Nocardioides daejeonensis sp. nov., a denitrifying bacterium isolated from sludge in a sewage-disposal plant

Sung-Geun Woo,^{1,2} Sathiyaraj Srinivasan,^{1,3} Jihoon Yang,^{1,2}
Yong-An Jung,⁴ Myung Kyum Kim³ and Myungjin Lee¹

Correspondence

Myungjin Lee
mgeneli@nate.com

¹Research and Development Division, H-Plus Eco Ltd, BVC 301, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Eoeun-dong, Yuseong-gu, Daejeon 305-333, Republic of Korea

²School of Civil and Environmental Engineering, Yonsei University, Seoul 120-749, Republic of Korea

³Department of Bio and Environmental Technology, Division of Environmental and Life Science, College of Natural Science, Seoul Women's University, 623 Hwarangno, Nowon-gu, Seoul 139-774, Republic of Korea

⁴Korea Testing Certification, Chemical Industry Team, 587-10, Sinsa-Dong, Gangnam-Gu, Seoul, Republic of Korea

Strain MJ31^T, a Gram-reaction-positive, aerobic, rod-shaped, non-motile bacterium, was isolated from a sludge sample collected at the Daejeon sewage-disposal plant, in South Korea, and characterized in order to determine its taxonomic position. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain MJ31^T belonged to the genus *Nocardioides*, appearing most closely related to *Nocardioides dubius* KSL-104^T (98.6 % sequence similarity), *Nocardioides jensenii* DSM 20641^T (97.6 %), *Nocardioides daedukensis* MDN22^T (97.2 %) and *Nocardioides mesophilus* MSL-22^T (97.0 %). The chemotaxonomic properties of strain MJ31^T were consistent with those of the genus *Nocardioides*: MK-8(H₄) was the predominant menaquinone, iso-C_{16:0}, iso-C_{17:0} and C_{18:1ω9c} were the predominant cellular fatty acids, and the cell-wall peptidoglycan was based on LL-2,6-diaminopimelic acid. The genomic DNA G + C content of strain MJ31^T was 71.2 mol%. Some differential phenotypic properties and low DNA–DNA relatedness values (<28 %) with the type strains of closely related species indicated that strain MJ31^T represents a novel species, for which the name *Nocardioides daejeonensis* sp. nov. is proposed. The type strain is MJ31^T (=KCTC 19772^T=JCM 16922^T).

The genus *Nocardioides* was first described by Prauser (1976) with a single species, *Nocardioides albus*. At the time of writing, the genus *Nocardioides* comprised 51 species, many of which were isolated from soils from different parts of the world (Prauser, 1976, 1984; O'Donnell *et al.*, 1982; Suzuki & Komagata, 1983). In the course of investigating the micro-organisms in the sludge at a sewage-disposal plant in South Korea, a Gram-reaction-positive, non-motile, aerobic bacterium, strain MJ31^T, was isolated. The

taxonomic position of this bacterium has now been investigated using a polyphasic approach.

Strain MJ31^T was isolated from sludge at the Daejeon sewage-disposal plant in South Korea. For the isolation, 1 g fresh sludge was mixed with 50 ml saline [0.85 % (w/v) NaCl], vortexed and serially diluted. A 100 µl sample of each dilution was then spread onto 0.1 × R2A agar (Difco) and incubated at 30 °C for 1 week. Single colonies of strain MJ31^T were transferred onto fresh plates and the purified colonies were tentatively identified by analysis of partial 16S rRNA gene sequences. The isolate was routinely cultured on nutrient agar (NA; Difco) at 30 °C and preserved, as a suspension in Luria–Bertani broth (LB broth; Difco) with 20 % (w/v) glycerol, at –70 °C.

Cell morphology and motility were observed under a light microscope (BH2; Olympus) and a transmission electron

Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MJ31^T is JF937066.

A supplementary table and three supplementary figures are available with the online version of this paper.

microscope (LEO912AB; Carl Zeiss), using cells grown for 24 h in nutrient broth at 30 °C. For electron microscopy, cells were gently resuspended in a drop of deionized water, placed on a carbon- and Formvar-coated nickel grid and allowed to settle for 30 s, before the grid was floated on a drop of 0.1 % (w/v) aqueous uranyl acetate, blotted dry and then viewed at 100 kV. The Gram reaction was performed using the non-staining (KOH) method, as described by Buck (1982). Catalase activity was assessed by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was assessed using 1 % (w/v) tetramethyl *p*-phenylenediamine (Cappuccino & Sherman, 2002). Anaerobic growth was examined in serum bottles, using nutrient broth with sodium thioglycolate (1 g l⁻¹) and replacing the air in the bottles with nitrogen.

Physiological characteristics were determined by using the API 20NE, API ID 32GN and API ZYM galleries (bioMérieux) according to the manufacturer's instructions. Growth at 4, 10, 20, 25, 28, 30, 35, 37, 40, 42 and 45 °C and pH 3.0–12.0 (at intervals of 0.5 pH unit) was assessed in LB broth for 5 days. The pH of the medium was adjusted using diluted NaOH or HCl. Salt tolerance was tested in R2A broth supplemented with 0–15 % (w/v) NaCl, with incubation for 5 days at 30 °C. Growth on trypticase soy agar (TSA; Difco), R2A agar and LB agar was also evaluated at 30 °C. Most of the phenotypic characteristics of strain MJ31^T are given in the species description; those that differentiated strain MJ31^T from closely related type strains are listed in Table 1.

The genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995) and then enzymatically degraded into nucleosides so that its G+C content could be determined as described by Mesbah *et al.* (1989) using reverse-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum and re-extracted in *n*-hexane/water (1:1, v/v). The crude *n*-hexane/quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by HPLC as previously described (Hiraishi *et al.*, 1996). In order to perform fatty acid methyl ester analysis, strain MJ31^T and closely related type strains were grown on TSA for 48 h at 30 °C. Two loopfuls of bacterial mass of each strain were collected and subjected to saponification, methylation and extraction using the methods of Kuykendall *et al.* (1988). The fatty acid methyl ester mixtures were separated using version 6.0 of the Sherlock Microbial Identification System (MIDI), analysed by GC (6890; Hewlett Packard) and identified with the Microbial Identification software package (Sasser, 1990). To determine the polar lipid profile of strain MJ31^T, the strain's total cellular polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1977).

Extraction of genomic DNA was performed with a commercial genomic DNA extraction kit (Solgent) before PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to

Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999) and evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were then constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms in the MEGA3 program (Kumar *et al.*, 2004). To obtain confidence levels for the branches of these trees, bootstrap analyses were conducted, with 1000 replicates (Felsenstein, 1985). A maximum-likelihood tree was also constructed, using the PHYLIP program (Choi *et al.*, 2000; Brinkman *et al.*, 2001).

DNA–DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and micro-dilution wells. The hybridizations were carried out reciprocally, with five replications per sample. The highest and lowest values obtained for each sample were excluded and the remaining three values were utilized in the calculation of each of the mean relatedness values that are reported below.

The 16S rRNA gene sequence of the strain MJ31^T determined in this study, which was a continuous stretch of 1468 bp, appeared most similar to the corresponding sequences of *Nocardioides dubius* KSL-104^T (98.6 % sequence similarity), *Nocardioides jensenii* DSM 20641^T (97.6 %), *Nocardioides daedukensis* MDN22^T (97.2 %) and *Nocardioides mesophilus* MSL-22^T (97.0 %). Sequence similarities between the novel strain and other members of the genus *Nocardioides* or members of the genus *Marmoricola* were all 97.0 % or lower. In the neighbour-joining phylogenetic tree (Fig. 1) strain MJ31^T was clustered with *N. dubius* KSL-104^T (with a bootstrap value of 97 %), close to species of *Marmoricola* and *N. mesophilus* MSL-22^T. In the maximum-parsimony and maximum-likelihood trees (Figs S1 and S2, available in IJSEM Online), strain MJ31^T was again clustered with *N. dubius* KSL-104^T but this cluster was closer to most other members of the genus *Nocardioides* than to species of *Marmoricola*.

The results obtained from the chemotaxonomic analyses were in agreement with the results of phylogenetic inference, indicating that strain MJ31^T belongs to the genus *Nocardioides*. The major menaquinone detected for this strain was MK-8(H₄). The overall fatty acid composition of strain MJ31^T (Table S1) was very similar to those of other members of the genus *Nocardioides*, being dominated by large amounts of iso-branched and unsaturated fatty acids, particularly iso-C_{16:0} (34.7 %), iso-C_{17:0} (15.4 %) and C_{18:1}ω9c (11.6 %). However, there were some qualitative and quantitative differences in the fatty acid contents of the novel strain and the closely related type strains. For example, although all the closely related type strains of *Nocardioides* species included in Table S1 contained iso-C_{14:0}, C_{18:1}ω7c and C_{16:0} 2-OH, none of these fatty acids was detected in

Table 1. Some selective phenotypic characteristics of strain MJ31^T and the type strains of related *Nocardioides* species

Strains: 1, MJ31^T; 2, *N. alkalitolerans* KCTC 19037^T; 3, *N. daedukensis* KCTC 19601^T; 4, *N. dubius* KCTC 9992^T; 5, *N. jensenii* KCTC 9134^T; 6, *N. mesophilus* KCTC 19310^T; 7, *N. salarius* DSM 18239^T. All data were obtained in the present study. +, Positive reaction; –, negative reaction; w, weakly positive reaction.

Characteristic	1	2	3	4	5	6	7
Motility	–	–	–	+	–	+	–
Cell length (µm)	0.8–2.0	1.5–2.0	0.8–3.0	1.5–2.5	3.0–7.0	0.9–1.4	0.6–1.6
Cell width (µm)	0.5–0.8	0.8–1.0	0.4–0.8	0.8–1.0	0.6–0.8	0.3–1.4	0.3–0.6
Colony colour	Pale yellow	Milky white	Yellowish white	Yellowish white	Yellowish white	Cream white	Cream
Optimum growth temperature (°C)	30	25	30	30	30	28	25
Temperature range (°C)	15–37	4–35	4–37	20–37	15–37	15–37	10–35
Optimum pH	7.5–8.0	7.0–9.0	7.0–8.0	7.0–8.5	7.0–7.5	7.0–7.5	6.0–7.0
pH range	5.5–9.0	5.5–12.0	5.5–8.5	6.0–8.5	5.5–8.0	6.0–9.0	6.0–9.5
NaCl range (%)	0–10	0–5	0–9	0–7	0–5	0–7	0–10
Enzyme activity:							
N-Acetyl-β-glucosaminidase	–	–	–	–	+	–	–
Acid phosphatase	+	+	–	–	w	+	–
Cystine arylamidase	w	–	w	+	–	–	w
Esterase (C4)	w	+	+	+	–	+	+
Esterase (C8)	+	+	+	+	–	+	+
α-Glucosidase (starch hydrolysis)	–	+	–	+	+	–	+
β-Glucosidase	+	–	w	+	–	–	–
β-Glucuronidase	–	–	–	–	–	–	–
Leucine arylamidase	+	+	+	+	+	–	+
Lipase (C14)	–	+	–	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	+	w	w	w	w	+	–
Trypsin	w	–	–	+	–	–	+
Valine arylamidase	+	w	+	+	w	–	w
Assimilation of:							
3-Hydroxybenzoate	+	–	+	+	–	+	–
4-Hydroxybenzoate	+	w	+	w	+	+	w
DL-3-Hydroxybutyrate	–	+	–	–	–	w	–
2-Ketogluconate (α)	+	+	+	–	+	w	+
Caprate	+	+	+	w	+	–	+
Citrate	+	+	+	–	+	+	+
Itaconate	w	–	w	+	–	–	–
DL-Lactate	+	–	+	–	–	+	+
Propionate	–	–	–	+	–	w	–
Suberate	–	+	–	+	–	–	–
Maltose	+	–	–	–	–	–	–
Melibiose	–	–	–	–	–	+	–
L-Rhamnose	+	+	–	–	+	+	–
D-Ribose	–	w	w	–	–	+	–
Sucrose	–	+	–	–	–	+	+
myo-Inositol	–	w	–	+	–	+	+
D-Mannitol	–	+	–	–	+	+	+
D-Sorbitol	–	+	–	–	w	+	–
L-Alanine	–	–	–	–	–	+	–
N-Acetyl-D-glucosamine	–	w	–	–	+	+	+
DNA G + C content (mol%)	71.2	73.6	68.7	70.6	68.8	78.7	73.0

strain MJ31^T. In terms of their fatty acids, strain MJ31^T and the type strains of *Nocardioides* species included in Table S1 were, however, clearly distinguishable from the type strains of *Marmoricola aurantiacus* (the type species of the genus *Marmoricola*) and *Marmoricola bigeumensis*.

Total lipids were observed in TLC after staining with 5 % ethanolic molybdophosphoric acid and ninhydrin reagent or with Zinzadze reagent (Fig. S3). Strain MJ31^T contained major amounts of diphosphatidylglycerol (DPG), moderate amounts of phosphatidylethanolamine (PE) and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain MJ31^T, *Nocardioide* species and other related genera. Percentage bootstrap values >50% (based on 1000 replications) are shown at branch points. *Terrabacter tumescens* ATCC 6947^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

phosphatidylglycerol (PG) and minor amounts of two unknown phospholipids (PL1 and PL2). The polar lipid profile of strain MJ31^T was similar to those of established *Nocardioide* species (Yoon *et al.*, 2010). The genomic DNA G+C content of strain MJ31^T was 71.2 mol%.

To differentiate strain MJ31^T from the closely related *Nocardioide* species, DNA–DNA hybridizations were performed. Strain MJ31^T exhibited relatively low DNA–DNA relatedness values with *Nocardioide alkalitolerans* KCTC 19037^T (7.5%), *N. daedukensis* KCTC 19601^T (18.2%), *N. dubius* KCTC 9992^T (28.2%), *N. jensenii* KCTC 9134^T (24.0%), *N. mesophilus* KCTC 19310^T (14.6%) and *Nocardioide salarius* DSM 18239^T (8.8%). These results indicated that strain MJ31^T is not related to any established species in the genus *Nocardioide* (Wayne *et al.*, 1987).

There is insufficient taxonomic evidence currently available to differentiate several *Nocardioide* species from the members of the genus *Marmoricola* (Yoon *et al.*, 2005; Yamamura *et al.*, 2011). In the present study, however, strain MJ31^T and the type strains of closely related *Nocardioide* species could be differentiated from members of the genus *Marmoricola* by their fatty acid profiles (Table S1) and by having rod/coccoid life cycles. It was possible to differentiate strain MJ31^T from the type strains of closely related species by using several phenotypic and chemotaxonomic characteristics. In addition, in the phylogenetic trees constructed using the maximum-parsimony and maximum-likelihood algorithms (Figs S1 and S2), strain MJ31^T formed a monophyletic clade with *N. dubius* and this clade formed

part of a coherent phylogenetic cluster with most other members of the genus *Nocardioide*. Based on this evidence, both strain MJ31^T and *N. dubius* belong to the genus *Nocardioide* and not to the genus *Marmoricola*.

On the basis of the phylogenetic, genetic, phenotypic and chemotaxonomic data presented, strain MJ31^T represents a novel species in the genus *Nocardioide*, for which the name *Nocardioide daejeonensis* sp. nov. is proposed.

Description of *Nocardioide daejeonensis* sp. nov.

Nocardioide daejeonensis (da.e.je.on.en'sis. N.L. masc. adj. *daejeonensis* of or pertaining to Daejeon, the city in South Korea where the type strain was isolated).

Cells are Gram-reaction-positive, aerobic, non-motile and rod-shaped, measuring about 0.5–0.8 µm in width and 0.8–2.0 µm in length. Cells showed rod-to-coccus morphogenesis between the early exponential phase and stationary phase. Colonies grown on NA for 2 days are circular, raised, smooth, and pale yellow in colour. Growth occurs at 15–37 °C (optimum about 30 °C), at pH values of 5.5–9.0 (optimum between 7.5 and 8.0), and in the presence of 0–10% (w/v) NaCl (optimum, 2%). Oxidase-negative and catalase-positive. Positive (API 20NE) for nitrate and nitrite reduction and aesculin hydrolysis but negative for indole production, acid production from glucose and arginine dihydrolase, urease and gelatinase activities. In API 20NE and API 32GN tests, L-arabinose, caprate, citrate,

3-hydroxybenzoate, 4-hydroxybenzoate, 2-ketogluconate, DL-lactate, maltose, malonate, L-rhamnose and L-serine are utilized but *N*-acetyl-D-glucosamine, adipate, L-alanine, L-fucose, gluconate, D-glucose, glycogen, L-histidine, 3-hydroxybutyrate, 5-ketogluconate, malate, D-mannose, D-mannitol, melibiose, *myo*-inositol, phenylacetate, L-proline, propionate, D-ribose, salicin, D-sorbitol, suberate, sucrose and *n*-valerate are not. Positive (API ZYM) for acid phosphatase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, β -glucosidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase, cystine arylamidase (weak), esterase (C4) (weak) and trypsin (weak) activities but negative for acetyl- β -glucosaminidase, α -chymotrypsin, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, *N*- α -mannosidase and lipase (C14). The cell-wall peptidoglycan is based on LL-2-6-diaminopimelic acid and MK-8(H₄) is the predominant menaquinone. The major cellular fatty acids are iso-C_{16:0}, iso-C_{17:0} and C_{18:1} ω 9c. The polar lipid profile contains major amounts of DPG and moderate amounts of PE and PG.

The type strain, MJ31^T (=KCTC 19772^T=JCM 16922^T), was isolated from sludge at the Daejeon sewage-disposal plant. The genomic DNA G+C content of the type strain is 71.2 mol%.

Acknowledgements

This work was supported by the GAIA Project (grant 173-101-034) of the Ministry of Environment, Republic of Korea.

References

- Brinkman, F. S., Wan, I., Hancock, R. E., Rose, A. M. & Jones, S. J. (2001). PhyloBLAST: facilitating phylogenetic analysis of BLAST results. *Bioinformatics* **17**, 385–387.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin Cummings.
- Choi, J. H., Jung, H. Y., Kim, H. S. & Cho, H. G. (2000). PhyloDraw: a phylogenetic tree drawing system. *Bioinformatics* **16**, 1056–1058.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996). Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β -glucosidase-producing bacterium in the family Sphingomonadaceae in α -4 subclass of the Proteobacteria. *J Microbiol* **43**, 152–157.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Moore, D. D. & Dowhan, D. (1995). Preparation and Analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. W. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- O'Donnell, A. G., Goodfellow, M. & Minnikin, D. E. (1982). Lipids in the classification of *Nocardioideae*: reclassification of *Arthrobacter simplex* (Jensen) Lochhead in the genus *Nocardioideae* (Prauser) emend. O'Donnell et al. as *Nocardioideae simplex* comb. nov. *Arch Microbiol* **133**, 323–329.
- Prauser, H. (1976). *Nocardioideae*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **26**, 58–65.
- Prauser, H. (1984). *Nocardioideae luteus* spec. nov. *Z Allg Mikrobiol* **24**, 647–648.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Suzuki, K. & Komagata, K. (1983). *Pimelobacter* gen. nov., a new genus of coryneform bacteria with LL-diaminopimelic acid in the cell wall. *J Gen Appl Microbiol* **29**, 59–71.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yamamura, H., Ohkubo, S. Y., Nakagawa, Y., Ishida, Y., Hamada, M., Ootoguro, M., Tamura, T. & Hayakawa, M. (2011). *Nocardioideae iriomotensis* sp. nov., an actinobacterium isolated from a forest soil. *Int J Syst Evol Microbiol* **61**, 2205–2209.
- Yoon, J. H., Lee, C. H. & Oh, T. K. (2005). *Nocardioideae dubius* sp. nov., isolated from an alkaline soil. *Int J Syst Evol Microbiol* **55**, 2209–2212.
- Yoon, J. H., Park, S., Jang, S. J., Lee, J. S., Lee, K. C. & Oh, T. K. (2010). *Nocardioideae daedukensis* sp. nov., a halotolerant bacterium isolated from soil. *Int J Syst Evol Microbiol* **60**, 1334–1338.