**Nocardioides rotundus** sp. nov., isolated from deep seawater

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A Gram-staining-positive, aerobic, coccoid-shaped, non-motile actinobacterium, designated strain GY0594T, was isolated from deep seawater of the western Pacific. Phylogenetic analyses based on 16S rRNA gene sequences showed that this strain was affiliated with the genus *Nocardioides* with low 16S rRNA gene sequence similarities (≤96.0 %) with members of the genus *Nocardioides*. Chemotaxonomic characterization of strain GYP0594T supported the result of the phylogenetic analysis. The diagnostic diamino acid in the cell-wall peptidoglycan was LL-2,6-diaminopimelic acid. The major menaquinone was MK-8(H4). The polar lipids detected were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unidentified lipid and six unidentified phospholipids. The major cellular fatty acids were iso-C₁₆ : ₀ and C₁₈ : ₁ω₉c. The DNA G+C content of strain GY0594T was determined to be 71.2 mol%. However, strain GY0594T could be distinguished from closely related species by cell morphology, nitrate reduction, aesculin hydrolysis, activity of urease, cystine arylamidase, trypsin and acid phosphatase, assimilation of N-acetylglucosamine, maltose, adipic acid, malic acid and phenylacetate, and significant differences in the proportions of several fatty acids.

In conclusion, based on the data presented, strain GY0594T should be placed in the genus *Nocardioides* as a representative of a novel species, for which the name *Nocardioides rotundus* sp. nov. is proposed. The type strain is GY0594T (=MCCC 1A10561T=KCTC 39638T).

Seawater samples were collected from the western Pacific (11° 0.8’ N 141° 57.3’ E; −7001 m), and spread on a modified ZoBell 2216E agar (MZ2216E; 1.0 g yeast extract, 5.0 g tryptone, 1 l clarified seawater and 15.0 g agar, pH 7.4–7.6). After 15 days of aerobic incubation at 30 °C, a creamy white colony, designated GY0594T, was picked. The isolate was purified by repeated restreaking. Purity was confirmed by the uniformity of cell morphology. Unless otherwise stated, the strain was routinely cultured on MZ2216E agar at 30 °C, and culture suspensions were stored in 20 % (v/v) glycerol at −80 °C.

Genomic DNA extraction of strain GY0594T was performed as described by Li et al. (2007). The 16S rRNA gene sequence was amplified by PCR using primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GTTACCTTGTAGACTT-3’). The PCR products were purified and directly sequenced by using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730xl; Applied Biosystems), with primers 802R (5’-TAC-CAGGGTAATCTAACCC-3’) and 518F (5’-CCAGCAGCCGC-GGTAAATACG-3’), 27F and 1492R. The 16S rRNA gene sequence of strain GY0594T was compared with those of species with validly published names from the GenBank database via the BLAST program and the EzTaxon-e server (Kim et al., 2012). All sequence alignments were analysed...
with the MEGA5 software package (Tamura et al., 2011). Phylogenetic trees were reconstructed using three tree-making algorithms: neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-likelihood (Felsenstein, 1981). The stability of relationships was evaluated by performing a bootstrap analysis based on 1000 replications (Felsenstein, 1985).

The almost-complete 16S rRNA gene sequence (1484 nt) of strain GY0594T was obtained. The isolate was closely related to Nocardioides marinus CL-DD14T with a 16S rRNA gene sequence similarity of 96.0 %. 16S rRNA gene similarities between strain GY0594T and the type strains of other related species of the genus Nocardioides were less than 96 %. The phylogenetic position of strain GY0594T, determined by using the various tree-making algorithms (neighbour-joining, minimum-evolution and maximum-likelihood), revealed that the isolate was a member of the genus Nocardioides and formed a distinct sublineage within the genus Nocardioides, supported by a high bootstrap value (Fig. 1 and Figs S1–S3, available in the online Supplementary Material). Thus, the low 16S rRNA gene sequence similarity value (i.e. ≤ 96.0 %) with recognized species of the genus Nocardioides and the phylogenetic position of strain GY0594T showed that the strain could be assigned to a novel species of the genus Nocardioides.

Morphological and physiological tests of strain GY0594T were performed as follows. Cell morphology was observed with a light microscope (BH-2; Olympus) and a transmission electron microscope (H-600; Hitachi) using cells that had been grown for 24–36 h on MZ2216E agar medium at 30 °C. Gram staining was carried out by the standard Gram’s reaction as described by Cerny (1978). Growth at different temperatures, salinities and pH were assessed using methods described by Yin et al. (2015). Cell motility was confirmed by the development of turbidity throughout a tube containing semisolid medium (Leifson, 1960). Growth under anaerobic conditions was determined after incubation on MZ2216E in an anaerobic chamber at 30 °C for about 1 month. Catalase activity was tested by bubble production upon addition of a drop of 3 % (v/v) H2O2, and oxidase activity was determined using 1 % (w/v) tetramethyl-p-phenylenediamine. Nitrate reduction, milk peptonization and coagulation, gelatin liquefaction and production of H2S and melanin were tested as described by Ruan & Huang (2011). Other physiological and biochemical characteristics were determined using the API 20NE and API ZYM systems (bioMérieux) and GP2 MicroPlate panels (Biolog), according to the instructions of the manufacturer. Nocardioides marinus JCM 15615T, obtained from the Japan Collection of Microorganisms (JCM), was used as a reference strain for morphological, physiological and biochemical analyses.

For chemotaxonomic analyses, biomass was obtained from cultures grown in shake flasks containing MZ2216E broth and incubated at 30 °C for 5 days. The isomer type of dia-
minopimelic acid was determined by one-dimensional TLC as described by Lechevalier & Lechevalier (1980). Polar lipids were extracted using the integrated procedure of Minnikin et al. (1984), separated by two-dimensional TLC and identified using the procedure described by Minnikin et al. (1977). Respiratory quinones were analysed using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) as described by Kaiser et al. (2012). For cellular fatty acid analyses, strain GY0594T and N. marinus JCM 15615T were grown on MA (Difco) medium for 3 days at 30 °C. Fatty acid methyl esters were prepared and analysed according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0) and identified by using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). The DNA G+C content was determined as described by Mesbah et al. (1989) using reversed-phase HPLC.

Cells of strain GY0594T were Gram-stain-positive, aerobic, non-motile and non-spore-forming cocci (diameter 0.7–0.9 μm). After incubation for 3 days on MZ2216E agar medium at 30 °C, colonies were observed to be opaque, convex and creamy white, and measured 0.5–1 mm in diameter. Growth of strain GY0594T occurred at 15–40 °C (optimum 30–35 °C), at pH 5–11 (optimum pH 8–9) and in the presence of 0–12 % (w/v) NaCl (optimum 0–6 %). According to API ZYM kits, strain GY0594T was positive for the activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, but negative for the activity of lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, trypsin, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase. In API 20NE strips, there were positive results for reduction of nitrate to nitrite, hydrolysis of gelatin and p-nitrophenyl-β-D-galactopyranoside, and assimilation of D-glucose, D-mannitol, potassium gluconate, adipic acid, malic acid and phenylacetic acid; weakly positive results for assimilation of N-acetylglucosamine; and negative results for indole production, aesculin hydrolysis, activity of arginine dihydrolase and urease, D-glucose fermentation and assimilation of L-arabinose, D-mannose, maltose, capric acid and trisodium citrate. D-Fructose, D-galacturonic acid, α-D-glucose, D-mannose, D-psicose, D-ribose, proionic acid, pyruvic acid, N-acetyl-L-glutamic acid, 2,3-butanediol, glycerol and D,L-α-glycerol phosphate can be utilized in the Biolog GP2 MicroPlate system. A comparison of selected morphological and physiological characteristics of strain GY0594T and N. marinus JCM 15615T is provided in Table 1.

The chemotaxonomic data supported the result of the phylogenetic analysis. Strain GY0594T was shown to possess chemical features consistent with those of the genus Nocardioides. The diagnostic diamino acid in the cell-wall peptidoglycan was LL-2,6-diaminopimelic acid. The major menaquinone was MK-8(H4). The polar lipid profile of strain GY0594T comprised diphostidylglycerol, phosphatidyglycerol, phosphatidylinositol, one unidentified lipid and six unidentified phospholipids (Fig. S4). The dominant fatty acids (>5 %) of strain GY0594T were iso-C16 : 0 (27.4 %), C18 : 1ω9c (20.9 %), iso-C15 : 0 (9.9 %), iso-C17 : 0 (7.7 %), summed feature 9 (iso-C17 : 1ω9c and/or 10-methyl C16 : 0) (5.9 %) and C17 : 1ω8c (5.7 %). The fatty acid iso-C16 : 0 which was typically found as a major component in members of the genus Nocardioides (Yoon et al., 2004, Zhang et al., 2012), was also detected in N. marinus JCM 15615T (Table S1). Significant differences in the proportions of several fatty acids were found between the isolate and the reference strain, such as strain GY0594T contained a lower amount of iso-C16 : 0 and higher amounts of C18 : 1ω9c, iso-C15 : 0 and iso-C17 : 0 than N. marinus JCM 15615T. Detailed fatty acid profiles of the isolate and the reference strain are presented in Table S1. The DNA G+C content of strain GY0594T was determined to be 71.2 mol%, within the range 64.9–74.9 mol% reported for recognized species of the genus Nocardioides (Cui et al., 2013, Lim et al., 2014).

On the basis of phenotypic, chemotaxonomic and phylogenetic analyses, strain GY0594T exhibited markers typical of members of the genus Nocardioides. Characteristics

### Table 1. Differential phenotypic characteristics of strain GY0594T and its closest phylogenetic neighbour, N. marinus JCM 15615T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
</tr>
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<tbody>
<tr>
<td>Cell morphology</td>
<td>Cocci</td>
<td>Rods, cocci</td>
</tr>
<tr>
<td>Growth conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature range (optimum) (°C)</td>
<td>15–40 (30–35)</td>
<td>10–40 (28–30)</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>5–11 (8–9)</td>
<td>5–9 (7–8)</td>
</tr>
<tr>
<td>Salt tolerance range (optimum) (%)</td>
<td>0–12 (0–6)</td>
<td>0–10 (0–3)</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Enzyme activities:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Trypsin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Adipic acid</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Malic acid</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Phenylacetate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>71.2</td>
<td>72.9*</td>
</tr>
</tbody>
</table>

*Data from Choi et al. (2007).
summarized in Tables 1 and S1, and the low 16S rRNA gene sequence similarities (≤96.0 %) with members of the genus Nocardioides strongly support the hypothesis that the isolate GY0594 T is representative of a novel species of the genus Nocardioides, for which the name Nocardioides rotundus sp. nov. is proposed.

**Description of Nocardioides rotundus sp. nov.**

*Nocardioides rotundus* (ro.tun’dus. L. masc. adj. rotundus round).

Cells are Gram-staining-positive, strictly aerobic, non-motile and non-spore-forming cocci (diameter 0.7–0.9 μm) without any flagella. After incubation for 3 days at 30 °C, colonies on MZ2216E agar medium are opaque, convex and creamy white, and measure 0.5–1 mm in diameter. On MA medium, colonies are 0.4–0.6 mm in diameter, circular, translucent, moist and faint yellow. Growth occurs at 15–40 °C (optimum 30–35 °C), at pH 5–11 (optimum pH 8–9) and in the presence of 0–12 % (w/v) NaCl (optimum 0–6 %). Oxidase-negative and catalase-positive. Positive for nitrate reduction, milk coagulation and peptonization, and hydrolysis of gelatin and casein. Negative for methyl red test, Voges–Proskauer reaction, starch hydrolysis and production of H2S and melain. Positive for acid phosphatase activity and assimilation of adipic acid, malic acid and phenylacetate. Negative for maltose assimilation and activity of urease, cystine arylamidase and trypsin. The major menaquinone is MK-8(H4). The cell wall contains LL-diaminopimelic acid. The polar lipids detected are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unidentified lipid and six unidentified phospholipids. The major fatty acids are iso-C16 : 0 C18 : 1ω9c, iso-C15 : 0 and/or 10-methyl C16 : 0 and C17 : 0ω8c.

The type strain, GY0594 T (=MCCC 1A10561 T=KCTC 39638 T), was isolated from a sample of deep seawater collected from the western Pacific Ocean. The DNA G+C content of the type strain is 71.2 mol%.

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**References**


