Sulfitobacter pseudonitzschiae sp. nov., isolated from the toxic marine diatom Pseudo-nitzschia multiseries

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A taxonomic study was carried out on bacterial strain H3T, which was isolated from the toxic marine diatom Pseudo-nitzschia multiseries. Cells of strain H3T were Gram-stain-negative, rod-shaped, non-motile and capable of reducing nitrate to nitrite, but not denitrification. Growth was observed at NaCl concentrations of 1–9 %, pH 6–12 and 10–37 °C. It was unable to degrade aesculin or gelatin. The dominant fatty acids (>10 %) were C18 : 1\(^v7\)/C16 : 0 (summed feature 8) and C16 : 0. The respiratory ubiquinone was Q10. The major lipids were phosphatidylethanolamine, phosphatidylglycerol, an aminolipid and one unknown lipid, and the minor lipids were two phospholipids and three unknown lipids. The G+C content of the chromosomal DNA was 61.7 mol%. 16S rRNA gene sequence comparison showed that strain H3T was related most closely to Sulfitobacter donghicola DSW-25T (97.3 % similarity) and levels of similarity with other species of the genus Sulfitobacter were 95.1–96.9 %. The mean (±SD) DNA–DNA hybridization value between strain H3T and Sulfitobacter donghicola DSW-25T was 18.0 ± 2.25 %. The average nucleotide identity between strain H3T and Sulfitobacter donghicola DSW-25T was 70.45 %. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain H3T formed a separate clade close to the genus Sulfitobacter and was distinguishable from phylogenetically related species by differences in several phenotypic properties. On the basis of the phenotypic and phylogenetic data, strain H3T represents a novel species of the genus Sulfitobacter, for which the name Sulfitobacter pseudonitzschiae is proposed (type strain H3T = DSM 26824T = MCCC 1A00686T).

The genus Sulfitobacter was erected by Sorokin (1995) to accommodate two strains of heterotrophic bacteria isolated from the Black Sea and, at the time of writing, comprised ten recognized species: Sulfitobacter pontiacus (Sorokin, 1995), Sulfitobacter mediterraneus (Pukall et al, 1999), Sulfitobacter brevis (Labrenz et al, 2000), Sulfitobacter delicatus and Sulfitobacter dubius (Ivanova et al, 2004), Sulfitobacter marinus (Yoon et al, 2007a), Sulfitobacter litoralis (Park et al, 2007), Sulfitobacter donghicola and Sulfitobacter guttiformis (Yoon et al, 2007b) and Sulfitobacter porphyrae (Fukui et al, 2014). The current study focuses on a new member of the genus Sulfitobacter, strain H3T, which was isolated from a toxic marine diatom. Accordingly, the aim of the present work was to determine the exact taxonomic position of strain H3T using a polyphasic characterization, including determination of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Strain H3T was isolated from a toxic marine diatom, Pseudo-nitzschia multiseries, obtained from the North Atlantic Ocean. Cultures were maintained in sterilized f/2 medium (Guillard, 1975) at 15 °C with an incident photon...
irradiance of 60 μmol m⁻² s⁻¹ under 12:12 h light/dark periods. To obtain bacterial isolates from algal cultures, 1 ml samples were serially diluted (10-fold dilution, using f/2 medium) and aliquots (0.1 ml) of each dilution were spread onto 2216E agar plates (per litre seawater: 1 g yeast extract, 5 g peptone, 0.1 g ferric phosphate, 10 g agar, pH 7.5; Zobell, 1941) and incubated for 7 days at 25 °C. Individual colonies showing unique traits were picked and purified by successive streaking and restreaking on fresh 2216E agar plates at 25 °C. One of the isolates, designated H3ₜ, has been characterized here.

Genomic DNA was prepared according to the method of Ausubel et al. (1995) and the 16S rRNA gene was amplified by PCR using primers and conditions that have been described previously (Liu & Shao, 2005). 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Phylogenetic trees were reconstructed on the basis of neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and minimum-evolution (Rzhetsky & Nei, 1992, 1993) algorithms using the MEGA 5 program (Tamura et al., 2011) with bootstrap resampling analysis of 1000 replicates.

The nearly full-length 16S rRNA gene sequence (1426 nt) from strain H3ₜ was determined. Phylogenetic analysis of strain H3ₜ indicated that it belonged to the family Rhodobacteraceae (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain H3ₜ formed a separate clade close to the genera Sulfitobacter and Roseobacter. This topology was confirmed in the maximum-likelihood and minimum-evolution trees (Figs S1 and S2, available in the online Supplementary Material). It shared highest 16S rRNA gene sequence similarity with Sulfitobacter donghicola DSW-25ₜ (97.3 %), followed by the type strains of other species of the genera Sulfitobacter (95.1–96.9 %), Shimia (95.6–96.5 %), Tropicibacter (94.9–96.5 %) and Oceanibulbus (96.4 %). Levels of similarity with all other species were below 96.0 %. Strain H3ₜ shared low similarity (both 95.6 %) with the type strains of two species of the genus Roseobacter. In addition, Roseobacter demitrichicans OCh 114T and Roseobacter litoralis OCh 149T shared high similarity with members of the genus Sulfitobacter (95.7–97.5 and 96.0–98.0 %, respectively). The genus Roseobacter also formed a clade close to the genus Sulfitobacter in all three phylogenetic trees, implying that the genus Roseobacter may need to be reclassified within the genus Sulfitobacter.

The draft genome sequences of strain H3ₜ and Sulfitobacter donghicola DSW-25ₜ were determined by Shanghai Majorbio Bio-pharm Technology (Shanghai, China), using Solexa paired-end (500 bp library) sequencing technology. Their genome sequences have been deposited in NCBI under accession numbers JAMD00000000 and JAMC00000000, respectively. DNA–DNA hybridization values among strain H3ₜ and Sulfitobacter donghicola DSW-25ₜ were estimated using the genome-to-genome distance calculator (GGDC2.0) (Auch et al., 2010a, b; Meier-Kolthoff et al., 2013). The mean (± SD) DNA–DNA hybridization value between strain H3ₜ and Sulfitobacter donghicola DSW-25ₜ was 18.0 ± 2.25 %. The average nucleotide identity (ANI) was calculated using the algorithm of Goris et al. (2007) using the EZGenome web service. The ANI value between strain H3ₜ and Sulfitobacter donghicola DSW-25ₜ was 70.45 %, below standard ANI criteria for species identity (95–96 %; Richter & Rossello-Móra, 2009). These data indicated that strain H3ₜ represents a novel species of the genus Sulfitobacter.

Tests for Gram-staining, catalase, oxidase and lipase (Tween 80) activity, temperature and pH range for growth, NaCl tolerance, general cell morphology and electron microscopy (JEM-1230, JEOL, Japan) were performed as previously described (Lai et al., 2009). Gliding motility and production of flexirubin-type pigments were tested as recommended by Bernardet et al. (2002) and degradation of agar and starch were tested using the methods described by Cowan & Steel (1993). Other biochemical tests were performed using API 20NE, API ZYM (bioMérieux) and Biolog GN2 strips according to the manufacturers’ instructions, except that the NaCl concentration was adjusted in all tests to 3.0 %. The type strains of species of the genus Sulfitobacter were tested at the same time for comparison. For morphological and biochemical characterization, strain H3ₜ was cultivated on marine agar 2216 (MA; BD) at 28 °C unless otherwise indicated. Nine type species of the genus Sulfitobacter were used as reference strains and cultured under the same conditions as strain H3ₜ. Sulfitobacter porphyrae was described in 2014 at the time of this writing, so it was not included in this study for comparative purposes.

Strain H3ₜ was a Gram-stain-negative, rod-shaped (0.6–0.7 × 1.8–2.0 μm in size) (Fig. S3), non-motile, moderately halophilic, non-spore-forming bacterium. The new isolate was positive for oxidase (weak) and catalase activity, but negative for amylase and agarase activity. Other physiological and biochemical characteristics of strain H3ₜ are given in the species description.

Fatty acids in whole cells grown on MA at 28 °C for 48 h were saponified, methylated and extracted using the standard MIDI protocol (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed by GC (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). As shown in Table S1, the major fatty acids (>10 %) in strain H3ₜ were C₁₈:1ω7c/ω6c (summed feature 8) (69.1 %) and C₁₆:0 (16.9 %). In addition, the major fatty acids (>10 %) of all recognized Sulfitobacter species were summed feature 8 (C₁₈:1ω7c/ω6c) (59.5–88.4 %). The fatty acid profile of strain H3ₜ was in good agreement with those of members of the genus Sulfitobacter, but it can be differentiated from other species by the percentage of C₁₈:1ω7c 11-methyl, C₁₆:0 C₁₈:0 C₁₀:0 3-OH and summed feature 8 (C₁₈:1ω7c/ω6c).

Analyses of respiratory quinones and polar lipids were carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ).
The respiratory quinone of strain H3T was ubiquinone Q10 (100%), which was in accordance with other members of the genus Sulfitobacter (Yoon et al., 2007b; Fukui et al., 2014). As shown in Fig. S4, the major polar lipids of strain H3T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), an aminolipid (AL) and one unknown lipid (L3); the minor lipids included two phospholipids and three unknown lipids. As shown in Table 1, phosphatidylethanolamine and phosphatidylglycerol were also the major lipids in its closest related type strain, Sulfitobacter donghicola DSW-25T and most species of the genus Sulfitobacter, but these taxa can be differentiated by the presence and absence of phosphatidylcholine. An aminolipid was the major lipid in strain H3T and Sulfitobacter mediterraneus DSM 12244T.

The G + C content of the chromosomal DNA of strain H3T was determined according to its draft genome sequence. The chromosomal DNA G + C content of strain H3T was 61.7 mol%, which is within the range reported for the genus Sulfitobacter (55.0–63.7 mol%) (Table 1).

The results of the phylogenetic analysis, phenotypic analysis and chemotaxonomic studies presented above...
Table 1. Differential phenotypic characteristics between strain H3T and closely related species

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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>Phosphatidylglycerol</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diposphatidylglycerol</td>
<td>ND</td>
<td>W</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Aminolipid</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td>Phospholipid</td>
<td>W</td>
<td>+</td>
<td>ND</td>
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</table>
support the conclusion that strain H3<sup>T</sup> should be assigned to the genus Sulfitobacter. However, strain H3<sup>T</sup> could be distinguished from all validly named members of the genus Sulfitobacter by several phenotypic characteristics (Table 1). Given its low DNA–DNA hybridization value (18.0 ± 2.25 %) when compared with its closest relative, the isolate cannot be assigned to any previously recognized species. Therefore, strain H3<sup>T</sup> represents a novel species of the genus Sulfitobacter, for which the name Sulfitobacter pseudonitzschiae sp. nov. is proposed.

**Description of Sulfitobacter pseudonitzschiae sp. nov.**

*Sulfitobacter pseudonitzschiae* (pseu.do.nitz’schi.ae. N.L. gen. n. pseudonitzschiae referring to the diatom *Pseudo-nitzschia multiseri*es, from which the type strain was isolated).

Cells are Gram-stain-negative, rod-shaped, non-motile, 1.8–2.0 μm long and 0.6–0.7 μm wide. Positive for oxidase (weak) and catalase, urea activity, reduction of nitrate to nitrite, fermentation of d-glucose (weak), and utilization of d-mannitol, potassium gluconate, adipic acid, malic acid, trisodium citrate, l-arabinose (weak) and phenylacetic acid (weak), but negative for hydrolysis of starch, denitrification, indole production, arginine dihydrolase, β-glucosidase (aesculin hydrolysis), gelatin hydrolysis, β-galactosidase, and utilization of d-mannose, N-acetylglucosamine, maltose and capric acid. On MA, strain H3<sup>T</sup> forms smooth white colonies with regular edges that are 2–3 mm in diameter after 72 h of incubation at 25 °C. With API ZYM test strips, positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine aminopeptidase and valine aminopeptidase; weakly positive for cystine aminopeptidase, lipase (C14) and naphthol-AS-BI-phosphoamidase; and negative for N-acetyl-β-glucosaminidase, trypsin, α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase and β-glucuronidase. Among 95 substrates in the Biolog GN2 system, positive for fermentation of acetic acid, bromosuccinic acid, cis-aconitic acid, citric acid, DL-lactic acid, D-alanine, D-arabitol, D-fructose, D-gluconic acid, D-mannitol, D-sorbitol, glycerol, glycy1 L-aspartic acid, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, L-histidine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine, methyl pyruvate, monomethyl succinate, propionic acid, succinic acid, succinamic acid, Tween 40, Tween 80, urocanic acid, α-D-glucose, α-hydroxybutyric acid, α-ketoglutaric acid and β-hydroxybutyric acid; and weakly positive for DL-carnitine, D-psicose, formic acid, glycy1 L-glutamic acid, itaconic acid, L-aspartic acid, ρ-hydroxyphenylacetic acid, ω-ketobutyric acid, γ-aminobutyric acid and γ-hydroxybutyric acid. The dominant fatty acids (>10 %) are summed feature 8 (C<sub>18:1</sub>ω7c,d06c) and C<sub>16:0</sub>. The sole respiratory quinone is ubiquinone is Q10. The major lipids are phosphatidylethanolamine, phosphatidyglycerol, an aminolipid and one unknown lipid; minor lipids are two phospholipids and three unknown lipids. Table 1 shows characteristics used to distinguish strain H3<sup>T</sup> from related species of the genus Sulfitobacter.

The type strain, H3<sup>T</sup> (=DSM 26824<sup>T</sup>=MCCC 1A00686<sup>T</sup>), was isolated from the toxic marine diatom *Pseudo-nitzschia multiseri*es. The draft genome sequence has been deposited in NCBI under accession number JAMD00000000. The G+C content of the chromosomal DNA of the type strain is 61.7 mol%.

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**References**


