Nocardioides iriomotensis sp. nov., an actinobacterium isolated from forest soil

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An actinomycete strain, designated IR27-S3T, was isolated from a forest soil sample collected from Iriomote Island, Okinawa, Japan. Cells of the isolate were Gram-stain-positive, aerobic, non-sporulating, non-motile coccoids or short rods. The strain grew in the presence of 0–7 % (w/v) NaCl, at pH 6–8 and at 12–37 °C, with optimum growth at 30 °C. Chemotaxonomically, the strain contained LL-diaminopimelic acid as the diagnostic diamino acid and MK-8(H4) as the predominant menaquinone. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolaminol and an unknown phospholipid. The major cellular fatty acids were iso-C16:0, C17:0 cis-9, C15:0 and iso-C15:0. The DNA G+C content was 73.7 mol%. On the basis of 16S rRNA gene sequence analysis, strain IR27-S3T was closely related to Nocardioides mesophilus MSL-22T (98.1 % 16S rRNA gene sequence similarity), Marmoricola bigeumensis MSL-05T (97.2 %) and Nocardioides jensenii DSM 20641T (96.5 %). On the basis of fatty acid analysis, phylogenetic analysis and phenotypic data, the isolate should be classified in a novel species of the genus Nocardioides, for which the name Nocardioides iriomotensis sp. nov. is proposed. The type strain is IR27-S3T (=NBRC 105384T =KACC 14926T).

The family Nocardioidaceae was proposed by Nesterenko et al. (1985) and currently encompasses six genera, including the genus Nocardioides. The genus Nocardioides was established by Prauser (1976) and currently encompasses at least 45 species with validly published names (Euzéby, 1997). Morphologically, members of the genus Nocardioides develop mycelium that fragments into irregular rods or coccus-like elements. Phylogenetic analyses of 16S rRNA gene sequences have shown that the genus Nocardioides is closely related to the genus Marmoricola (Urzi et al., 2000), but they can be differentiated chemotaxonomically by their fatty acid profiles (Yoon & Park, 2006; Kim et al., 2009; Yoon et al., 2010). In recent years, several novel Nocardioides species have been isolated from terrestrial and aquatic environments, including soil (Zhang et al., 2009; Cho et al., 2010; Yoon et al., 2010), sand (Kim et al., 2009; Park et al., 2008), wastewater (Yoon et al., 2009) and sediment (Dastager et al., 2009). As one strain of the genus Nocardioides has been reported to produce the bioactive agent sandramycin (Matson & Bush, 1989), the discovery of congeneric species is likely to contribute positively towards understanding both the ecology of the genus and its potential as a bioresource for industrial applications.

A forest soil sample from Iriomote Island, Okinawa, Japan, was screened for the presence of actinomycete strains. Strain IR27-S3T was isolated by the serial dilution-plating method on humic acid-vitamin agar (Hayakawa & Nonomura, 1987) containing (l-arginine, 20 mg nalidixic acid, 1 mg cycloheximide and 300 g sorbitol after incubation at 30 °C for 2 weeks. Colony morphology was examined after incubation on tryptic soy agar (Difco) at 28 °C for 14 days. Cell morphology was examined by light and scanning electron microscopy. Cell motility was examined in hanging drops by light microscopy. Gram-staining was performed using Hucker's method (Gerhardt, 1981). Tests for aesculin degradation (Williams et al., 1983) and nitrate reduction (Gordon & Mihm, 1962) were performed using established procedures. Lipolysis was assessed on the medium described by Sierra (1957) using Tween 80 as a substrate. Melanin production was determined after 1–4 days on ISP media 6 and 7 (Shirling & Gottlieb, 1966). Conditions for growth were examined on tryptic soy agar as follows. To determine temperature and pH for growth, the strain was incubated at 5, 12, 20, 25, 30, 37 and 45 °C.
for 7 days (6 weeks for 12 °C) and at pH 4–12 (in increments of 1 pH unit) at 30 °C for 14 days. Growth with 0–7% (w/v) NaCl (in increments of 1%) was evaluated after 14 and 21 days at 30 °C (Williams et al., 1983). API ZYM kits (bioMérieux) were used to investigate several physiological and biochemical characteristics, according to the manufacturer's instructions.

Biomass for chemotaxonomic studies was obtained by growing the isolate in shake flasks of tryptic soy broth for 5 days at 30 °C and harvesting by centrifugation; the resultant pellets were washed twice with distilled water. Diaminopimelic acid isomers and sugars in whole-cell hydrolysates were analysed using the methods established by Hasegawa et al. (1983) and Schaal (1985), respectively. Cellular fatty acids were processed and analysed as methyl esters, using the protocol of the MIDI Sherlock Microbial Identification System version 4 (Sasser, 1990) with the ACTINO method. Standard procedures were also used for the extraction and analysis of isoprenoid quinones and polar lipids (Minnikin et al., 1984) and the results were compared with appropriate controls. Chromosomal DNA from strain IR27-S3T was isolated and purified by the method of Saito & Miura (1963) with a minor modification (Hatano et al., 2003). The DNA G+C content of strain IR27-S3T was determined by HPLC, as described by Mesbah et al. (1989). DNA–DNA hybridization was performed as described by Kusunoki et al. (1991) using biotinylated DNA.

PCR amplification of the 16S rRNA gene from strain IR27-S3T was performed according to the procedures described by Tamura & Hatano (2001) and the resulting PCR product was directly sequenced using an ABI Prism BigDye

### Table 1. Differential phenotypic characteristics of strain IR27-S3T and its closest phylogenetic relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Colony colour*</td>
<td>CY</td>
<td>CW</td>
<td>LY</td>
</tr>
<tr>
<td>Cell morphology†</td>
<td>C or ISR</td>
<td>C or SR</td>
<td>C</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12–37</td>
<td>20–37</td>
<td>20–37</td>
</tr>
<tr>
<td>Optimum</td>
<td>30</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>pH for growth</td>
<td>6–8</td>
<td>ND</td>
<td>6–12</td>
</tr>
<tr>
<td>NaCl concentration for growth (% w/v)</td>
<td>0–7</td>
<td>ND</td>
<td>0–7</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>W</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xanthine</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Major fatty acids§</td>
<td>C17:1,</td>
<td>i-C14:0 C15:0,</td>
<td>i-C15:0</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>73.7</td>
<td>68.7</td>
<td>72.9</td>
</tr>
</tbody>
</table>

*CW, Cream–white; CY, cream–yellow; LY, lemon yellow.
†, Cocc; ISR, irregular short rods; SR, short rods.
‡Data from this study.
§ai, Anteiso-branched; i, iso-branched.
Terminator Cycle Sequencing kit and an automated DNA sequencer (model 3730 Genetic Analyzer; Applied Biosystems). Calculation of pairwise 16S rRNA gene sequence similarities was conducted using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). The 16S rRNA gene sequence was aligned with reference sequences from the genera *Nocardioides* and *Marmoricola* available in GenBank using CLUSTAL X (Thompson et al., 1997). Phylogenetic trees were constructed with MEGA version 4.0 (Tamura et al., 2007) and CLUSTAL X (Thompson et al., 1997) using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Takahashi & Nei, 2000) methods. The PHYML software package (Guindon et al., 2005) was used to construct a maximum-likelihood tree. The robustness of the constructed tree topologies was evaluated by bootstrap analysis (Felsenstein, 1985) with 1000 resamplings (neighbour joining and maximum parsimony) or 500 resamplings (maximum likelihood).

Cells of strain IR27-S3\(^T\) were short rods or coccioids, 0.4–0.5 \(\mu\text{m}\) wide and 0.4–1.0 \(\mu\text{m}\) long, occurring either singly or in pairs (Supplementary Fig. S1, available in IJSEM Online). The results of physiological and biochemical analyses are summarized in the species description and Table 1.

The chemotaxonomic and morphological characteristics of strain IR27-S3\(^T\) were consistent with its assignment to the genus *Nocardioides* (Yoon & Park, 2006; Kim et al., 2009; Chun et al., 2007).

**Fig. 1.** Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of strain IR27-S3\(^T\) with members of the genus *Nocardioides* and related taxa. Bootstrap values (\(>50\%\)) based on 1000 resamplings are shown at branch nodes. Asterisks indicate that the corresponding nodes were also recovered in trees generated using the maximum-parsimony and maximum-likelihood algorithms. Bar, 0.005 substitutions per nucleotide position.
Yoon et al., 2010). The whole-cell hydrolysate contained L-l-diaminopimelic acid and displayed no diagnostic sugars (wall chemotype III sensu Lechevalier & Lechevalier, 1970). The major menaquinone was MK-8(H4) and the major polar lipids were diphasphatidylglycerol, phosphatidylglycerol, phosphatidylglycositol and an unknown phospholipid (phospholipid type PI sensu Lechevalier et al., 1977). The major cellular fatty acids were iso-C16:0 (32.7 %), C17:1ω9c (18.1 %), C15:0ω6c (12.6 %) and iso-C15:0ω5c (12.5 %). The fatty acid profile of strain IR27-S3T was similar to those of other members of the genus Nocardioides, including Nocardioides mesophilus MSL-22T, Nocardioides jenseni DSM 20641T and Nocardioides daeudakensis MDN22T, in which iso-C15:0ω5c is also the predominant fatty acid (Yoon & Park, 2006; Kim et al., 2009; Yoon et al., 2010).

The nearly complete 16S rRNA gene sequence (1463 nt) of strain IR27-S3T was compared with sequences of members of the genus Nocardioides and related taxa. Strain IR27-S3T exhibited 98.1, 97.2 and 96.5 % 16S rRNA gene sequence similarity with N. mesophilus MSL-22T, Marmoricola bigeumensis MSL-05T and Nocardioides jenseni DSM 20641T, respectively. The range of 16S rRNA gene sequence similarities between the isolate and members of the genus Nocardioides was 92.2–98.1 %. In addition, the neighbour-joining tree (Fig. 1) showed that strain IR27-S3T formed a separate lineage within the cluster containing N. mesophilus MSL-22T and M. bigeumensis MSL-05T; interestingly, this topology was also seen using other algorithms (Fig. 1). Strain IR27-S3T showed 38.8 ± 2.7 % DNA–DNA relatedness with N. mesophilus NBRC 107606T. This value was well below the 70 % cut-off point recommended for the assignment of bacterial strains to the same genomic species (Wayne et al., 1987). Strain IR27-S3T was also distinguished from its phylogenetic neighbours by comparison of phenotypic and chemotaxonomic characteristics (Table 1).

On the basis of fatty acid analysis and phenotypic and genotypic characteristics, strain IR27-S3T represents a novel species within the genus Nocardioides, for which the name Nocardioides iriomotensis sp. nov. is proposed.

Description of Nocardioides iriomotensis sp. nov.

Nocardioides iriomotensis (i.ri.o.mo.ten’sis. N.L. masc. adj. iriomotensis pertaining to Iriomote Island, Okinawa, Japan, where the type strain was isolated).

Aerobic, Gram-stain-positive, non-motile actinomycete, with coccoid or irregular short rod-shaped cells, 0.4–0.5 μm wide and 0.4–1.0 μm long, that occur singly or in pairs. Colonies are entire, convex and cream yellow. Melanoid pigments are not produced on ISP 6 or 7. Aesculin is hydrolysed and nitrate is reduced. Degrades casein, gelatin and starch, but does not degrade l-tyrosine, hypoxanthine or xanthine. Growth occurs at 12–37 °C (optimum 30 °C), but not at 5 or 45 °C, with 0–7 % (w/v) NaCl and at pH 6–8 (optimum pH 7). With API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and α- and β-glucosidases, but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Whole-cell hydrolysates contain L-l-diaminopimelic acid and glucose and galactose. The major fatty acids are iso-C16:0ω9c, C17:1ω9c and iso-C15:0ω5c. The polar lipid profile consists of diphasphatidylglycerol, phosphatidylglycerol, phosphatidylglycositol and an unknown phospholipid. MK-8(H4) is the predominant menaquinone. The DNA G + C content of the type strain is 73.7 mol %.

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Acknowledgements

We are grateful to Miss Haruna Ashizawa and Mr Yuya Sakuraki for their technical assistance. This study was supported in part by a research grant from the Institute for Fermentation, Osaka (IFO).

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