Maribaculum marinum gen. nov., sp. nov., isolated from deep seawater

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A taxonomic study was carried out on strain P38T, which was isolated from an enriched polycyclic aromatic hydrocarbon-degrading consortium from a deep seawater sample collected from the Indian Ocean. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain P38T formed a distinct evolutionary lineage within the family Hyphomonadaceae. The family Hyphomonadaceae was first proposed by Lee et al. (2005) based on phylogenetic analyses of 16S rRNA gene sequences. At the time of writing, the genus contains six genera: Hyphomonas (Moore et al., 1984), Hirschia (Schlesner et al., 1990), Maricaulis (Abraham et al., 2002), Oceanicaulis (Strömpl et al., 2003), Robignitomaculum (Lee et al., 2007) and Hellea (Alain et al., 2008). Another genus, Woodsholea (Abraham et al., 2004), should be assigned to the family Hyphomonadaceae (Alain et al., 2008). Accordingly, the aim of the present work was to determine the exact taxonomic position of strain P38T.

Deep sea water was sampled at a depth of 2914 m (200 m above the sea floor) at the site of IR-CTD13 (24.2822°S 69.7944°E) on the south-west Indian Ridge during cruise

In an attempt to investigate polycyclic aromatic hydrocarbon-degrading bacteria in deep seawater of the Indian Ocean, many bacterial strains were isolated and characterized taxonomically (Lai et al., 2009). Comparative 16S rRNA gene sequence analysis indicated that one of these isolates, designated strain P38T, formed a deep branch within the family Hyphomonadaceae. The family Hyphomonadaceae was first proposed by Lee et al. (2005) based on phylogenetic analyses of 16S rRNA gene sequences. At the time of writing, the genus contains six genera: Hyphomonas (Moore et al., 1984), Hirschia (Schlesner et al., 1990), Maricaulis (Abraham et al., 2002), Oceanicaulis (Strömpl et al., 2003), Robignitomaculum (Lee et al., 2007) and Hellea (Alain et al., 2008). Another genus, Woodsholea (Abraham et al., 2004), should be assigned to the family Hyphomonadaceae (Alain et al., 2008). Accordingly, the aim of the present work was to determine the exact taxonomic position of strain P38T.

Deep sea water was sampled at a depth of 2914 m (200 m above the sea floor) at the site of IR-CTD13 (24.2822°S 69.7944°E) on the south-west Indian Ridge during cruise

Abbreviation: UPGMA, unweighted pair group method with arithmetic means.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P38 T is EU819081.

A transmission electron micrograph of strain P38T and a table detailing the API ZYM characteristics of strain P38T and related genera are available with the online version of this paper.

DY-105A of R/V Da-Yang Yi-Hao in December 2005. The sample was enriched with polycyclic aromatic hydrocarbons and strains were isolated on 216L marine agar medium following the method described by Lai et al. (2009). For morphological and biochemical characterization, strain P38T was cultivated on 216L agar.

General cell morphology of strain P38T was studied under an Olympus inverted microscope using a 1-day-old culture. For electron microscopy, exponential-phase cells were harvested, suspended and absorbed onto a Formvar-carbon-coated grid and stained with phosphotungstic acid (Supplementary Fig. S1, available in IJSEM Online). The Gram reaction and tests for catalase and oxidase activities, lipase (Tween 80), amylase and hydrolysis of aesculin were carried out according to Dong & Cai (2001). The optimal growth temperature was determined on 216L agar over the range 4–55°C. Tolerance of NaCl was tested by using Luria–Bertani medium (Sambrook et al., 1989) supplemented with NaCl (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18 and 20%, w/v). Antibiotic susceptibility tests were performed by the disc diffusion method as described by Shieh et al. (2003) with Oxoid discs. Other biochemical tests were performed with API 20NE and API ZYM strips (bioMérieux) and GN2 MicroPlates (Biolog), according to the manufacturers’ instructions with the adjustment of NaCl to 3.0%. To detect bacteriochlorophyll a and carotenoids, pigments of strain P38T were extracted with acetone/methanol (1:1, v/v) and absorption spectra were recorded using a scanning UV/visible spectrophotometer.
(SmartSpec Plus; Bio-Rad). Additionally, the genetic potential for anoxygenic phototrophy was determined by PCR amplification of the photosynthetic reaction centre genes (pufLM) using the pufLF and pufMR primer set (Béja et al., 2002). The results are given in the genus and species descriptions and Table 1.

Genomic DNA was prepared according to the method of Ausubel et al. (1995) and the 16S rRNA gene was amplified by PCR with primers that have been described previously (Liu & Shao, 2005). A nearly full-length 16S rRNA gene by PCR with primers that have been described previously was determined. Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 4 (Tamura et al., 2002). The results are given in the genus and species descriptions and Table 1.

Phylogenetic analyses showed that strain P38T formed a distinct evolutionary lineage within the family Hyphomonadaceae (Fig. 1). Strain P38T showed the highest 16S rRNA gene sequence similarity (>97%) to sequences from strains that have not yet been assigned to any species. Of the sequences from recognized species, strain P38T was most closely related to those from the genera Hyphomonas (92.3–93.5%), Hirschia (88.8%), Maricaulis (88.3–88.6%), Hellea (87.5%), Oceanicaulis (87.4%) and Robiginitomaculum (86.7%), which belong to the family Hyphomonadaceae. All of the 16S rRNA gene sequence divergences between strain P38T and recognized species were greater than 6.5% and the distinct phylogenetic relationships revealed that strain P38T could not be assigned to any of the recognized genera. Consequently, strain P38T should be considered to represent a novel species in a new genus in the family Hyphomonadaceae. In addition, we found that the genus Woodsholea (Abraham...

Table 1. Characteristics that differentiate strain P38T from related genera of the family Hyphomonadaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>-</td>
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<tr>
<td>Flagella</td>
<td>-</td>
<td>+*</td>
<td>+</td>
<td>+†</td>
<td>+†</td>
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<td>+</td>
<td>+†</td>
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<td>ND</td>
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<td>+</td>
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<td>16–54</td>
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<td>G + C content (mol%)</td>
<td>61.0</td>
<td>57–64</td>
<td>45.6</td>
<td>62.5–65.2</td>
<td>61.8</td>
<td>60.3</td>
<td>65.2</td>
<td>46.8</td>
</tr>
</tbody>
</table>

*Data for Hyphomonas adhaerens, Hyphomonas johnsonii and Hyphomonas rosenbergii.
†Data for Maricaulis maris.
§Some cells have a prostheca with a thin, tapered end.
§§All species are positive except Hyphomonas polymorpha.
||Data for all species except Maricaulis maris.
‡‡All species are positive except Hyphomonas polymorpha.
et al., 2004) probably belongs to the family Hyphomonadaceae, as shown previously by Alain et al. (2008).

The G+C content of the chromosomal DNA was determined according to the methods described by Mesbah & Whitman (1989) using reversed-phase HPLC. The DNA G+C content of strain P38T was 61.0 mol%, which is similar to values reported for the genera Hyphomonas (57–64 mol%), Maricaulis (62.5–65.2 mol%), Oceanicaulis (61.8 mol%), and Robiginitomaculum (60.3 mol%), but differs by more than 10 mol% from values reported for the genera Hirschia (45.6 mol%) and Hellea (46.8 mol%).

Fatty acids from whole cells grown on marine agar 2216 (BD Difco) at 28°C for 48 h were extracted, saponified, and esterified. The fatty acid methyl esters were analysed by GC according to the instructions of the MIDI system (Sasser, 1997). The major fatty acids of strain P38T were C₁₆:0 (20%), C₁₇:0 (3.7%), C₁₈:1ω7c (37.7%), C₁₈:0 (6.3%) and C₁₈:1ω7c 11-methyl (7.1%). Minor amounts of C₁₂:0 3-OH (3.7%), C₁₄:0 (0.9%), C₁₆:1ω5c (4.4%), C₁₇:1ω8c (4.8%), C₁₇:1ω6c (4.1%), C₁₉:0 cyclo ω8c (0.9%), C₁₈:1ω7c 2-OH (2.6%) and C₂₀:0 (1.4%) were also found in strain P38T. These results differentiated strain P38T from members of the genus Hyphomonas, which do not contain C₁₈:1ω7c (Lee et al., 2007), although it should be noted that Abraham et al. (2004) detected C₁₈:0 in Hyphomonas polymorpha DSM 2665T (trace) and Hyphomonas jannaschiana ATCC 33833T (3.7%).

Although strain P38T is related most closely to members of the genus Hyphomonas (92.3–93.5% 16S rRNA gene sequence similarity), with the highest similarity to Hyphomonas oceanisit SCH89T (93.5%), it cannot be affiliated to the genus Hyphomonas because it is non-budding and does not produce protoplasts and is non-motile and oxidative-negative. The low levels of 16S rRNA gene sequence similarity between strain P38T and all of the other members of the family Hyphomonadaceae, together with the differential phenotypic properties shown in Table 1, suggest that strain P38T represents a novel species in a new genus within the family Hyphomonadaceae, for which the name Maribaculum marinus gen. nov., sp. nov. is proposed.

**Description of Maribaculum marinus gen. nov.**

*Maribaculum* (Ma.ri.ba’c.u.lum. L. neut. n. mare the sea; L. neut. n. baculum a stick or rod; N.L. neut. n. Maribaculum rod from the sea).

Cells are Gram-negative-staining, short rods or ovoid, non-motile, non-budding and non-protoplast-producing, oxidative-negative and catalase-positive. Multiplication occurs by binary fission. Flagella and holdfasts are not present. Carotenoid, bacteriochlorophyll a and the genes for anoxygenic photosynthesis (pufLM) are not found. Chemoheterotrophic. The dominant fatty acids are C₁₆:0 and C₁₈:1ω7c. The DNA G+C content of the type strain of the type species is 61.0 mol%. The genus is assigned phylogenetically to the family Hyphomonadaceae in the order Rhodobacterales. The type species is Maribaculum marinus.

**Description of Maribaculum marinus sp. nov.**

*Maribaculum marinus* (ma.rí.‘num. L. neut. adj. marinus of the sea, marine).

Displays the following properties in addition to those given for the genus. Cells are 1.2–1.4 μm long and 0.8–0.9 μm wide. Negative for lipase (Twen 80), amylase, urease, gelatinase, arginine dihydrolase and indole, hydrolysis of ascelin and reduction of nitrate to nitrite. On 216L agar, forms smooth, grey colonies with regular edges that are 2–12 mm in diameter after 72 h incubation at 28°C, unable to ferment glucose. The predominant fatty acids are C₁₆:0, C₁₇:0, and C₁₈:1ω7c. The DNA G+C content of the type strain of the type species is 61.0 mol%.
Resistant to cephalixin (30), cephalolin (30), cefobid (30), clindamycin (2), furazolidone (15), lincomycin (2), metronidazole (5), norfloxacin (10), oxacillin (1), polymyxin B (30 U) and vancomycin (30). With API ZYM, positive for acid phosphatase, alkaline phosphatase, cystine aminopeptidase, leucine aminopeptidase, naphthol-AS-Bl-phosphoamidase, trypsin, valine aminopeptidase, $\text{z}$-chymotrypsin and $\text{z}$-glucosidase; weakly positive for esterase (C4), esterase lipase (C8) and lipase (C14); negative for N-acetyl-$\beta$-glucosaminidase, $\text{z}$-fucosidase, $\text{z}$- and $\beta$-galactosidase, $\text{z}$-mannosidase, $\beta$-glucosidase and $\beta$-glucuronidase. With API 20NE, does not utilize adipic acid, capric acid, D-glucose, maltose, D-mannitol, D-mannose, L-arabinose, malic acid, N-acetylglucosamine, phenylacetic acid, potassium gluconate or trisodium citrate. With GN2 MicroPlates, positive for utilization of L-ascorbic acid, L-glutamic acid, L-ornithine, L-threonine, Tween 40 and 80, $\text{z}$-ketobutyric acid, $\text{z}$-ketoglutaric acid, $\text{z}$-ketovaleric acid and $\beta$-hydroxybutyric acid; weakly positive for utilization of acetic acid, cis-aconitic acid, citric acid, D-alanine, D-arabinitol, cellobiose, dextrin, D-galacturonic acid, D-serine, glucuronamide, glycerogen, glycol, L-ascorbic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, lactulose, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-leucine, L-serine, maltose, pyruvic acid methyl ester, succinic acid monomethyl ester, phenylethylamine, proline, quinic acid, $\text{z}$-D-glucose, $\text{z}$-aminobutyric acid and $\gamma$-hydroxybutyric acid; negative for utilization of 2,3-butanediol, 2-aminoethanol, adonitol, bromosuccinic acid, D-lactate, D-lactic acid, DL-$\text{z}$-glycerol phosphate, D-fructose, D-galactonic acid lactone, D-galactose, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D-mannitol, D-mannose, melibiose, D-psicose, raffinose, D-saccharic acid, D-sorbitol, trehalose, formic acid, gentiobiose, $\text{z}$-D-glucose 1-phosphate, D-glucose 6-phosphate, glycerol, hydroxy-L-proline, i-erythritol, inosine, itaconic acid, L-arabinose, L-fucose, L-histidine, L-phenylalanine, L-proline, L-pyrrol glutamic acid, L-ribose, malonic acid, myo-inositol, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, $\beta$-phenylpropionylglycerol acid, putrescine, sebacic acid, succinamic acid, succinic acid, sucrrose, thymidine, turanose, uridine, urocanic acid, xylitol, $\text{z}$-cyclodextrin, $\text{z}$-lactose, $\text{z}$-hydroxybutyric acid and methyl $\text{z}$-D-glucoside. Table I and Supplementary Table S1 show characteristics that can be used to distinguish the type strain from related species.

The type strain, P38$^T$ (= CCTCC AB 208227$^T$ = LMG 24711$^T$ = MCCC 1A01086$^T$), was isolated from deep seawater of the Indian Ocean.

Acknowledgements

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References


