marine sediment sample from the coast of Weihai, China (122°03’ 44.01”E 37°32’ 01.93”N), and a dead shark (Cetorhinus maximus) that caught by fishermen from the Yellow Sea, China, respectively, using an enrichment culture technique. The medium consisted of the following ingredients in 1000 ml seawater: 0.1 % NH4Cl, 0.2 % CH3COONa, 0.02 % MgSO4.7H2O, 0.02 % yeast extract, 0.02 % peptone. The pH of the medium was adjusted to pH 7.5 and then autoclaved. A 10 % (w/v) NaHCO3 solution was filtered and a 2 % (w/v)
KH$_2$PO$_4$ solution was autoclaved. Each of the two solutions was added to the autoclaved media (10 ml per litre). Incubation was performed at 25 °C for 21 days with a 500 ml sealed glass bottle (filled with medium and 10 g sample was added). The bottle was shaken twice a day and kept sealed during the incubation. The anaerobic enrichment cultures were diluted with 9 ml sterile seawater and spread onto 2216 marine agar (MA; Hopebio). The plates were incubated aerobically at 28 °C for 5 days. Strains FH5$^T$ and SS4 were obtained in pure culture after three successive transfers to fresh MA plates and stored at −80 °C in 20 % (v/v) glycerol.

Routine tests, including Gram staining and agarase, amylase, urease, catalase, gelatinase and oxidase activities, were carried out as described by Smibert & Krieg (1994). Cell morphology and flagella were observed by transmission electron microscopy (Jem-1200; Jeol). Colony morphology was observed on MA after 4 days of incubation at 28 °C. Antibiotic sensitivity was assessed as described by the Clinical and Laboratory Standards Institute (CLSI, 2012): a cell suspension (McFarland standard 0.5) was swabbed over the surface of Iso-Sensitest agar (Oxoid) plates to create a uniform lawn before aseptic placement of antibiotic discs onto the agar surface. Inoculated plates were incubated at 28 °C for up to 7 days. The effects of different temperatures on growth were assessed on MA plates with incubation at 20 °C, 25 °C, and 30 °C for 21 days with a 500 ml sealed glass bottle (filled with medium and 10 g sample was added). The bottle was shaken twice a day and kept sealed during the incubation. The anaerobic enrichment cultures were diluted with 9 ml sterile seawater and spread onto 2216 marine agar (MA; Hopebio). The plates were incubated aerobically at 28 °C for 5 days. Strains FH5$^T$ and SS4 were obtained in pure culture after three successive transfers to fresh MA plates and stored at −80 °C in 20 % (v/v) glycerol.

DNA was extracted and purified using a bacteria genomic DNA Mini kit (TaKaRa Bio) following the manufacturer’s protocol. The gene encoding 16S rRNA was amplified by PCR with two universal primers, 27f and 1492r (Jordan et al., 2007). The purified PCR product was ligated to the vector pMD 18-T (TaKaRa Bio) and cloned according to the manufacturer’s instructions. 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 nearly complete 16S rRNA gene sequence of strains FH5$^T$ and SS4 was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. 16S rRNA gene sequences of several closely related species were first aligned by using CLUSTAL X (version 1.81) (Thompson et al., 1997), and then the alignments were corrected manually. A phylogeny of the taxa was inferred by using the neighbour-joining method implemented in the computer program MEGA version 5.2.1 (Tamura et al., 2011), and statistical reliability was assessed from 1000 bootstrap replicates.

For DNA–DNA hybridization, genomic DNA from all strains was extracted from cultured cells using the Qiagen DNAeasy Midi kit (Qiagen) and subsequently quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen). The DNA G + C content (mol%) of the study strains was determined using the method of Gonzalez & Saiz-Jimenez (2002). The type strains Marinifilum fragile NRRL B-59702$^T$, Wandonia haliotis NRRL B-59700$^T$, Escherichia coli NRRL B-3054, Deinococcus radiodurans NRRL B-2906$^T$, Streptosporangium roseum NRRL 2505$^T$, Bacillus subtilis subsp. subtilis NRRL NRS-1315$^T$, Streptomyces coelicolor NRRL B-2812$^T$ and Clostridium amino philum NRRL B-23502$^T$ were used as reference taxa to generate a calibrated regression equation from which G + C mol% could be determined. Subsequent DNA–DNA hybridization experiments were conducted using the fluorometric method of Gonzalez & Saiz-Jimenez (2005). Prior to analysis, isolated DNA was renatured at the optimum temperature for renaturation ($T_{on}$) as described by De Ley et al. (1970). Renaturation conditions consisted of a denaturation step of 99 °C for 10 min, followed by an annealing period of 8 h at $T_{on}$, which was approximated according to the method of De Ley et al. (1970) using the...
equation $T_m = 0.51(\% G + C) + 47.0$. This was followed by progressive 60 min steps, each at 10 °C below the previous one, until room temperature was reached. Renatured DNA was diluted to 10 ng ul$^{-1}$ in 0.1 × SSC, pH 8.0, containing 1 × SYBR Green (Molecular Probes). Fluorescence measurements were recorded with a Rotor-gene Q (Qiagen) instrument. The thermal profile consisted of a 15 min hold at 25 °C followed by a 25–99 °C ramp in 0.2 °C steps with a 5 s hold. The mean of three or more replicates was used to determine the $T_m$ (melt temperature). $T_m$ values of DNA from homologous and hybrid DNA solutions were calculated as the temperatures corresponding to a 50 % decrease in fluorescence. Percentage similarity was estimated based on the $\Delta T_m$ values (Gonzalez & Saiz-Jimenez, 2005).

After 3 weeks of enrichment culture at 25 °C, strains FH5$^T$ and SS4 were isolated. The two isolates exhibited almost identical characteristics and both produced colonies on 2216 MA that were circular with entire edge, light pink to tawny in colour and nearly opaque. Microscopic examination suggested that the cells of the two strains were straight to slightly curved rods, and flagella were not observed. Cells were non-motile and non-endospore-forming. Both strains contained MK-7 (100 %) as the sole menaquinone. The predominant cellular fatty acids of strain FH5$^T$ were anteiso-C$_{15}$ : 0 (25.6 %), iso-C$_{15}$ : 0 (17.1 %), C$_{17}$ : 0 2-OH (9.7 %) and iso-C$_{17}$ : 0 3-OH (6.3 %). The complete morphological, physiological and biochemical characteristics of strain FH5$^T$ are given in the species description. The characteristics that differentiate strain FH5$^T$ from related genera of the class Bacteroidia are shown in Table 1.

The DNA G + C contents of strains FH5$^T$ and SS4 were both determined to be 42.0 %. The results of DNA–DNA hybridization studies indicated that the FH5$^T$ and SS4 genomes are highly similar with a $\Delta T_m < 1$ °C, which corresponds to an overall relatedness greater than 95 % based on the method of Gonzalez & Saiz-Jimenez (2005). $\Delta T_m$ values between homologous and hybrid DNA of 5 °C or lower are considered as corresponding to the same microbial species (Rossello-Mora & Amann, 2001; Wayne et al., 1987), indicating that strains FH5$^T$ and SS4 are thus conspecific. We compared these strains to the most closely related strains that we identified, _W. haliotis_ and _Marinifilum fragile_, using the EzTaxon-e 16S rRNA gene sequence database (Kim et al., 2012), and which we could obtain from public culture collections. The results of these analyses suggested that FH5$^T$ and SS4 represent a distinct species from _Marinifilum fragile_ with $\Delta T_m$ values of 7.4 and 8.2 °C, respectively, corresponding to an overall genome relatedness of ~60–65 %. FH5$^T$ and SS4 were also found to represent a distinct species from _W. haliotis_ with $\Delta T_m$ values of 7.4 and 7.6 °C, respectively, suggesting an overall genome relatedness of ~60–65 %.

The 16S rRNA gene analysis placed strain FH5$^T$ within the class _Bacteroidia_. Similarity values calculated for isolate FH5$^T$ indicated a remote relationship (less than 90 % similarity) with members of the class _Bacteroidia_. 16S rRNA gene sequence similarity calculations indicated that strain FH5$^T$ showed the greatest degree of similarity to _Marinifilum fragile_ JC2469$^T$ (GenBank accession no. FJ394546; 89.4 %); other closely related strains produced similar values, including _P. bellariivora_ F2$^T$ (AY918928; 89.0 %) and _Sunxiuqinia elliptica_ DQHS-4$^T$ (GQ200190; 88.4 %). Phylogenetic trees obtained by using the neighbour-joining method (Fig. 1) revealed clear affiliation of the novel isolates to the class _Bacteroidia_, being positioned on a separate branch within this class. This topology was also supported by the minimum-evolution and maximum-likelihood algorithms (data not shown). The _Draconibacterium_ clade was also shown to be a well separated lineage with less than 90 % 16S rRNA gene sequence similarity to the other recognized genera within the class _Bacteroidia_. We therefore propose to create a new genus, namely _Draconibacterium_ gen. nov. Strain FH5$^T$ represents the type strain of the type species, _Draconibacterium orientale_ sp. nov.

The proposed genus _Draconibacterium_ can be readily distinguished from the genus _Marinifilum_ because members of the latter can hydrolyse starch, cannot grow at pH 5.5 and have negative catalase activity; it can be distinguished from the genus _Prolixibacter_ because members of the latter can grow at 4 °C, hydrolyse starch and have MK7(H6) as one of the respiratory quinones. The proposed genus _Draconibacterium_ can be distinguished from the genus _Sunxiuqinia_ by the ability to hydrolyse gelatin, and can be distinguished clearly from the genus _Mangrovibacterium_ because members of the latter contain the respiratory quinones MK-7(H2), MK-7(H6) and MK-9 in addition to MK7. Strains FH5$^T$ and SS4 can also be distinguished from strains belonging to the family _Marinilabiliaceae_ by their lack of gliding motility, and from strains of the families _Bacteroidaceae_, _Rikenellaceae_, _Prevotellaceae_ and _Porphyromonadaceae_ by their marine origin and ability to grow aerobically. Based on the distinct phylogenetic position of the _Draconibacterium_ gen. nov. lineage in the class _Bacteroidia_ and the large degree of divergence revealed through 16S rRNA gene sequence comparisons (strain FH5$^T$ formed a deep branch in class _Bacteroidia_, clearly distinguished from other described families of the class), a novel family, _Draconibacteriaceae_ fam. nov. is proposed.

**Description of Draconibacterium gen. nov.**

_Draconibacterium_ (Dra.co.ni.bac.te.ri.um. L. masc. n. _dra.co.ni._97; L. n. _bac.te.ri._um. L. masc. n. _dra.co.ni._98; L. masc. n. _dra.co.ni._99; L. masc. n. _dra.co.ni._100)

Cells are Gram-stain-negative, straight to slightly curved rods, facultatively anaerobic, non-motile or non-gliding, and non-endospore-forming. Chemo-organotrophic. Oxidase- and...
**Table 1. Differential characteristics of strain FH5ᵀ and related genera of the class Bacteroidia**

Genera: 1, *Draconibacterium* gen. nov. (strain FH5ᵀ); 2, *Marinifilum* (data from Na et al., 2009; Ruvira et al., 2013); 3, *Prolificibacter* (Holmes et al., 2007; Huang et al., 2013); 4, *Sunxiuqinia* (Qu et al., 2011; Takai et al., 2013); 5, *Mangrovibacterium* (Huang et al., 2013); 6, *Marinilabilia* (Krieg, 2011; Shalley et al., 2013); 7, *Bacteroides* (Lan et al., 2006; Robert et al., 2007; Hayashi et al., 2007; Chassard et al., 2008; Ueki et al., 2008, 2011; Nishiyama et al., 2009; Watanabe et al., 2010; Kim et al., 2010; Kitahara et al., 2011, 2012; Krieg, 2011; Sakamoto & Ohkuma, 2013). + , Positive; −, negative; V, variable; ND, no data available; A, aerobic; F, facultatively anaerobic; An, strictly anaerobic.

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<th>7</th>
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<tr>
<td>Pigment</td>
<td>Light pink to tawny</td>
<td>Ivory or brownish ivory/unpigmented</td>
<td>White</td>
<td>White to orange-red</td>
<td>Orange-red</td>
<td>Pink to salmon/ reddish-orange</td>
<td>White to grey*</td>
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<td>O₂ metabolism</td>
<td>F</td>
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<td>F</td>
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<td>−</td>
<td>V</td>
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<td>Catalase</td>
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<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
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<td>+</td>
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<td>Starch</td>
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<td>+</td>
<td>−</td>
<td>−</td>
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<td>+</td>
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<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>−</td>
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<td>Utilization of:</td>
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<td>−</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
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<td>V</td>
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<tr>
<td>Arabinose</td>
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<td>+</td>
<td>−</td>
<td>ND</td>
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<td>+</td>
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<td>Respiratory quinones</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7, MK-7(H6)</td>
<td>MK-7</td>
<td>MK-7, MK-7(H2), MK-7(H6), MK-9</td>
<td>MK-7</td>
<td>MK8, MK-9, MK-10, MK-11, MK12</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>42.0</td>
<td>36</td>
<td>44.9</td>
<td>41.8–43.5</td>
<td>43.28</td>
<td>37–41</td>
<td>36.4–49</td>
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</table>

*Bacteroides faecis* was pale yellow.
†>85 % Negative/positive.
catalase-positive. NaCl is required for growth. The principal menaquinone is MK-7. The predominant cellular fatty acids are anteiso-C15:0 and iso-C15:0. The major polar lipids are phosphatidylethanolamine, an unknown phospholipid and an unknown lipid.

The type species is *Draconibacterium orientale*.

**Description of *Draconibacterium orientale* sp. nov.**

*Draconibacterium orientale* (o.eu.en.ta.'le. L. neut. adj. orientale of or belonging to the east, oriental).

Displays the following properties in addition to those given in the genus description. Cells are 1.3–1.8 μm in length and 0.3–0.5 μm in width. Colonies on marine agar 2216 are light pink to tawny, smooth, circular, convex and 1.0–1.5 mm in diameter after 4 days of incubation at 28 °C. Growth occurs at 20–40 °C and pH 5.5–9.0 and in the presence of 1–7 % (w/v) NaCl. Optimal growth is observed at 28–32 °C, pH 7.0–7.5 and with 2–4 % (w/v) NaCl. No growth is observed without NaCl. Fermentation of glucose occurs under anaerobic conditions with acid production, but no gas. Tween 80 is hydrolysed, but CM-cellulose, starch, sodium alginate and agar are not. In API 20E tests, negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, H2S production and Voges–Proskauer reaction, but positive for gelatinase, tryptophan deaminase, indole production and Simmons’ citrate utilization. Acid is produced from D-arabinose, L-arabinose, D-fructose, D-galactose, D-mannose, trehalose, N-acetylglucosamine, cellobiose, starch, glycogen, melibiose, D-xylose, aesculin ferric citrate, inulin, sucrose, turanose, raffinose, melezitose, gentiobiose, potassium 5-keto-D-gluconate, methyl α-D-glucoside and methyl α-D-mannoside in API 50CHB strips. In API ZYM kits, activities of alkaline phosphatase, esterase lipase (C8), trypsin, x-galactosidase, β-galactosidase, naphthol-AS-BI-phosphohydrolase, x-glucosidase, β-glucosidase, β-fructosidase, D-fructosidase and N-acetyl-β-glucosaminidase are positive and activities of leucine arylamidase and acid phosphatase are weakly positive. Negative for activities of esterase (C4), lipase (C14), valine arylamidase, cysteine arylamidase, chymotrypsin, β-glucuronidase and α-mannosidase. The
following results for carbon source assimilation are positive in Biolog GEN III MicroPlates: trehalose, cellobiose, sucrose, turanose, raffinose, d-fructose, l-fucose, l-rhamnose, d-sorbitol, l-lactic acid, α-ketobutyric acid, N-acetylneuraminic acid, gelatin, l-serine, propionic acid and acetic acid. The sole menaquinone is MK-7. The predominant cellular fatty acids are anteiso-C15:0, iso-C15:0 C17:0 2-0H and iso-C17:0 3-0H. In addition to the major polar lipids in the genus description, aminolipid and one unknown lipid are present in moderate and minor amounts.

The type strain, FH5T (=DSM 25947T=CICC 10585T), was isolated from a sediment sample collected from the coastal area of Weihai, China. The genomic DNA G + C content of the type strain is 42.0 mol%. The type strain is resistant to nalidixic acid (30 μg), cotrimoxazole (25 μg), gentamicin (10 μg), kanamycin (30 μg), tobramycin (10 μg), neomycin (30 μg), polymyxin B (30 μg), amikacin (30 μg) and oxacillin (1 μg), but sensitive to nitrofurantoin (30 μg), acetylsalicycymycin (30 μg), midecamycin (30 μg), vancomycin (30 μg), chloramphenicol (30 μg), rocephin (30 μg), furazolidone (300 μg), novobiocin (30 μg), cephalaxin (30 μg) and rifampicin (5 μg) as determined by antibiotic discs.

**Description of Draconibacteriaceae fam. nov.**

*Draconibacterium* (Dra.co.ni.bac.te.ri.a.ce.ae. N.L. neut. n. Draconibacterium type genus of the family; suff. -aceae ending to denote family; N.L. pl. n. Draconibacteriaceae the Draconibacterium family).

Cells are gram-stain-negative, straight or curved rods, mesophilic, non-motile and non-endospore-forming. Chemoorganotrophic. The major respiratory quinone is MK-7. Phosphatidylethanolamine and at least one lipid are present as major polar lipids. Segregation of these organisms into a new family is justified by their distinct phyletic lineage based on 16S rRNA gene sequences and chemotaxonomic analyses for menaquinone. At present the family only contains the type genus *Draconibacterium*.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


