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

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A Gram-staining-positive, aerobic, coccoid-shaped, non-motile actinobacterium, designated strain **GY0594**T, 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 **GY0594**T 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 diphosphatidyglycerol, 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 **GY0594**T was determined to be 71.2 mol%. However, strain **GY0594**T 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 **GY0594**T 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 **GY0594**T (=MCCC 1A10561T =KCTC 39638T).

The genus **Nocardioides** was first proposed by Prauser (1976) with the description of **Nocardioides albus** as the type species. At the time of writing, the genus comprises 76 species with validly published names (http://www.bacterio.net/nocardioides.html). Among them, four species have been isolated from seawater: **Nocardioides marinus** (Choi et al., 2007), **Nocardioides salarius** (Kim et al., 2008), **Nocardioides mariniquilinus** (Cho et al., 2013a) and **Nocardioides salsibiostraticola** (Cho et al., 2013b). In this study, another novel **Nocardioides** strain (**GY0594**T) was isolated from deep seawater of the western Pacific. Based on data obtained using a polyphasic approach, we propose that the isolate represents a novel species of the genus **Nocardioides**.

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 **GY0594**T, 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 **GY0594**T 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’-GGTTACCTGTAGACCTT-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-CAGGGTATCTAATCC-3’]), 518F ([5’-CCAGCAGCGCC-GGTATACG-3’]), 27F and 1492R. The 16S rRNA gene sequence of strain **GY0594**T 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 GY0594\textsuperscript{T} was obtained. The isolate was closely related to Nocardioides marinus CL-DD14\textsuperscript{T} with a 16S rRNA gene sequence similarity of 96.0 %. 16S rRNA gene similarities between strain GY0594\textsuperscript{T} and the type strains of other related species of the genus Nocardioides were less than 96 %. The phylogenetic position of strain GY0594\textsuperscript{T}, 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 GY0594\textsuperscript{T} showed that the strain could be assigned to a novel species of the genus Nocardioides.

Morphological and physiological tests of strain GY0594\textsuperscript{T} 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) H\textsubscript{2}O\textsubscript{2}, and oxidase activity was determined using 1 % (w/v) tetramethyl-p-phenylenediamine. Nitrate reduction, milk peptonization and coagulation, gelatin liquefaction and production of H\textsubscript{2}S 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 15615\textsuperscript{T}, 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-gluconisidase, 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, propionic acid, pyruvic acid, N-acetyl-L-glutamic acid, 2,3-butanediol, glycerol and DL-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 Nocardiooides. 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 diphosphatidylglycerol, phosphatidylglycerol, 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 Nocardiooides (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 Nocardiooides (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 Nocardiooides. 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>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Cell morphology</td>
<td>Cocci</td>
<td>Rods, cocci</td>
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<tr>
<td>Growth conditions</td>
<td></td>
<td></td>
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<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>
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<tr>
<td>Urease</td>
<td>–</td>
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<tr>
<td>Cystine arylamidase</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Trypsin</td>
<td>–</td>
<td>+</td>
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<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>
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<tr>
<td>Maltose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Malic acid</td>
<td>–</td>
<td>+</td>
</tr>
<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 GY0594T 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 M2216E 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 melanin. 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, iso-C₁₇ : ₀ summed feature 9 (iso-C₁₇ : ₁ω₉c and/or 10-methyl C₁₆ : ₁₀) and C₁₇ : ₁ω₈c.

The type strain, GY0594T (=MCCC 1A10561T=KCTC 39638T), 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**


