Motilibacter peucedani gen. nov., sp. nov., isolated from rhizosphere soil

Soon Dong Lee

Correspondence

Soon Dong Lee

sdlee@jejunu.ac.kr

Department of Science Education, Jeju National University, Jeju 690-756, Republic of Korea

A novel actinomycete strain, designated RP-AC37^T, was isolated from rhizosphere soil collected on Mara Island of Jeju, Republic of Korea. Cells were aerobic, Gram-positive, oxidase-negative, catalase-positive, non-mycelium-forming and motile rods (0.6–0.7×1.9–2.4 µm). Phylogenetic analysis based on 16S rRNA gene sequences showed that the organism formed a distinct clade within the radiation occupied by the suborder *Frankineae*. 16S rRNA gene similarity values were less than 93.2 % to members of the suborder *Frankineae* and related taxa. The diamino acid isomer in the cell-wall peptidoglycan was LL-diaminopimelic acid. The major whole-cell sugars were glucose, galactose and xylose. The major menaquinone was MK-9(H₄). The polar lipids were diphosphatidylglycerol, phosphatidylcholine and phosphatidylinositol. The cellular fatty acids were straight-chain, unsaturated and saturated, with a significant amount of tuberculostearic acid (10-methyl-C_{18:0}). The DNA G+C content was 73.2 mol%. The combination of morphological, chemotaxonomic and phylogenetic data clearly separate the isolate from members of known genera of the suborder *Frankineae* and related taxa, suggesting that the isolate represents a novel species in a new genus in this suborder, for which the name *Motilibacter peucedani* gen. nov., sp. nov. is proposed; the type strain is RP-AC37^T (=KCTC 19630^T=DSM 45328^T).

The suborder *Frankineae*, proposed by Stackebrandt *et al.* (1997) and emended by Zhi *et al.* (2009) currently encompasses six families: *Frankiaceae*, *Acidothermaceae*, *Cryptosporangiaceae*, *Geodermatophilaceae*, *Nakamurellaceae* and *Sporichthyaceae*. Eleven genera are assigned to this suborder and members have been isolated from various sources (plants, hot springs, stone surfaces, activated sludge, soils and a medieval mine): *Frankia* (Brunchorst, 1886), *Acidothermus* (Mohagheghi *et al.*, 1986), *Cryptosporangium* (Tamura *et al.*, 1998), *Geodermatophilus* (Luedemann, 1968), *Blastococcus* (Ahrens & Moll, 1970), *Modestobacter* (Mevs *et al.*, 2000), *Nakamurella* (Tao *et al.*, 2004), *Humicoccus* (Yoon *et al.*, 2007), *Saxeibacter* (Lee *et al.*, 2008), *Sporichthya* (Lechevalier *et al.*, 1968) and *Fodinicola* (a genus without a family; Carlsohn *et al.*, 2008). Three genera, *Kineococcus* (Yokota *et al.*, 1993), *Kineosporia* (Pagani & Parenti, 1978) and *Quadrisphaera* (Maszenan *et al.*, 2005), have been assigned to the families 'Kineosporiaceae' and *Nakamurellaceae* (Garrity *et al.*, 2007) of this suborder, but were recently transferred to the family *Kineosporiaceae*, suborder *Kineosporiineae* (Zhi *et al.*, 2009). Recently, a new genus, *Angustibacter* (Tamura *et al.*, 2010), was included as a member of the suborder

Kineosporiineae. This paper describes the classification and identification of an actinomycete strain from rhizosphere soil by a polyphasic taxonomic approach. The organism is phylogenetically remotely related to members of the suborder *Frankineae*.

Strain RP-AC37^T was isolated from rhizosphere soil of a plant (*Peucedanum japonicum* Thunb.) collected on Mara Island of Jeju, Republic of Korea. Treatment of the soil sample and the medium for bacterial isolation were as described by Lee (2009). After incubation for 14 days at 30 °C, the isolate was maintained as 20 % (v/v) glycerol suspensions at –20 and –80 °C.

Cell morphology and motility were observed using phase-contrast and transmission electron microscopy, with cells grown on International *Streptomyces* Project (ISP) 2 medium (Shirling & Gottlieb, 1966) for 3 days at 30 °C. Cells were negatively stained with 1 % phosphotungstic acid before observation. Colony morphology and pigmentation were observed on ISP 2 medium incubated for 5 days at 30 °C. Oxidase activity was checked with tetramethyl-*p*-phenylenediamine. Gram staining was carried out using the Gram 2 kit (bioMérieux) following the instructions of the manufacturer. Catalase activity was checked with a solution of 3 % (v/v) H₂O₂. Anaerobic growth was tested by using a GasPak EZ Anaerobe Pouch System (BD). Growth of cells was examined on ISP 2 medium, nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco). Growth was tested at different temperatures (4, 10, 25, 30, 37 and 42 °C) over

Abbreviations: DAP, diaminopimelic acid; ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of RP-AC37^T is FM998003.

Two supplementary figures are available with the online version of this paper.

7 days and at pH 4.1–12.1 for 7 days at 30 °C. The medium pH was adjusted at intervals of 1.0 pH unit with 6 M HCl or 10 M NaOH, after autoclaving. Tolerance to NaCl was examined on ISP 2 medium supplemented with 1–9 % (w/v) NaCl (at intervals of 1 %) and the results were recorded after incubation for 7 days at 30 °C. Decomposition of hypoxanthine, DL-tyrosine and xanthine was determined according to the method of Gordon *et al.* (1974). Degradation of carboxymethyl-cellulose (0.5 %, w/v), casein [1 % (w/v) skim milk] and soluble starch (1 %, w/v) was tested using ISP 2 medium as the basal medium. Hydrolysis of DNA was assessed using DNase test agar (Difco). Urease activity, nitrate reduction, gelatin liquefaction and hydrolysis of aesculin were assessed by using the methods of MacFaddin (1980). Utilization of carbohydrates was determined on ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with filter-sterilized carbon sources. Acid production from carbohydrates and alcohols was determined by using Bacto OF basal medium (Difco) as described previously (Lee, 2007). Other physiological and biochemical tests were performed using API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions.

Cells were strictly aerobic, Gram-positive, oxidase-negative, catalase-positive, non-mycelium-forming rods (0.6–0.7 × 1.9–2.4 µm), motile by means of a polar flagellum (see Supplementary Fig. S1 in IJSEM Online). Colonies were orange-coloured, circular, opaque, pulvinate with entire margins and reached 1.0–2.0 mm in diameter after 5 days of incubation at 30 °C on ISP 2 medium. Other physiological and biochemical data are given in the species description.

Extraction and purification of genomic DNA were performed as described by Hopwood *et al.* (1985). Amplification and purification of the 16S rRNA gene and direct sequencing of the PCR product were performed as described by Lee (2009). The 16S rRNA gene sequence of strain RP-AC37^T (1424 nt) was aligned to actinobacterial sequences retrieved from public databases by using the CLUSTAL_X algorithm (Thompson *et al.*, 1997). Evolutionary distances between pairs of micro-organisms were calculated by using the model of Jukes & Cantor (1969). Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. To determine the stability of tree topologies, bootstrap analysis (Felsenstein, 1985) was performed using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE contained in the PHYLIP package (Felsenstein, 1993), with 1000 neighbour-joining datasets.

A total of 1288 nt unambiguously aligned positions of the 16S rRNA genes were used for tree construction. A neighbour-joining tree (Fig. 1) based on evolutionary distances calculated by the model of Jukes & Cantor (1969) revealed that the isolate belonged to the suborder *Frankineae* with a bootstrap support value of 81 %, but formed a separate line of descent between two clades, one occupied by members of the family *Nakamurellaceae* and the

other occupied by members of the genus *Fodinicola* and the families *Frankiaceae*, *Acidothermaceae*, *Cryptosporangiaceae*, *Geodermatophilaceae* and *Sporichthyaceae*. In a sequence-based maximum-parsimony tree, the isolates formed deep branching points at the base of a radiation encompassing all members of the suborder *Frankineae* (data not shown). Strain RP-AC37^T showed 16S rRNA gene sequence similarity values of less than 93.2 % to members of other taxa of the order *Actinomycetales*. Highest sequence similarity values to members of the suborder *Frankineae* were found with strains of members of the genera *Blastococcus* (92.3–93.2 %) and *Geodermatophilus* (91.8–93.2 %) (family *Geodermatophilaceae*) and *Cryptosporangium* (92.1–93.0 %) (family *Cryptosporangiaceae*). Strain RP-AC37^T was slightly less closely related to *Sporichthya* strains (92.5–92.9 %) (family *Sporichthyaceae*, suborder *Frankineae*) and *Kineococcus* strains (92.5–92.9 %) (family *Kineosporiaceae*, suborder *Kineosporiineae*). Levels of 16S rRNA gene sequence similarity between the novel isolate and other strains of the suborder *Frankineae* and related taxa were less than 92.5 %.

For analyses of chemotaxonomic characteristics, strain RP-AC37^T was grown in shake-flasks of ISP 2 broth for 3 days at 30 °C; cells were then washed with distilled water and freeze-dried. The diamino acid isomer in the cell-wall peptidoglycan was determined by the method of Stanek & Roberts (1974). The *N*-acyl type of the murein in the cell-wall peptidoglycan was determined according to the method of Uchida & Aida (1984). Analysis of whole-cell sugars was carried out by GC as described by Saddler *et al.* (1991). Isoprenoid quinones were extracted by using the method of Minnikin *et al.* (1984) and identified by HPLC (Kroppenstedt, 1985). Polar lipids were analysed by two-dimensional TLC (Minnikin *et al.*, 1977). Mycolic acids were extracted and analysed as described by Minnikin *et al.* (1980). The DNA G + C content was determined by HPLC (Mesbah *et al.*, 1989).

The diagnostic diamino acid in the cell-wall peptidoglycan was LL-diaminopimelic acid (LL-DAP). The acyl type of the muramic acid residue was *N*-glycolylated. The major whole-cell sugars of the isolate were glucose, galactose and xylose, with small amounts of ribose and mannose. The major menaquinone was MK-9(H₄). Mycolic acids were absent. Diphosphatidylglycerol, phosphatidylcholine and phosphatidylinositol were detected as the major polar lipids. Ninhydrin-positive phospholipids or glycolipids were not present (see Supplementary Fig. S2 in IJSEM Online). The DNA G + C content was 73.2 mol%.

For cellular fatty acid analysis, cells of strain RP-AC37^T were grown on ISP 2 medium for 3 days at 30 °C. Fatty acid methyl esters were prepared and analysed according to the instructions of the Sherlock Microbial Identification System (version 2.11; MIDI), using the AEROBE package including TSBA (version 3.9), CLIN (version 3.9) and MI7H10 (version 3.8) databases for identification of fatty acids.

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships between strain RP-AC37^T and members of the suborder Frankineae and related taxa, based on 1288 unambiguously aligned nucleotides present in all strains. Asterisks indicate corresponding branches also found in a maximum-parsimony (Fitch, 1971) tree. Values at nodes represent bootstrap support values (>50%) based on 1000 replications. Bar, 1 substitution per 100 nt positions.

The cellular fatty acid profile of strain RP-AC37^T was represented by the presence of considerable amounts of unsaturated, saturated and 10-methyl-branched fatty acids with moderate amounts of iso-branched fatty acids, and contained C_{18:1}ω9c (23.9 %), C_{16:0} (25.1 %), 10-methyl-C_{18:0} (tuberculostearic acid; 15.8 %), C_{18:0} (10.7 %), iso-C_{16:0} (4.7 %), C_{17:0} (4.6 %), C_{17:1}ω8c (4.2 %), C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (3.8 %), C_{14:0} (2.7 %), C_{15:0} (2.4 %) and 10-methyl-C_{17:0} (2.1 %).

Assignment of strain RP-AC37^T to the suborder *Frankineae* is supported by the possession of most of the 16S rRNA gene sequence signatures defined for the suborder *Frankineae* (Zhi *et al.*, 2009), with the exception of A→U instead of G→C at position 127:234. However, the combined data (Table 1) obtained from morphological and chemotaxonomic characterization indicate that strain RP-AC37^T forms a separate line of descent in the 16S rRNA gene sequence-based phylogenetic tree for the suborder *Frankineae* and related taxa. The presence of LL-DAP in the cell-wall peptidoglycan of strain RP-AC37^T is a key character that can differentiate the organism from members of most genera of the suborder *Frankineae*, with the exception of *Sporichthya* and *Acidothermus*. *Sporichthya* strains possess the same DAP isomer (LL-DAP) in the peptidoglycan as the isolate, but they differ with respect to menaquinones, polar lipids and cellular fatty acids. Furthermore, *Sporichthya* strains produce aerial hyphae, but strain RP-AC37^T does not. Although the diamino acid isomer was not defined for *Acidothermus cellulolyticus*, this species differs from the isolate in terms of cell motility and DNA G+C content. Strain RP-AC37^T forms motile rods and its DNA G+C content is 73.2 mol%, whereas the type strain of *A. cellulolyticus* is a non-motile rod with a DNA G+C content of 61.0 mol%.

Besides the DAP isomer type in the peptidoglycan, phylogenetically close neighbours and strains of *Blastococcus* and *Geodermatophilus* (family *Geodermatophilaceae*) and of *Cryptosporangium* (family *Cryptosporangiaceae*) can be clearly distinguished from strain RP-AC37^T on the basis of their polar lipid and cellular fatty acid profiles (Table 1). They contain phosphatidylethanolamine as the diagnostic phospholipid and iso-branched fatty acids among the predominant components, whereas strain RP-AC37^T has phosphatidylcholine as a diagnostic polar lipid and lacks phosphatidylethanolamine. With respect to the cellular fatty acid profile, strain RP-AC37^T is characterized by the predominance of saturated and unsaturated fatty acids together with a significant proportion of tuberculostearic acid (10-methyl-C_{18:0}). Furthermore, the formation of spherical to irregularly shaped sporangia by *Cryptosporangium* strains serves to differentiate the novel isolate from members of that genus (Tamura *et al.*, 1998).

Of the remotely related neighbours, *Kineosporia* strains (suborder *Kineosporiineae*) are similar to strain RP-AC37^T in that they possess MK-9(H₄) as the predominant menaquinone and contain phosphatidylcholine as the diagnostic phospholipid, but they produce substrate mycelia

and contain *meso*-DAP and LL-DAP, or lysine (*Kineosporia mesophila*) as the diamino acid(s) depending on the species. Furthermore, iso- and anteiso-branched fatty acids, which are present as moderate amount in the isolate, are not present in *Kineosporia* strains (Itoh *et al.*, 1989; Kudo *et al.*, 1998; Li *et al.*, 2009; Sakiyama *et al.*, 2009). It is evident from the phenotypic, genetic and phylogenetic studies that strain RP-AC37^T can be clearly differentiated from members of all genera of the suborder *Frankineae* and related taxa.

The data presented here from our polyphasic studies indicate that the isolate represents a novel species in a new genus of the suborder *Frankineae*, for which the name *Motilibacter peucedani* gen. nov., sp. nov. is proposed.

Description of *Motilibacter* gen. nov.

Motilibacter (Mo.ti.li.bac'ter. L. adj. *motilis* motile; N.L. masc. n. *bacter* the masculine equivalent of the Gr. neut. n. *bakterion* a rod; N.L. masc. n. *Motilibacter* a motile rod).

Cells are aerobic, Gram-positive, oxidase-negative, catalase-positive, non-mycelium-forming, motile rods. The diamino acid in the cell-wall peptidoglycan is LL-DAP. The major whole-cell sugars are glucose, galactose and xylose. The major menaquinone is MK-9(H₄). The polar lipids contain diphosphatidylglycerol, phosphatidylcholine and phosphatidylinositol. The acyl type of the murein is *N*-glycolylated. Mycolic acids are absent. The cellular fatty acids are represented by unsaturated and saturated fatty acids together with tuberculostearic acid (10-methyl-C_{18:0}). Based on 16S rRNA gene sequence studies, the genus belongs to the suborder *Frankineae*. The type species of the genus is *Motilibacter peucedani*.

Description of *Motilibacter peucedani* sp. nov.

Motilibacter peucedani (peu.ce'da.ni. N.L. n. *peucedanum* the plant hog's-fennel or sulphurwort and also a botanical genus name; L. gen. n. *peucedani* of *Peucedanum*, isolated from *Peucedanum japonicum* Thunb.).

General morphological and chemotaxonomic characteristics are as given above in the genus description. Cells are rod-shaped (0.6–0.7 × 1.9–2.4 µm). Colonies are orange-coloured, circular, opaque, pulvinate with entire margins and reach 1.0–2.0 mm in diameter after 5 days incubation at 30 °C on ISP 2 medium. Good growth occurs on ISP 2 medium and NA, but growth does not occur on TSA. Growth occurs at 20–37 °C (optimum at 30 °C) and pH 5.1–7.1 (optimum at pH 6.1–7.1). Growth does not occur in the presence of ≥1 % (w/v) NaCl. Decomposition of hypoxanthine, DL-tyrosine and xanthine does not occur. Aesculin is hydrolysed, but carboxymethyl-cellulose, casein, DNA, starch and urea are not. The following tests (API 20NE strip) are negative: nitrate reduction, gelatin liquefaction, indole and H₂S production, glucose fermentation, arginine dihydrolase and urease. Tests for alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase,

Table 1. Differential characters of *Motilibacter* gen. nov. and related genera of the suborder *Frankineae*

Data for reference genera are from the following: *Acidothermus* (Mohagheghi *et al.*, 1986), *Blastococcus* (Urzi *et al.*, 2004), *Cryptosporangium* (Tamura *et al.*, 1998), *Fodinicola* (Carlsohn *et al.*, 2008), *Frankia* (Lechevalier, 1994; Mirza *et al.*, 1991), *Geodermatophilus* (Luedemann & Fonseca, 1989; Zhang *et al.*, 2010), *Humicoccus* (Yoon *et al.*, 2007), *Modestobacter* (Mevs *et al.*, 2000), *Nakamurella* (Yoshimi *et al.*, 1996; Yoon *et al.*, 2007), *Saxeibacter* (Lee *et al.*, 2008) and *Sporichthya* (Rainey *et al.*, 1993; Tamura *et al.*, 1999). +, Present; –, absent; +/–, variable; ND, no data available.

Taxon	Cellular morphology	Spore/bud formation	Motility	Cell-wall diamino acid*	Major menaquinone(s)	Polar lipid(s)†	Predominant fatty acid(s)‡	DNA G + C content (mol%)
<i>Motilibacter</i>	Rods	–	+	LL-DAP	MK-9(H ₄)	DPG, PC, PI	C _{18:1} , C _{16:0} , TBSA, C _{18:0}	73.2
Acidothermaceae								
<i>Acidothermus</i>	Slender rods, filaments	–	–	DAP	ND	ND	ND	61
Cryptosporangiaceae								
<i>Cryptosporangium</i>	Substrate and aerial mycelia; sporangia	Sporangiospores	+	meso-DAP	MK-9(H ₆), MK-9(H ₄), MK-9(H ₈)	PE	i-C _{16:0} , C _{17:1} , C _{18:1}	70–76
Frankiaceae								
<i>Frankia</i>	Substrate hyphae; no aerial mycelium; multilocular sporangia	Sporangiospores	–	meso-DAP	MK-9(H ₄), MK-9(H ₆), MK-9(H ₈)	DPG, PI, PIM	i-C _{15:0} , i-C _{16:0} , C _{17:1}	66–71
Geodermatophilaceae								
<i>Geodermatophilus</i>	Thallus consisting of cuboid to oval cells; multilocular sporangia; rudimentary hyphae; no aerial mycelium	Zoospores	+/–	meso-DAP	MK-9(H ₄), MK-9	DPG, PE, PI, PIM	i-C _{16:0} , i-C _{15:0} , C _{17:1} , C _{18:1}	72.8–75.0
<i>Blastococcus</i>	Cocci, rods, vibrios, pairs, tetrads, clusters	Buds	+/–	meso-DAP	MK-9(H ₄), MK-9	DPG, PE, PG, PI	i-C _{16:0} , i-C _{16:1} , i-C _{15:0} , C _{18:1} , C _{17:1} , C _{17:0}	74
<i>Modestobacter</i>	Rods, cocci	Buds	+/–	meso-DAP	MK-9(H ₄)	DPG, PE, PG, PI	C _{18:1} , i-C _{16:0} , ai-C _{17:0}	68–73
Nakamurellaceae								
<i>Nakamurella</i>	Cocci, pairs, clusters	–	–	meso-DAP	MK-8(H ₄)	DPG, PE, PME	i-C _{16:0} , i-C _{15:0} , C _{18:1}	68
<i>Humicoccus</i>	Cocci	–	–	meso-DAP	MK-8(H ₄), MK-9(H ₄)	DPG, PE, PME	ai-C _{15:0} , i-C _{15:0} , C _{17:0}	73
<i>Saxeibacter</i>	Rods	–	–	meso-DAP	MK-8(H ₄)	DPG, PME	ai-C _{15:0} , C _{16:0} , i-C _{16:0} , ai-C _{17:0}	74.3
Sporichthyaceae								
<i>Sporichthya</i>	Short aerial hyphae; no substrate mycelium	Coccoid to rod-shaped spores	+	LL-DAP	MK-9(H ₈), MK-9(H ₆), MK-8(H ₆)	DPG, PG, PI, PL	C _{16:0} , i-C _{16:0} , C _{17:1} , C _{17:0}	71
Unclassified Frankineae								
<i>Fodinicola</i>	Substrate and aerial mycelia	– (fragmentation of aerial hyphae)	–	meso-DAP	MK-9(H ₄), MK-9(H ₆), MK-9(H ₈)	DPG, PE, PI, PL, PS, GL	i-C _{16:0} , 10-methyl-C _{17:0} , C _{17:1}	65

*DAP, Diaminopimelic acid.

†DPG, Diphosphatidylglycerol; GL, unknown glycolipid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid(s); PME, phosphatidylmethylethanolamine; PS, phosphatidylserine.

‡TBSA, Tuberculostearic acid (10-methyl-C_{18:0}); i-, iso-; ai-, anteiso.

α -glucosidase and β -glucosidase are positive, but tests for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are negative. Enzyme activity for β -glucuronidase is variable (API ZYM). Melezitose is used as a sole carbon and energy source. Acid is produced from methyl α -D-glucoside, sucrose and trehalose, but not from D-arabinose, L-arabinose, cellobiose, dextran, D-fructose, D-glucose, lactose, maltose, D-mannose, melezitose, melibiose, methyl α -D-mannoside, raffinose, L-rhamnose, L-ribose, salicin, L-sorbose, D-xylose, adonitol, dulcitol, *meso*-erythritol, glycerol, *myo*-inositol, D-mannitol or D-xylitol. Acid production from D-galactose and D-sorbitol is variable. The predominant fatty acids are C_{18:1} ω 9c, C_{16:0}, 10-methyl-C_{18:0} (tuberculostearic acid) and C_{18:0}.

The type strain is RP-AC37^T (=KCTC 19630^T=DSM 45328^T), isolated from rhizosphere soil of a wild plant on Mara Island in Jeju, Republic of Korea. The DNA G+C content of the type strain is 73.2 mol%.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology, Republic of Korea.

References

- Ahrens, R. & Moll, G. (1970). Ein neues knospendes Bakterium aus der Ostee. *Arch Mikrobiol* **70**, 243–265 (in German).
- Brunchorst, J. (1886). Über einige Wurzelanschwellungen besonders diejenigen von Alnus und den Elaeagnaceen. *Bot Inst Tubingen* **2**, 151–177 (in German).
- Carlsohn, M. R., Groth, I., Saluz, H.-P., Schumann, P. & Stackebrandt, E. (2008). *Fodinicola feengrottensis* gen. nov., sp. nov., an actinomycete isolated from a medieval mine. *Int J Syst Evol Microbiol* **58**, 1529–1536.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package) version 3.5.1. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Towards defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Garrrity, G. M., Lilburn, T. G., Cole, J. R., Harrison, S. H., Euzéby, J. & Tindall, B. J. (2007). *The Taxonomic Outline of Bacteria and Archaea*. Part 10. The Bacteria: phylum Actinobacteria, class “Actinobacteria”. TOBA Release 7.7. <http://www.taxonomicoutline.org>
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardia strain. *Int J Syst Bacteriol* **24**, 54–63.
- Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P., Ward, J. M. & Schrempf, H. (editors) (1985). *Genetic Manipulation of Streptomyces. A Laboratory Manual*. Norwich: John Innes Foundation.
- Itoh, T., Kudo, T., Parenti, F. & Seino, A. (1989). Amended description of the genus *Kineosporia*, based on chemotaxonomic and morphological studies. *Int J Bacteriol* **39**, 168–173.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics (Society for Applied Bacteriology Technical Series vol. 20)*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Kudo, T., Matsushima, K., Itoh, T., Sasaki, J. & Suzuki, K.-I. (1998). Description of four new species of the genus *Kineosporia*: *Kineosporia succinea* sp. nov., *Kineosporia rhizophila* sp. nov., *Kineosporia mikuniensis* sp. nov. and *Kineosporia rhamnosa* sp. nov., isolated from plant samples, and amended description of the genus *Kineosporia*. *Int J Syst Bacteriol* **48**, 1245–1255.
- Lechevalier, M. P. (1994). Taxonomy of the genus *Frankia* (Actinomycetales). *Int J Syst Bacteriol* **44**, 1–8.
- Lechevalier, M. P., Lechevalier, H. A. & Holbert, P. E. (1968). *Sporichthya*, un nouveau genre de Streptomycetaceae. *Ann Inst Pasteur (Paris)* **114**, 277–286 (in French).
- Lee, S. D. (2007). *Marmoricola aequoreus* sp. nov., a novel actinobacterium isolated from marine sediment. *Int J Syst Evol Microbiol* **57**, 1391–1395.
- Lee, S. D. (2009). *Amycolatopsis ultiminotia* sp. nov., isolated from rhizosphere soil, and emended description of the genus *Amycolatopsis*. *Int J Syst Evol Microbiol* **59**, 1401–1404.
- Lee, S. D., Park, S. K., Yun, Y.-W. & Lee, D. W. (2008). *Saxeibacter lacteus* gen. nov., sp. nov., an actinobacterium isolated from rock. *Int J Syst Evol Microbiol* **58**, 906–909.
- Li, J., Zhao, G.-Z., Huang, H.-Y., Qin, S., Zhu, W.-Y., Xu, L.-H. & Li, W.-J. (2009). *Kineosporia mesophila* sp. nov., isolated from surface-sterilized stems of *Tripterygium wilfordii*. *Int J Syst Evol Microbiol* **59**, 3150–3154.
- Luedemann, G. M. (1968). *Geodermatophilus*, a new genus of the Dermatophilaceae (Actinomycetales). *J Bacteriol* **96**, 1848–1858.
- Luedemann, G. M. & Fonseca, A. F. (1989). Genus *Geodermatophilus* Luedemann 1968, 1857^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2406–2409. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- MacFaddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Maszenan, A. M., Tay, J.-H., Schumann, P., Jiang, H.-L. & Tay, S. T.-L. (2005). *Quadrifphaera granulorum* gen. nov., sp. nov., a Gram-positive polyphosphate-accumulating coccus in tetrads or aggregates isolated from aerobic granules. *Int J Syst Evol Microbiol* **55**, 1771–1777.
- 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.
- Mevs, U., Stackebrandt, E., Schumann, P., Gallikowski, C. A. & Hirsch, P. (2000). *Modestobacter multiseptatus* gen. nov., sp. nov., a budding actinomycete from soils of the Asgard Range (Transantarctic Mountains). *Int J Syst Evol Microbiol* **50**, 337–346.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1977). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin layer chromatographic analysis of whole-cell methanolsates. *J Gen Microbiol* **88**, 200–204.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin layer chromatography of methanolsates of mycolic acid-containing bacteria. *J Chromatogr A* **188**, 221–233.

- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Mirza, M. S., Janse, J. D., Hahn, D. & Akkermans, A. D. L. (1991). Identification of atypical *Frankia* strains by fatty acid analysis. *FEMS Microbiol Lett* **83**, 91–98.
- Mohagheghi, A., Grohmann, K., Himmel, M., Leighton, L. & Updegraff, D. M. (1986). Isolation and characterization of *Acidothermus cellulolyticus* gen. nov., sp. nov., a new genus of thermophilic, acidophilic, cellulolytic bacteria. *Int J Syst Bacteriol* **36**, 435–443.
- Pagani, H. & Parenti, F. (1978). *Kineosporia*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **28**, 401–406.
- Rainey, F. A., Schumann, P., Prauser, H., Toalster, R. & Stackebrandt, E. (1993). *Sporichthya polymorpha* represents a novel line of descent within the order Actinomycetales. *FEMS Microbiol Lett* **109**, 263–267.
- Saddler, G. S., Tavecchia, P., Lociuoro, S., Zanol, M., Colombo, E. & Selva, E. (1991). Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J Microbiol Methods* **14**, 185–191.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sakiyama, Y., Thao, N. K., Giang, N. M., Miyadoh, S., Hop, D. V. & Ando, K. (2009). *Kineosporia babensis* sp. nov., isolated from plant litter in Vietnam. *Int J Syst Evol Microbiol* **59**, 550–554.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis. nov. *Int J Syst Bacteriol* **47**, 479–491.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Tamura, T., Hayakawa, M. & Hatano, K. (1998). A new genus of the order Actinomycetales, *Cryptosporangium* gen. nov., with descriptions of *Cryptosporangium arvum* sp. nov. and *Cryptosporangium japonicum* sp. nov. *Int J Syst Bacteriol* **48**, 995–1005.
- Tamura, T., Hayakawa, M. & Hatano, K. (1999). *Sporichthya brevicatena* sp. nov. *Int J Syst Bacteriol* **49**, 1779–1784.
- Tamura, T., Ishida, Y., Otoguro, M., Yamamura, H., Hayakawa, M. & Suzuki, K. (2010). *Angustibacter luteus* gen. nov., sp. nov., isolated from subarctic forest soil. *Int J Syst Evol Microbiol* **60**, 2441–2445.
- Tao, T.-S., Yue, Y.-Y., Chen, W.-X. & Chen, W.-F. (2004). Proposal of *Nakamurella* gen. nov. as a substitute for the bacterial genus *Microsphaera* Yoshimi *et al.* 1996 and *Nakamurellaceae* fam. nov. as a substitute for the illegitimate bacterial family *Microsphaeraceae* Rainey *et al.* 1997. *Int J Syst Evol Microbiol* **54**, 999–1000.
- 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.
- Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.
- Urzi, C., Salamone, P., Schumann, P., Rohde, M. & Stackebrandt, E. (2004). *Blastococcus saxobidensis* sp. nov., and emended descriptions of the genus *Blastococcus* Ahrens and Moll 1970 and *Blastococcus aggregatus* Ahrens and Moll 1970. *Int J Syst Evol Microbiol* **54**, 253–259.
- Yokota, A., Tamura, T., Nishii, T. & Hasegawa, T. (1993). *Kineococcus aurantiacus* gen. nov., a new aerobic, gram-positive, motile coccus with meso-diaminopimelic acid and arabinogalactan in the cell wall. *Int J Syst Bacteriol* **43**, 52–57.
- Yoon, J.-H., Kang, S.-J., Jung, S.-Y. & Oh, T.-K. (2007). *Humicoccus flavidus* gen. nov., sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 56–59.
- Yoshimi, Y., Hiraishi, A. & Nakamura, K. (1996). Isolation and characterization of *Microsphaera multipartida* gen. nov., sp. nov., a polysaccharide-accumulating Gram-positive bacterium from activated sludge. *Int J Syst Bacteriol* **46**, 519–525.
- Zhang, Y.-Q., Chen, J., Liu, H.-Y., Zhang, Y.-Q., Li, W.-J. & Yu, L.-Y. (2010). *Geodermatophilus ruber* sp. nov., isolated from the rhizosphere soil of a medicinal plant. *Int J Syst Evol Microbiol* **60**, 190–193.
- Zhi, X.-Y., Li, W.-J. & Stackebrandt, E. (2009). An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* **59**, 589–608.