

Nocardioides zeae sp. nov., isolated from the stem of *Zea mays*

Stefanie P. Glaeser,¹ John A. McInroy,² Hans-Jürgen Busse³ and Peter Kämpfer¹

Correspondence

Peter Kämpfer
peter.kaempfer@umwelt.uni-giessen.de

¹Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

²Auburn University, Department of Entomology and Plant Pathology, Auburn, Alabama 36849, USA

³Abteilung für Klinische Mikrobiologie und Infektionsbiologie, Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität Wien, A-1210 Wien, Austria

A Gram-stain-positive aerobic organism, isolated from the healthy stem of a *Zea mays* plant was studied for its taxonomic position. Based on 16S rRNA gene sequence analysis strain JM-1068^T was most closely related to *Nocardioides alkalitolerans* (97.2%). The 16S rRNA gene sequence similarity to all other species of the genus *Nocardioides* was ≤96.1%. The quinone system of strain JM-1068^T contained the major menaquinone MK-8(H₄). The diagnostic diamino acid of the peptidoglycan was LL-diaminopimelic acid. In the polar lipid profile, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and two unidentified phospholipids were predominant. The polyamine pattern contained predominantly spermidine and spermine. The fatty acid profile was composed of iso-C_{16:0} and C_{18:1ω9c} in addition to C_{16:0}, C_{17:0} and C_{17:1ω8c} and low amounts of C_{16:0} 2-OH and C_{17:0} 2-OH. This supported the allocation of the strain to the genus *Nocardioides*. In addition, the results of physiological and biochemical tests also allowed phenotypic differentiation of strain JM-1068^T from *N. alkalitolerans*. It is concluded that JM-1068^T represents a novel species of the genus *Nocardioides*, for which we propose the name *Nocardioides zeae* sp. nov., with JM-1068^T (=CIP 110696^T=LMG 28079^T) as the type strain.

The genus *Nocardioides* was proposed by Prauser (1976) with *Nocardioides albus* as the type species. The genus comprises Gram-stain-positive, non-acid-fast, catalase-positive, aerobic and mesophilic nocardioform actinomycetes, which may develop a mycelium that easily fragments into irregular rod- to coccus-like elements. Chemotaxonomically the species are characterized by a quinone system with the major menaquinone MK-8(H₄) and LL-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan. The fatty acid profile contains both branched and straight chain fatty acids (O'Donnell *et al.*, 1982). At the time of writing, the genus *Nocardioides* comprised 68 species with validly published names (<http://www.bacterio.net/nocardioides.html>; Euzéby, 1997), many of them isolated from a wide variety of sources, like soils, sediments, sand, water, herbage, an oil shale column and glacier cryoconite. Some species of the genus *Nocardioides* have been isolated as endophytes from different plant species, e.g. *Nocardioides caricicola* from the halophyte, *Carex scabrifolia* (Song *et al.*, 2011), *Nocardioides panzhihuensis*

from the medicinal plant, *Jatropha curcas* (Qin *et al.*, 2012), *Nocardioides perillae* from surface-sterilized roots of *Perilla frutescens* (Du *et al.*, 2013) and just recently *Nocardioides endophyticus* and *Nocardioides conyzicola* from surface-sterilized roots of mugwort (*Artemisia princeps*) and horseweed (*Conyza canadensis*) (Han *et al.*, 2013).

Strain JM-1068^T was isolated in July 1990 from internal stem tissue of healthy corn (*Zea mays*, cultivar 'Sweet Belle') 10 weeks after planting in the field at the Plant Breeding Unit facility, E.V. Smith Research Center in Tallassee, Alabama, USA as described in detail previously (Kämpfer *et al.*, 2014). The strain showed single cells forming small pale yellow colonies (<0.5 mm) with a smooth surface after 48 h at 28 °C on Tryptone Soy Agar (TSA, Oxoid). Cell morphology was investigated for cells grown on TSA at 28 °C by phase-contrast microscopy. During exponential growth rod-shaped to coccoide cells of strain JM-1068^T were 0.9–1.5 µm wide and 1.5–2.5 µm long and showed no motility in the early exponential growth phase. Cells stained Gram-positive (analysed as described by Gerhardt *et al.*, 1994) and were also positive for cytochrome oxidase, determined by using an oxidase test (Merck). Endospores could not be detected. Temperature dependent growth was determined at

Abbreviation: OH-PG, phosphatidylglycerol containing OH group(s).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JM-1068^T is KF900163.

TSA at 4, 15, 25, 28, 32, 37 and 42 °C. Salinity and pH dependent growth were analysed in tryptic soy broth (Difco) either supplemented with 1–10 % (w/v) NaCl (increasing in 0.5 % steps) or adjusted to pH values between pH 4 and pH 12 (increasing in 0.5 pH-unit increments by the addition of HCl or NaOH); both were cultured at 28 °C.

Phylogenetic analysis was performed in ARB release 5.2 (Ludwig *et al.*, 2004) using the 'All-Species Living Tree' Project (LTP) (Yarza *et al.*, 2008) database release s111 (February 2013). Sequences not included in the LTP database were aligned with SINA (v1.2.9; Pruesse *et al.*, 2012) according to the SILVA seed alignment (<http://www.arb-silva.de>; Pruesse *et al.*, 2007) and implemented in the ARB database. The alignment was checked manually, based on secondary structure information. Pairwise sequence similarities were calculated in ARB without the use of an evolutionary substitution model. Phylogenetic trees were

reconstructed with the maximum-likelihood method using RAXML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis and the neighbour-joining method (ARB neighbour-joining) with the Jukes–Cantor correction (Jukes & Cantor, 1969). Phylogenetic trees were calculated with 100 resamplings for bootstrap analysis (Felsenstein, 1985) and based on 16S rRNA gene sequences between positions 125 and 1385 (according to *Escherichia coli* numbering, Brosius *et al.*, 1978).

The 16S rRNA gene sequence of strain JM-1068^T is a continuous stretch of 1438 nt spanning *E. coli* positions 18 to 1474 (*E. coli* numbering). Phylogenetic analysis placed strain JM-1068^T into the genus *Nocardioide*s with highest 16S rRNA gene sequence similarity to the type strain of *Nocardioide*s *alkalitolerans* (97.2 %). The 16S rRNA gene sequence similarities to all other type strains of species of the genus *Nocardioide*s were between 93 and 96.1 %.

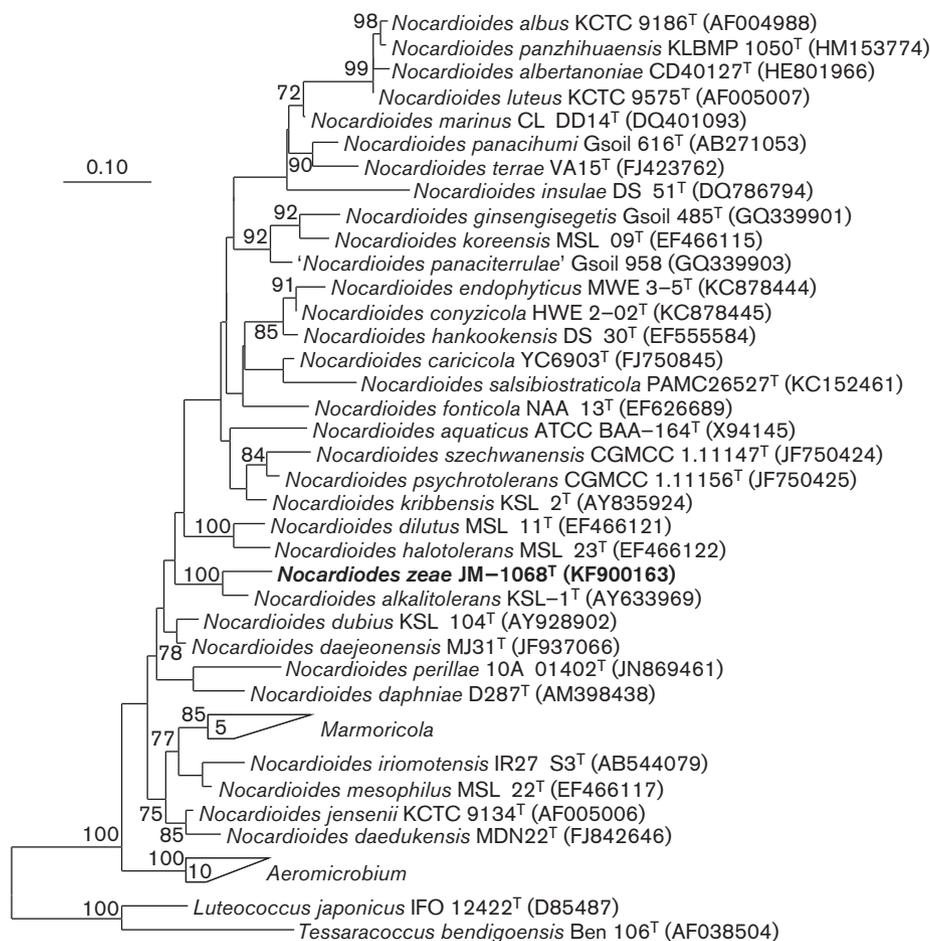


Fig. 1. Reduced maximum-likelihood tree showing the phylogenetic position of strain JM-1068^T among type strains of species of the genus *Nocardioide*s and type strains of species of the most closely related genera. The tree was generated in ARB using RAXML (GTR-GAMMA, rapid bootstrap analysis) and based on nucleotide sequences among 16S rRNA gene sequence positions 125–1385 (according to *E. coli* numbering). Bootstrap values ≥ 70 % given in the tree. *Luteococcus japonicus* IFO 12422^T and *Tessaracoccus bendigoensis* Ben 106^T were used as outgroup. Bar, 0.1 substitutions per nucleotide position. Several of the type strains of species of the genus *Nocardioide*s, which were not directly related to JM-1068^T, were removed from the tree after tree reconstruction.

Phylogenetic trees showed that strain JM-1068^T formed a distinct cluster with the type strain of *Nocardiooides alkalitolerans* (bootstrap support of 100 %) (Fig. 1), independent of the applied treeing method,

Biomass subjected to analyses of polyamines, diamino acid, quinones and polar lipids was grown in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 28 °C. For polyamine analysis biomass was harvested at the late exponential growth phase as recommended by Altenburger *et al.* (1997) whereas biomasses use for extraction of diamino acids, quinones and polar lipids were harvested at the stationary growth phase. Polyamines were extracted as reported by Busse & Auling (1988) and Altenburger *et al.* (1997) and analysed using HPLC conditions described by Busse *et al.* (1997). Diamino acid extraction was carried out according to the protocol of Schumann (2011). Quinones and polar lipids were extracted and analysed as described by Tindall (1990a, b) and Altenburger *et al.* (1996). The HPLC apparatus used was described by Stolz *et al.* (2007). The diagnostic diamino acid of the peptidoglycan was LL-diaminopimelic acid. Strain JM-1068^T showed a complex quinone system which contains 75.6 % menaquinone MK-8(H₄), 10.5 % MK-7(H₄), 5.8 % MK-7(H₂), 2.4 % MK-9(H₄), 2.2 % MK-9, 1.8 % MK-8(H₂) and traces (<1 %) of MK-7, MK-8 and MK-9(H₂). The polar lipid profile (Fig. 2) consisted of the major lipids diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, two unidentified phospholipids (PL1, 2),

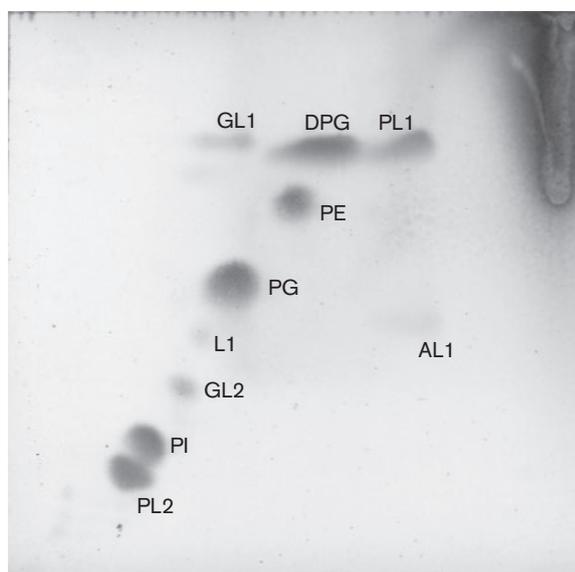


Fig. 2. Polar lipid profile of strain JM-1068^T after two-dimensional TLC and detection with molybdato-phosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; GL1, 2, unidentified glycolipids; AL1, unidentified aminolipid; PL1, PL2, unidentified phospholipids; L1, unidentified polar lipid. First dimension, →; second dimension, ↑.

moderate amounts of two unidentified glycolipids (GL1, 2), an unidentified aminolipid (AL1) and traces of a lipid not containing a sugar residue, an amino group or a phosphate group. Collins *et al.* (1983) reported the presence of two phosphatidylglycerols containing OH groups (OH-PGs) in *N. albus* and *N. luteus*, which show a chromatographic motility corresponding to phosphatidylinositol (identified by co-chromatography of authentic standard and extract on the same TLC plate; results not shown) and PL2 in Fig. 2. Since Collins *et al.* (1983) concluded the identity of the two lipid spots only on the basis of positive reactions to the lipid phosphate and periodate-Schiff reagents it appears to be possible that at least the OH-PG

Table 1. Major fatty acid composition of strain JM-1068^T and the type strains of the most closely related species *N. alkalitolerans* and the type species *N. albus*

Strains: 1, JM-1068^T; 2, *Nocardiooides alkalitolerans* DSM 16699^T [data from this study (data in parentheses from Yoon *et al.*, 2005)]; 3, *Nocardiooides albus* DSM 43109^T (data from Alias-Villegas *et al.*, 2013). All data were obtained from cells grown on TSA after 3 days at 28 °C.

Fatty acids	1	2	3
Saturated			
C _{15:0}	2.4	1.1	–
C _{16:0}	12.2	6.4 (5.9)	2.9
C _{17:0}	12.2	6.0 (3.1)	1.4
C _{18:0}	7.7	3.4 (5.5)	2.2
Unsaturated			
C _{17:1ω6c}	4.3	9.2 (10.0)	4.5
C _{17:1ω8c}	7.7	3.7 (1.6)	1.5
C _{18:1ω7c}	–	0.8 (1.0)	–
C _{18:1ω9c}	23.8	9.6 (12.2)	5.8
Branched			
iso-C _{14:0}	–	0.8	1.1
iso-C _{15:0}	–	0.5 (1.1)	1.2
iso-C _{16:0}	14.7	37.8 (20.7)	58.0
iso-C _{16:1H}	–	–	0.7
iso-C _{17:0}	–	0.5 (1.3)	–
anteiso-C _{17:0}	–	(0.8)	1.1
iso-C _{18:0}	–	3.6 (5.2)	–
iso-C _{18:1}	–	–	1.7
Hydroxy			
C _{16:0} 2-OH	2.9	1.5 (2.1)	–
C _{17:0} 2-OH	2.1	1.3 (0.8)	–
10-Methyl			
C _{16:0} 10-methyl	–	0.5	2.6
C _{17:0} 10-methyl	3.0	4.5 (2.8)	4.0
C _{18:0} 10-methyl (TBSA)	6.8	7.3 (23.2)	8.3
Summed features*			
Summed feature 3	–	0.7	1.0
Summed feature 6	–	0.8	–

*Summed feature 3 comprises C_{16:1ω7c} and/or iso-C_{15:0} 2-OH; summed feature 6 comprises C_{19:1ω9c} and/or C_{19:1ω11c}.

lipid spot corresponding to phosphatidylinositol in our analysis was misidentified. The polyamine pattern contains [$\mu\text{mol (g dry weight)}^{-1}$]: 8.04 spermidine, 3.90 spermine, 0.61 putrescine, 0.33 tyramine, 0.20 cadaverine, 0.16 *sym*-homospermidine and 0.03 1,3-diaminopropane. The detection of LL-diaminopimelic acid in the peptidoglycan and the quinone system consisting predominantly of menaquinone MK-8(H₄) is well in agreement with the description of the genus *Nocardioides* (O'Donnell *et al.*, 1982). The polar lipid profile of strain JM-1068^T shows some similarities to those of other species of the genus *Nocardioides* in respect of the presence of phospholipids, including the type species of the genus, *N. albus*, *N. luteus*, *N. simplex* (O'Donnell *et al.*,

1982; Collins *et al.*, 1983) and *N. dubius* (Yoon *et al.*, 2005) the latter species being one of the closest relatives of strain JM-1068^T (Fig. 1). However, among 68 established species, the results of polar lipid analysis of only 21 species have been reported and only the presence of phosphatidylglycerol is common to all other species in the genus. The presence of diphosphatidylglycerol was reported for the majority of species, phosphatidylinositol was present in half of the tested species and phosphatidylethanolamine and phosphatidylcholine in three and four species, respectively. This intra-generic heterogeneity in polar lipid analyses to some degree is also indicated by low 16S rRNA gene sequence similarities between the most distantly related species (approximately

Table 2. Differential phenotypic characteristics of JM-1068^T and the type strains of the closest related species *N. alkalitolerans* and the type species *N. albus*

Strains: 1, JM-1068^T; 2, *N. alkalitolerans* DSM 16699^T (data from this study, data in parentheses from Yoon *et al.*, 2005); 3, *N. albus* DSM 43109^T (data are from Yoon *et al.*, 2004). +, Positive; -, negative; ND, not determined; w, weakly positive; v, variable; data in parentheses are for the type strain. All strains are positive for Gram staining and catalase. All strains were negative for the utilization of inositol, lactose and D-ribose.

Characteristic	1	2	3
Cell morphology	Rods, cocci	Rods, cocci	Hyphae
Cell size (μm)	0.9–1.5 \times 1.5–2.5	0.8–1.0 \times 1.5–2.0	0.5–1.0
Motility	–	–	–
Colony colour*	Beige to yellowish	Milky white	Whitish to faintly brownish
Temperature range ($^{\circ}\text{C}$)	15–36	25–30	28
Hydrolysis of:			
Aesculin	–	–*	(w)
Casein	+	+*	(+)
Hypoxanthine	–	–*	(+)
Xanthine	–	–*	(+)
Tyrosine	–	+*	(+)
Utilization of:			
L-Arabinose	–	+*	+
Cellobiose	–	+*	(+)
D-Fructose	+	–†	+
D-Galactose	–	–*	(+)
D-Glucose	+	–*	+
Maltose	–	v(–)*	ND
D-Mannitol	–	–*	+
D-Mannose	–	–	(+)
Melibiose	–	–*	ND
L-Rhamnose	+	–*	+
Sucrose	+	–*	v (+)‡
Trehalose	+	+*	ND
D-Xylose	+	–*	+
Enzyme activities:			
Alkaline phosphatase	–	+*	(+)
β -Galactosidase	–	–*	(v)
α -Glucosidase	+	+*	(+)
β -Glucosidase	–	–*	(w)
DNA G + C content (mol%)	ND	72.4–73.6	67
Isolation source	Plant	Alkaline soil	Soil

*Identical results in this study and the study of Yoon *et al.* (2005).

†Result differs from that obtained by Yi & Chun (2004).

‡Data from Prauser (1976, 1984), Collins *et al.* (1994), Yoon *et al.* (1997; 1999; 2004) and Lawson *et al.* (2000).

92%), which may indicate that the genus *Nocardiooides* should be split into several genera. The polyamine pattern of strain JM-1068^T with the major compounds spermidine and spermine clearly differentiates it from *N. albus*, *N. luteus*, *N. jensenii* and *N. plantarum*, which were reported to contain polyamine patterns with the major compounds putrescine and cadaverine or cadaverine and spermine (Busse & Schumann, 1999). In addition, these variations in polyamine patterns may indicate that the genus should be divided into several genera.

Fatty acids analysis of cells, grown in TSA at 28 °C was done as described by Kämpfer & Kroppenstedt (1996). The fatty acid profile comprised predominant unsaturated fatty acids (mainly C_{18:1}ω9), an iso-branched fatty acid (iso-C_{16:0}), and saturated fatty acids (mainly C_{16:0} and C_{17:0}) and was similar to the closest related species, *N. alkalitolerans*. It was obvious, that strain JM-1068^T produced, like *N. alkalitolerans*, the hydroxylated fatty acids C_{16:0} 2-OH and C_{17:0} 2-OH, which were not found in the other species of the genus *Nocardiooides*, as shown for the type strain of the type species in Table 1. However, previously Collins *et al.* (1983) reported in *N. albus* the presence of 9% iso-C_{16:0} 2-OH. Hence, the presence of hydroxylated fatty acids is not an unusual trait within the genus. The detailed fatty acid profiles are shown in Table 1.

The results of the physiological characterization, performed using methods described previously (Kämpfer, 1990; Kämpfer *et al.*, 1991), are given in Table 2 and in the species description. Strain JM-1068^T was able to utilize several sugars or sugar-related compounds. A distinct physiological biochemical profile allowed differentiation of the strain from the type strain of *N. alkalitolerans*. Based on the low 16S rRNA gene sequence similarities (<96.1%) to all other species of the genus with validly published names, DNA–DNA hybridizations were not performed. From the results of the phylogenetic and chemotaxonomic analyses, it is obvious that strain JM-1068^T represents a novel species, which is allocated to the genus *Nocardiooides*. For this species, we propose the name *Nocardiooides zeae*.

Description of *Nocardiooides zeae* sp. nov.

Nocardiooides zeae (ze'ae. L. gen. n. zeae, of spelt, of *Zea mays*).

Cells are Gram-stain-positive, strictly aerobic rods (0.9–1.5 × 1.5–2.5 μm) and non-motile. Colonies grown on TSA are circular, convex and beige to yellowish. Optimal temperature for growth is 28 °C; growth occurs at 15–36 °C but not at 10 °C and 50 °C on TS agar. Optimal pH for growth is 7.0; growth occurs at pH 5.5–10.5. Growth occurs in the presence of 1–8% NaCl but not at higher concentrations in TS bouillon. The result of the test for catalase is positive, oxidase activity is weakly positive. No acid formation from sugars can be observed from D-glucose, D-xylose, lactose, sucrose, D-mannitol, dulcitol, salicin, D-adonitol, i-inositol, D-sorbitol, L-arabinose, raffinose, L-rhamnose, maltose, trehalose, cellobiose, erythritol,

melibiose and D-arabitol. Several sugar compounds are utilized: D-fructose, D-gluconate, D-glucose, L-rhamnose, sucrose, trehalose and D-xylose. L-Arabinose, N-acetyl-D-glucosamine, arbutin, cellobiose, D-galactose, gluconate, maltose, D-adonitol, myo-inositol, lactose, melibiose, ribose, D-sorbitol, salicin, D-maltitol, D-mannitol and D-mannose are not utilized. Major fatty acids are iso-C_{16:0} and C_{18:1}ω9c in addition to C_{16:0}, C_{17:0} and C_{17:1}ω8c. In addition, C_{16:0} 2-OH and C_{17:0} 2-OH are detected. The diagnostic diamino acid of the peptidoglycan is LL-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and two unidentified phospholipids. Moderate amounts of two unidentified glycolipids, an unidentified aminolipid and traces of a polar lipid not containing a sugar, phosphate or amino residue are present. The major quinone is menaquinone MK-8(H₄). In the polyamine pattern, spermidine and spermine are predominant.

The type strain JM-1068^T (=CIP 110696^T=LMG 28079^T) was isolated in July 1990 as an endophyte from internal stem tissue of healthy corn (*Zea mays*, cultivar 'Sweet Belle') 10 weeks after planting in the field at the Plant Breeding Unit facility, E.V. Smith Research Center in Tallassee, Alabama, USA.

References

- Alias-Villegas, C., Jurado, V., Laiz, L., Miller, A. Z. & Saiz-Jimenez, C. (2013). *Nocardiooides albertanoniae* sp. nov., isolated from Roman catacombs. *Int J Syst Evol Microbiol* **63**, 1280–1284.
- Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.
- Altenburger, P., Kämpfer, P., Akimov, V. N., Lubitz, W. & Busse, H.-J. (1997). Polyamine distribution in actinomycetes with group B peptidoglycan and species of the genera *Brevibacterium*, *Corynebacterium*, and *Tsukamurella*. *Int J Syst Bacteriol* **47**, 270–277.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Busse, H.-J. & Schumann, P. (1999). Polyamine profiles within genera of the class *Actinobacteria* with LL-diaminopimelic acid in the peptidoglycan. *Int J Syst Bacteriol* **49**, 179–184.
- Busse, H.-J., Bunka, S., Hensel, A. & Lubitz, W. (1997). Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* **47**, 698–708.
- Collins, M. D., Keddie, R. M. & Kroppenstedt, R. M. (1983). Lipid composition of *Arthrobacter simplex*, *Arthrobacter tumescens* and possibly related taxa. *Syst Appl Microbiol* **4**, 18–26.
- Collins, M. D., Cockcroft, S. & Wallbanks, S. (1994). Phylogenetic analysis of a new LL-diaminopimelic acid-containing coryneform bacterium from herbage, *Nocardiooides plantarum* sp. nov. *Int J Syst Bacteriol* **44**, 523–526.
- Du, H. J., Wei, Y. Z., Su, J., Liu, H. Y., Ma, B. P., Guo, B. L., Zhang, Y. Q. & Yu, L. Y. (2013). *Nocardiooides perillae* sp. nov., isolated from

- surface-sterilized roots of *Perilla frutescens*. *Int J Syst Evol Microbiol* **63**, 1068–1072.
- Euzéby, J. P. (1997)**. List of bacterial names with standing in nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* **47**, 590–592. <http://www.bacterio.cict.fr>
- Felsenstein, J. (1985)**. Confidence limits of phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994)**. *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Han, J. H., Kim, T. S., Joung, Y., Kim, M. N., Shin, K. S., Bae, T. & Kim, S. B. (2013)**. *Nocardioides endophyticus* sp. nov. and *Nocardioides conyzicola* sp. nov., isolated from herbaceous plant roots. *Int J Syst Evol Microbiol* **63**, 4730–4734.
- Jukes, T. H. & Cantor, C. R. (1969)**. Evolution of the protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P. (1990)**. Evaluation of the Titertek-Enterobac-Automated System (TTE-AS) for identification of members of the family *Enterobacteriaceae*. *Zentralbl Bakteriol* **273**, 164–172.
- Kämpfer, P. & Kroppenstedt, R. M. (1996)**. Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991)**. Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., McInroy, J. A. & Glaeser, S. P. (2014)**. *Chryseobacterium zaeae* sp. nov., *Chryseobacterium arachidis* sp. nov., and *Chryseobacterium geocarposphaerae* sp. nov. isolated from the rhizosphere environment. *Antonie van Leeuwenhoek* **105**, 491–500.
- Lawson, P. A., Collins, M. D., Schumann, P., Tindall, B. J., Hirsch, P. & Labrenz, M. (2000)**. New LL-diaminopimelic acid-containing actinomycetes from hypersaline, heliothermal and meromictic Antarctic Ekho Lake: *Nocardioides aquaticus* sp. nov. and *Friedmanniella lacustris* sp. nov. *Syst Appl Microbiol* **23**, 219–229.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004)**. ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- O'Donnell, A. G., Goodfellow, M. & Minnikin, D. E. (1982)**. Lipids in the classification of *Nocardioides*: reclassification of *Arthrobacter simplex* (Jensen) lochhead in the genus *Nocardioides* (Prauser) emend. O'Donnell et al. as *Nocardioides simplex* comb. nov. *Arch Microbiol* **133**, 323–329.
- Prauser, H. (1976)**. *Nocardioides*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **26**, 58–65.
- Prauser, H. (1984)**. *Nocardioides luteus* spec. nov. *Z Allg Mikrobiol* **24**, 647–648.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J. & Glöckner, F. O. (2007)**. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**, 7188–7196.
- Pruesse, E., Peplies, J. & Glöckner, F. O. (2012)**. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823–1829.
- Qin, S., Yuan, B., Zhang, Y. J., Bian, G. K., Tamura, T., Sun, B. Z., Li, W. J. & Jiang, J. H. (2012)**. *Nocardioides panzhuhuaensis* sp. nov., a novel endophytic actinomycete isolated from medicinal plant *Jatropha curcas* L. *Antonie van Leeuwenhoek* **102**, 353–360.
- Schumann, P. (2011)**. Peptidoglykan structure. In *Methods in Microbiology (Taxonomy of Prokaryotes)*, vol. 38, pp. 101–129. Edited by F. A. Rainey & A. Oren. London: Academic Press.
- Song, G. C., Yasir, M., Bibi, F., Chung, E. J., Jeon, C. O. & Chung, Y. R. (2011)**. *Nocardioides caricicola* sp. nov., an endophytic bacterium isolated from a halophyte, *Carex scabrifolia* Steud. *Int J Syst Evol Microbiol* **61**, 105–109.
- Stamatakis, A. (2006)**. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.
- Stolz, A., Busse, H.-J. & Kämpfer, P. (2007)**. *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.
- Tindall, B. J. (1990a)**. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tindall, B. J. (1990b)**. A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Yarza, P., Richter, M., Peplies, J., Euzéby, J., Amann, R., Schleifer, K. H., Ludwig, W., Glöckner, F. O. & Rosselló-Móra, R. (2008)**. The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst Appl Microbiol* **31**, 241–250.
- Yi, H. & Chun, J. (2004)**. *Nocardioides ganghwensis* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **54**, 1295–1299.
- Yoon, J. H., Rhee, S. K., Lee, J. S., Park, Y. H. & Lee, S. T. (1997)**. *Nocardioides pyridinoliticus* sp. nov., a pyridine-degrading bacterium isolated from the oxic zone of an oil shale column. *Int J Syst Bacteriol* **47**, 933–938.
- Yoon, J.-H., Cho, Y.-G., Lee, S. T., Suzuki, K., Nakase, T. & Park, Y.-H. (1999)**. *Nocardioides nitrophenolicus* sp. nov., a *p*-nitrophenol-degrading bacterium. *Int J Syst Bacteriol* **49**, 675–680.
- Yoon, J.-H., Kim, I.-G., Kang, K.-H., Oh, T. K. & Park, Y. H. (2004)**. *Nocardioides aquiterrae* sp. nov., isolated from groundwater in Korea. *Int J Syst Evol Microbiol* **54**, 71–75.
- Yoon, J.-H., Kim, I.-G., Lee, M.-H., Lee, C.-H. & Oh, T. K. (2005)**. *Nocardioides alkalitolerans* sp. nov., isolated from an alkaline serpentinite soil in Korea. *Int J Syst Evol Microbiol* **55**, 809–814.