**Tropicibacter naphthalenivorans** gen. nov., sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from Semarang Port in Indonesia

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An aerobic, Gram-negative, motile bacterium, strain C02T, was isolated from seawater obtained from Semarang Port in Indonesia. Cells of strain C02T were peritrichously flagellated and rod-shaped. Strain C02T was able to degrade naphthalene, alkynaphthalenes and phenanthrene. 16S rRNA gene sequence analysis revealed that this strain was affiliated with the family *Rhodobacteraceae* in the class *Alphaproteobacteria* and was related most closely to *Marinovum algicola* FF3T (95.7% similarity) and *Thalassobius aestuarii* JC2049T (95.2%). The DNA G+C content of strain C02T was 64.6 mol%. The major cellular fatty acids were C19:0ω7c (50.9% of the total), C18:1ω7c (14.7%), C18:1ω9c (2.9%) and C19:0 cyclo ω8c (2.4%), and the predominant respiratory lipoquinone was ubiquinone-10. Based on physiological, chemotaxonomic and phylogenetic data, strain C02T is suggested to represent a novel species of a new genus, for which the name *Tropicibacter naphthalenivorans* gen. nov., sp. nov. is proposed. The type strain of *Tropicibacter naphthalenivorans* is C02T (=JCM 14838T=DSM 19561T).

Contamination of the marine environment with petroleum hydrocarbons is of great public concern owing to their toxicity to humans and marine organisms (Malins et al., 1985; Meador et al., 1995). A number of hydrocarbon-degrading bacteria have been isolated from tropical waters (Chaillan et al., 2002a, b; Ozaki et al., 2006), although information regarding hydrocarbon-degrading bacteria from tropical waters is relatively scarce (Chaillan et al., 2004; Zhuang et al., 2003; Zinjarde & Pant, 2002). We have recently isolated a substantial number of marine bacteria from seawater obtained from Semarang Port in Indonesia, and demonstrated that some of the isolates were capable of degrading hydrocarbons (Harwati et al., 2007). The present study characterizes one of these Indonesian isolates, designated strain C02T, affiliated with the class *Alphaproteobacteria*. Based on the results of polyphasic examinations, including phenotypic, chemotaxonomic and phylogenetic analyses, we propose that this strain represents a novel species of a new genus.

The ability of strain C02T to degrade hydrocarbons in crude oil was examined in 10 ml ONR7a medium (Dyksterhouse et al., 1995) supplemented with 1 mg heat-treated Arabian Light crude oil ml⁻¹ (Dutta & Harayama, 2000). Cells of strain C02T grown in 10 ml marine broth 2216 (MB; Difco) up to an optical density at 600 nm of approximately 1 were harvested by centrifugation (8000 g, 10 min), washed twice with ONR7a medium and inoculated on to the medium in 50-ml tubes fitted with Teflon-lined caps. The tubes were incubated at 30 °C on a reciprocal shaker (at 90 r.p.m.) for 4 weeks. Cultures were prepared in triplicate. Non-inoculated samples were incubated similarly and served as controls. Following incubation, oil components were extracted by using chloroform, and hydrocarbon losses were analysed via GC-MS (GC-MS-QP5000; Shimadzu) as described by Kasai et al. (2002b). The percentage biodegradation was calculated as described by Dutta & Harayama (2000). Strain C02T degraded 88.1 ± 1.1% (mean ± se) of total naphthalene, 56.0 ± 10.4% of total C1-alkynaphthalenes, 22.5 ± 8.6% of total C2-alkynaphthalenes and 14.5 ± 2.7% phenanthrene, while alkanes, C5–4-alkynaphthalenes, C6–4-alkylbenzenothiophenes, C5–6-alkylphenanthrenes and C6–3-alkylfluorenes were not significantly degraded (<10%) (C0–C6 represent total carbon numbers of branched alkyl groups).

The cell morphology of strain C02T was examined by transmission electron microscopy (Beveridge et al., 1994) and motility was examined under a phase-contrast microscope. Gram staining and oxidase and catalase tests...
were performed according to the procedures of Smibert & Krieg (1994). Growth was tested at 30 °C in MB unless otherwise stated. Salinity requirements were tested by using modified MB (Sohn et al., 2004) supplemented with 0–20 % (w/v) NaCl at 30 °C. The pH range for optimal growth was determined on solid media containing MB whose pH was adjusted to 5.5–9.5. Solid media contained 1.5 % (w/v) Bactoagar (Difco). The presence of poly-β-hydroxyalkanoate was detected by using Sudan Black according to the procedures of de Lima et al. (1998). Susceptibility to antibiotics was determined on agar plates containing MB (MA plates) in the presence of the following antibiotics (concentrations given in parentheses; μg ml⁻¹): ampicillin (50, 100, 150 and 200), chloramphenicol (20), gentamicin (50), kanamycin sulfate (20), nalidixic acid (20, 50, 100 and 200), spectinomycin (7.5, 15 and 20), streptomycin (20) and tetracycline (10). API ZYM, API 20NE (bioMérieux) and Microlog GN2 microplates (Biolog) were used for physiological and biochemical characterization according to the manufacturers’ instructions.

Cells of strain C02T were Gram-negative rods (1.1–3.4 μm in length and 0.1–0.7 μm in width), motile by means of peritrichous flagella (Fig. 1). Strain C02T formed white colonies on MA plates. The strain was oxidase-positive but catalase-negative. It reduced nitrate to nitrite. Cells contained poly-β-hydroxyalkanoate. Growth of strain C02T was observed between 10 and 43 °C, with optimum growth at 37 °C. It grew within a pH range of 6.5–8.5, with optimum growth at pH 7.6. Strain C02T showed essential requirements for NaCl, as no growth was observed in media lacking NaCl. It grew at NaCl concentrations from 1 to 15 %, with optimum growth at 5 % NaCl. The physiological and biochemical characteristics of strain C02T are presented in detail in the species description below. It was susceptible to ampicillin, chloramphenicol, gentamicin, nalidixic acid, kanamycin, spectinomycin, streptomycin and tetracycline.

Table 1. Cellular fatty acid contents of strain C02T and closely related taxa

<table>
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<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>ND</td>
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<td>2.1</td>
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<td>ND</td>
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<tr>
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<td>ND</td>
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<tr>
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<td>17.9</td>
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<td>8.6</td>
<td>6.9</td>
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<td>6.8</td>
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<td>2.2</td>
<td>8.6</td>
</tr>
<tr>
<td>C16:0</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>C18:0</td>
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<td>1.0</td>
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<td>0.8</td>
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<tr>
<td>C16:1ω7c</td>
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<td>*</td>
<td>71.2</td>
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<td>ND</td>
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<tr>
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<td>ND</td>
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<td>ND</td>
<td>2.9</td>
<td>ND</td>
</tr>
<tr>
<td>C16:0-2-OH</td>
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<td>ND</td>
<td>ND</td>
<td>3.4</td>
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<tr>
<td>11 Methyl C16:1ω7c</td>
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<td>ND</td>
<td>ND</td>
<td>7.1</td>
<td>3.2</td>
<td>12.0</td>
<td>ND</td>
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<tr>
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</table>

*Major fatty acid, but not quantified.
Cellular fatty acids and quinone were analysed at the TechnoSuruga Laboratory Co., Ltd from cells grown in MB for 24 h. The major cellular fatty acids of strain C02T were C18:1ω7c (50.9 % of the total), C16:0 (17.9 %), 11 methyl C18:1ω7c (14.7 %), C18:1ω9c (2.9 %) and C19:0 cyclo ω8c (2.4 %) (detailed results are given in Table 1). The major lipoquinone was ubiquinone-10 (approximately 88 % of the total).

The 16S rRNA gene sequence of strain C02T (1363 bp) was determined previously (Harwati et al., 2007). Searches for similar 16S rRNA gene sequences were conducted by using the GenBank and RDP (Maidak et al., 1999) databases. Phylogenetic analysis was performed by using CLUSTAL_X (version 1.83) (Thompson et al., 1997), and a phylogenetic tree was constructed by using the neighbour-joining plot program within the MEGA software package (version 3.0) (Kumar et al., 2004). In the neighbour-joining phylogenetic tree, strain C02T formed a separate branch within the family Rhodobacteraceae (Fig. 2). 16S rRNA gene sequence analysis revealed that strain C02T was related most closely to Marinovum algicola FF3T (95.7 % similarity) and Thalassobius aestuarii JC2049T (95.2 %). The latter two taxa belong to the Roseobacter clade, which is known as one of the most abundant groups in the marine environment (Buchan et al., 2005). The DNA G+C content of strain C02T as determined by the method of Katayama-Fujimura et al. (1984) was 64.6 mol%.

Phenotypic characteristics of strain C02T that can be used to differentiate it from closely related members of the Roseobacter clade are detailed in Table 2. Based on phylogeny, fatty acid composition and phenotypic characteristics, we conclude that strain C02T represents a novel species of a new genus, for which the name Tropicibacter naphthalenivorans gen. nov., sp. nov. is proposed.
Description of *Tropicibacter naphthalenivorans* gen. nov., sp. nov.

*Tropicibacter* [Trop.ic.i.bac.ter. L. adj. *tropicus* tropical, pertaining to the tropical zone of the Earth; N.L. masc. n. *bacter* (from Gr. n. *bakterion*) rod; N.L. masc. n. *Tropicibacter* a rod belonging to the tropical zone].

Cells are Gram-negative, motile by means of peritrichous flagella and rod-shaped (1.1–3.4 μm long and 0.1–0.7 μm wide). Require sodium ions for growth. Positive for oxidative and nitrate reduction. Contain poly-β-hydroxyalkanoate. The major ubiquinone is Q-10. The predominant fatty acids are C18:1, C16:0 and 11 methyl C18:1. The type species is *Tropicibacter naphthalenivorans*.

Description of *Tropicibacter naphthalenivorans* sp. nov.


The description is identical to that for the genus, with the following additions. Colonies on MA are circular, slightly convex, smooth, yellowish-white and 2.0–3.0 mm in diameter after 3 days incubation at 37 °C. Growth occurs at temperatures of 10–43 °C (optimum 37 °C), at pH 6.5–8.5 and at NaCl concentrations of 1–7 % (optimum 3 %). Able to degrade C0.2-alkynaphthalene and phenanthrene. Susceptible to (μg ml⁻¹) ampicillin (50), chloramphenicol (20), gentamicin (50), kanamycin (20), nalidixic acid (20), spectinomycin (7.5), streptomycin (20) and tetracycline (10). Positive for nitrate reduction, protease, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-glucuronidase, α-mannosidase and α-fucosidase. The following Biolog GN2 test substrates score positive: dextrin, Tween 80, L-arabinose, D-fructose, D-glucose, D-lactose, maltose, D-mannose, D-sorbitol, sucrose, lactic acid, succinic acid, L-aspartic acid, L-alanine, L-proline, serine, inosine, uridine, turanose, xyitol, trehalose, inosine, uridine, cellobiose, melibiose, L-rhamnose and acetic acid. The predominant fatty acids are C18:1, C16:0 and 11 methyl C18:1. The type strain is *Tropicibacter naphthalenivorans* C02T (=JCM 14838T=DSM 19561T), was isolated from seawater in Semarang Port, Java, Indonesia. The DNA G+C content of the type strain is 64.6 mol%.

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References


