Actinomadura geliboluensis sp. nov., isolated from soil

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A novel actinobacterium, strain A8036^T, isolated from soil, was investigated by using a polyphasic taxonomic approach. The organism formed extensively branched substrate hyphae that generated spiral chains of spores with irregular surfaces. The cell wall contained *meso*-diaminopimelic acid (type III) and cell-wall sugars were glucose, madurose, mannose and ribose. The predominant menaquinones were MK-9(H₆) and MK-9(H₄). The phospholipids were diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The major cellular fatty acids were iso-C_{16:0}, C_{17:1}*cis*9, C_{16:0}, C_{15:0} and 10-methyl C_{17:0}. Based on 16S rRNA gene sequence analysis, the closest phylogenetic neighbours of strain A8036^T were *Actinomadura meyeriae* DSM 44715^T (99.23 % similarity), *Actinomadura bangladeshensis* DSM 45347^T (98.9 %) and *Actinomadura chokoriensis* DSM 45346^T (98.3 %). However, DNA–DNA relatedness and phenotypic data demonstrated that strain A8036^T could be clearly distinguished from the type strains of all closely related *Actinomadura* species. Strain A8036^T is therefore considered to represent a novel species of the genus *Actinomadura*, for which the name *Actinomadura geliboluensis* sp. nov. is proposed. The type strain is A8036^T (=DSM 45508^T=KCTC 19868^T).

The genus *Actinomadura* was proposed by Lechevalier & Lechevalier (1970), and revised descriptions have been provided by Zhang *et al.* (2001) and Miyadoh & Miyara (2001). Members of the genus are aerobic, Gram-positive, non-acid-fast actinomycetes that form non-fragmenting branched substrate mycelia and aerial hyphae bearing chains of up to 15 spores. Spore chains have various shapes and ornamentation. Members of the genus *Actinomadura* are also characterized by a number of chemical markers, including the presence of *meso*-diaminopimelic acid (cell wall type III), the acyl group of the muramic acid is of the acetyl type, and most species contain madurose as the characteristic whole-cell sugar. The predominant menaquinone types are MK-9(H₄), MK-9(H₆) and MK-9(H₈). The phospholipid pattern is of type PI *sensu* Lechevalier *et al.* (1981), in which no nitrogenous phospholipids are present (Kroppenstedt *et al.*, 1990; Kroppenstedt & Goodfellow, 2006), and the fatty acid pattern is of type 3a *sensu* Kroppenstedt *et al.* (1990) and Kroppenstedt & Goodfellow (1991), with the exception of the thermophilic actinomadurae, which differ in their fatty acid composition.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A8036^T is HQ157187.

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

The primary reservoir of actinomadurae is soil (Quintana *et al.*, 2003; Cook *et al.*, 2005; Lee & Jeong, 2006; le Roes & Meyers, 2007; Wang *et al.*, 2007; Ara *et al.*, 2008), a few have been isolated from clinical materials (Trujillo & Goodfellow, 1997, 2003; Yassin *et al.*, 2010), an endophytic species was isolated from the leaves of *Maytenus austroyunnanensis* (Qin *et al.*, 2009) and one strain was isolated from a hive of the European honey bee in Thailand (Promnuan *et al.*, 2009).

Strain A8036^T was isolated from soil samples collected in Canakkale, Turkey, by using Gauze's medium no. 2 (per litre: 10 g glucose, 5 g peptone, 3 g tryptone, 5 g NaCl and 15 g agar; pH 7.0) (Gauze *et al.*, 1957) supplemented with cycloheximide (50 µg ml⁻¹), nystatin (50 µg ml⁻¹), nalidixic acid (10 µg ml⁻¹) and novobiocin (10 µg ml⁻¹), after 21 days incubation at 28 °C. Following isolation, strain A8036^T was maintained on glucose-yeast extract agar (GYEA) slopes (Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20 %, v/v) at –20 °C.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product were carried out following Chun & Goodfellow (1995). The almost-complete (1475 bp) 16S rRNA gene sequence of strain A8036^T was determined by using an ABI PRISM 3730 XL automatic sequencer. Identification of phylogenetic

neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved via the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The program CLUSTAL W version 1.8 (Thompson *et al.*, 1994) was used to align the sequences of strain A8036^T and related taxa retrieved from public databases. Phylogenetic trees were inferred with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) tree-making algorithms from the MEGA version 3 program (Kumar *et al.*, 2004), and the maximum-likelihood method (Felsenstein, 1981) from the PHYLIP suite of programs (Felsenstein, 1993). The evolutionary distance model of Jukes & Cantor (1969) was used to generate evolutionary distance matrices for the neighbour-joining algorithm. Topologies of the resultant trees were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 re-samplings of the neighbour-joining dataset by using the CONSENSE and SEQBOOT options from the PHYLIP package.

For DNA–DNA hybridization experiments, DNA was isolated with a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridizations were carried out as described by De Ley *et al.* (1970), under consideration of the modifications described by Huß *et al.* (1983), by using a Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multi-cell changer and a temperature controller with an *in situ* temperature probe (Varian).

Biomass for chemotaxonomic studies was prepared by growing strain A8036^T in glucose-yeast extract-malt extract broth at 160 r.p.m. for 10 days at 28 °C; cells were harvested by centrifugation, washed in distilled water, recentrifuged and freeze-dried. Whole-cell amino acids and sugars were prepared according to Lechevalier & Lechevalier (1970) and analysed by TLC (Staneck & Roberts, 1974). Polar lipids were extracted and analysed according to the method of Minnikin *et al.* (1984) as modified by Kroppenstedt & Goodfellow (2006). Isoprenoid quinones were extracted and purified by using the method of Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1982). For extraction of whole-cell fatty acids, cells were grown in 20 ml trypticase soy broth (TSB) at 28 °C with shaking at 150 r.p.m. After 5 days incubation, 5 ml seed culture was inoculated into 50 ml TSB. The inoculated flask was incubated as before for 5 days. After harvesting by cellulose membrane filtration (0.45 µm), wet cells (200 mg) were placed in an extraction tube. Cellular fatty acids were extracted, derivatized to their fatty acid methyl esters and separated via the Microbial Identification System (MIDI; Microbial ID), utilizing an Agilent Technologies 6890N GC with a G2614A autosampler and a 6783 injector (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Fatty acid methyl ester peaks were analysed by using TSBA 5.0. The DNA G + C content of strain A8036^T was determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

The morphological and physiological characteristics of strain A8036^T were determined in comparison with the

type strains of its phylogenetically closest relatives, namely *Actinomadura meyeræ* DSM 44715^T, *Actinomadura bangladeshensis* DSM 45347^T and *Actinomadura chokoriensis* DSM 45346^T. Cultural characteristics of strain A8036^T were determined after incubation at 28 °C for 14 days on various media as described by Shirling & Gottlieb (1966): yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salt-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7), modified Bennett's agar (Jones, 1949) and nutrient agar (Difco). *National Bureau of Standards Color Name Charts* (Kelly, 1964) were used for colour designation. Growth was tested at 4–60 °C, at pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0, and in the presence of 0–5 % (w/v) NaCl on ISP 2. Decomposition of various compounds was examined by using GYEA as recommended by Athalye *et al.* (1985). In addition, degradation of Tweens 20, 40 and 80 (1 %, w/v) was examined by using Sierra medium (Sierra, 1957). Carbon source utilization was tested with carbon source utilization (ISP 9) medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the tested carbon sources and 0.1 % succinic acid. Nitrogen source utilization was examined on the basal medium recommended by Williams *et al.* (1983) supplemented with a final concentration of 0.1 % of the tested nitrogen sources. Tests in the commercial API 20E and API ZYM (bioMérieux) systems were performed according to the manufacturer's instructions. Colony morphology and micromorphological properties of strain A8036^T were determined by examining gold-coated dehydrated specimens of 14-day cultures from ISP 2 medium by using a Cambridge Stereoscan 240 instrument.

The almost-complete 16S rRNA gene sequence (1475 nt) of strain A8036^T determined in this study was compared with the corresponding sequences of members of the genus *Actinomadura*. A neighbour-joining tree based on 16S rRNA gene sequences showed that strain A8036^T formed a cluster with *A. meyeræ* DSM 44715^T (Fig. 1). 16S rRNA gene sequence similarity calculations indicated that the closest relatives of strain A8036^T were *A. meyeræ* DSM 44715^T (99.23 %; 11 nt differences at 1437 locations), *A. bangladeshensis* DSM 45347^T (98.9 %; 16 nt differences at 1455 locations), *Actinomadura formosensis* DSM 43997^T (98.76 %; 18 nt differences at 1452 locations), *A. chokoriensis* DSM 45346^T (98.35 %; 24 nt differences at 1456 locations), *Actinomadura maduræ* DSM 43067^T (98.13 %; 27 nt differences at 1445 locations), *Actinomadura nitritigenes* DSM 44137^T (98.05 %; 28 nt differences at 1436 locations) and *Actinomadura mexicana* DSM 44485^T (98.03 %; 28 nt differences at 1421 locations). Lower levels of 16S rRNA gene sequence similarity (<98.0 %) were found with the type strains of all other recognized species of the genus *Actinomadura*.

Levels of DNA–DNA relatedness between strain A8036^T and its closest phylogenetic neighbours, namely *A. meyeræ* DSM 44715^T, *A. bangladeshensis* DSM 45347^T and *A. formosensis* DSM 43997^T, were 19.5, 19.2 and 12.8 %, respectively,

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the relationship between strain A8036^T and the type strains of closely related species of the genus *Actinomadura* in the family *Thermomonosporaceae*. Asterisks indicate branches of the tree that were also recovered with the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Numbers at nodes indicate levels of bootstrap support (percentages of 1000 resamplings); only values above 50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site.

clearly below the 70 % value considered to be the threshold for the definition of bacterial species (Wayne *et al.*, 1987).

The morphological characteristics of strain A8036^T were consistent with those of members of the genus *Actinomadura*. Strain A8036^T grew well on all of the media tested. It produced well-developed, non-fragmenting and extensively branched substrate hyphae that often formed spiral chains of irregular-surfaced spores (Fig. S1, available in IJSEM Online). Whitish aerial mycelia formed on ISP 3, 4, 5 and 7, glucose-yeast extract-malt extract, modified Bennett's and nutrient agar. The colour of the substrate mycelia was orange-yellow. Diffusible pigments were not produced. Melanoid pigments were not produced on ISP 6 or ISP 7. The cultural characteristics of strain A8036^T and the type strains of closely related *Actinomadura* species in all tested media are presented in Table 1. The physiological properties that distinguish strain A8036^T from closely related species of the genus *Actinomadura* are listed in Table 2.

Strain A8036^T also possessed chemical markers that are typical of members of the genus *Actinomadura*. It

contained *meso*-diaminopimelic acid (Type III; Lechevalier & Lechevalier, 1970) as the cell-wall diamino acid and whole-cell sugars including glucose, ribose (major component) mannose and madurose (Type B; madurose was the diagnostic sugar) (Fig. S2). The polar lipids included diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides (Fig. S3). The predominant menaquinones were MK-9(H₆) (61 %) and MK-9(H₄) (21 %); MK-9(H₈) (8 %) and MK-9(H₂) (3 %) were also detected as minor components (Fig. S4). The major cellular fatty acids of strain A8036^T were iso-C_{16:0} (19.93 %), C_{17:1}*cis*9 (16.53 %), C_{16:0} (13.28 %), C_{15:0} (11.60 %), 10-methyl C_{17:0} (11.59 %) and C_{17:0} (7.27 %); iso-C_{11:0} 3-OH (6.09 %), C_{18:1}*cis*9 (4.26 %), 10-methyl C_{18:0} (tuberculostearic acid; 3.81 %), C_{16:1}*cis*9 (2.68 %), C_{14:0} (1.37 %) and C_{18:0} (1.57 %) were also present as minor components. The G + C content of the DNA of strain A8036^T was 70.8 mol%.

It is evident from the genotypic and phenotypic data presented that strain A8036^T represents a novel species of

Table 1. Cultural characteristics of strain A8036^T and the type strains of closely related species of the genus *Actinomadura*

Strains: 1, A8036^T; 2, *A. meyeriae* DSM 44715^T; 3, *A. bangladeshensis* DSM 45347^T; 4, *A. chokoriensis* DSM 45346^T. + + +, Abundant; + +, moderate; +, poor; –, no growth.

Medium	Growth and cultural characteristics	1	2	3	4
ISP 2	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	White	None	White	None
	Reverse colour	Strong orange–yellow	Light yellow	Brilliant orange–yellow	Greyish brown
	Soluble pigment	None	None	None	None
ISP 3	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	White	None	Moderate red	White
	Reverse colour	Moderate orange	Pale yellow	Dark reddish brown	Moderate yellowish brown
	Soluble pigment	None	Light yellow	Greyish reddish orange	None
ISP 4	Growth	+ + +	None	+ + +	+ + +
	Aerial mycelium	White	–	Dark pink	None
	Reverse colour	Moderate orange	–	Moderate reddish orange	Light yellowish brown
	Soluble pigment	None	–	None	None
ISP 5	Growth	+ + +	None	+ + +	+ + +
	Aerial mycelium	White	–	White	White
	Reverse colour	Light yellow	–	Pale yellow	Pale orange–yellow
	Soluble pigment	None	–	None	None
ISP 6	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	White	None	None	None
	Reverse colour	Strong orange–yellow	Strong orange–yellow	Deep orange–yellow	Light orange–yellow
	Soluble pigment	None	None	None	None
ISP 7	Growth	+ + +	–	+ + +	+ + +
	Aerial mycelium	White	–	White	White
	Reverse colour	Light yellow	–	Brownish orange	Moderate orange
	Soluble pigment	None	–	None	Light orange
Modified Bennett's agar	Growth	+ + +	+ + +	+ + +	+ +
	Aerial mycelium	White	None	None	None
	Reverse colour	Light yellow	Pale yellow	Light yellow	Pale yellow
	Soluble pigment	None	None	None	None
Glucose-yeast extract-malt extract agar	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	White	None	White	None
	Reverse colour	Strong orange–yellow	Light yellow	Brilliant orange–yellow	Moderate brown
	Soluble pigment	None	None	None	None
Nutrient agar	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	White	None	White	None
	Reverse colour	Brilliant orange–yellow	Light yellow	Brilliant orange–yellow	Light yellow
	Soluble pigment	None	None	None	None
Trypticase soy agar	Growth	+ + +	+ + +	+ + +	+ + +
	Aerial mycelium	White	None	White	None
	Reverse colour	Brilliant orange–yellow	Strong orange–yellow	Deep orange–yellow	Moderate orange–yellow
	Soluble pigment	None	None	None	None

Table 2. Differential phenotypic characteristics between strain A8036^T and the type strains of closely related species of the genus *Actinomadura*

Strains: 1, A8036^T; 2, *A. meyeri* DSM 44715^T; 3, *A. bangladeshensis* DSM 45347^T; 4, *A. chokoriensis* DSM 45346^T.

Characteristic	1	2	3	4
Biochemical tests				
Aesculin hydrolysis	–	+	–	–
Arbutin hydrolysis	+	+	–	+
Nitrate reduction	+	+	–	+
Degradation tests				
Elastin (0.3 %)	+	–	–	–
Gelatin (0.5 %)	–	+	–	–
Hypoxanthine (0.4 %)	–	+	–	–
Starch (1.0 %)	+	+	–	+
Testosterone (0.1 %)	+	–	+	–
Xylan (0.4 %)	–	–	+	+
Growth on sole carbon sources (1.0 %, w/v)				
(–)-D-Sorbitol	+	–	+	+
(+)-D-Galactose	+	–	–	–
(+)-D-Mannose	–	+	–	+
Melezitose	+	–	+	+
Melibiose	+	–	+	+
D-Mannitol	+	–	+	+
Dextrin	+	–	+	+
Dextran	+	–	–	–
Inulin	+	–	–	–
(–)-L-Sorbose	+	–	–	–
(+)-L-Rhamnose	+	+	–	+
Lactose	+	–	–	–
L-Glutamate	–	–	+	+
myo-Inositol	+	–	–	–
Sucrose	+	–	–	+
Succinic acid (0.1 %)	+	–	+	–
Growth on sole nitrogen sources (0.1 %, w/v)				
D-Phenylalanine	–	–	–	+
L-Hydroxyproline	–	+	–	–
L-Methionine	–	–	+	+
L-Phenylalanine	–	+	+	+
L-Proline	+	–	+	+
L-Serine	–	+	+	+
L-Threonine	–	–	–	+
Growth at/with:				
pH 11.0	+	–	–	–
45 °C	+	+	–	+
4 % NaCl	+	+	+	–
5 % NaCl	+	+	+	–
API 20E results				
Arginine dihydrolase	+	–	–	–
Citrate	+	–	–	+
H ₂ S production	–	–	–	+
Tryptophan deaminase	–	–	–	+
Amygdalin	+	+	+	–
Catalase	+	–	+	+
API ZYM results				
Esterase	–	–	+	–
Cystine arylamidase	–	–	+	–

Table 2. cont.

Characteristic	1	2	3	4
Trypsin	+	–	+	–
Chymotrypsin	+	–	–	–
Naphthol-AS-BI-phosphohydrolase	+	–	–	+
β-Glucosidase	+	–	+	+
N-Acetyl-β-glucosaminidase	+	–	–	–

the genus *Actinomadura*, for which the name *Actinomadura geliboluensis* sp. nov. is proposed.

Description of *Actinomadura geliboluensis* sp. nov.

Actinomadura geliboluensis (ge.li.bo.lu.en'sis. N.L. fem. adj. *geliboluensis* of or belonging to Gelibolu, Canakkale, Turkey, from where the type strain was isolated).

Aerobic, Gram-positive, non-acid-alcohol-fast, non-motile actinomycete that forms extensively branched, non-fragmenting, orange-yellow substrate mycelia that bear white aerial mycelia on several tested media. Aerial mycelia bear spiral chains of spores with irregular surfaces. Diffusible pigments are not produced. Melanoid pigment is not produced on ISP 6 or ISP 7 agars. Growth occurs at pH 5.0–11.0 and at 20–45 °C, but not at pH 4.0, or at 10 or 50 °C. Optimal growth occurs at 30 °C and at pH 7.0. Growth is observed with 0–5 % (w/v) NaCl. Positive for acid phosphatase, alkaline phosphatase, arginine dihydrolase, α-glucosidase, β-glucosidase, catalase, chymotrypsin, citrate, esterase lipase, leucine arylamidase, N-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but negative for α-galactosidase, β-galactosidase, cystine arylamidase, esterase, lipase, α-fucosidase, α-mannosidase, β-glucuronidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, indole and H₂S production. Positive for arbutin hydrolysis and nitrate reduction but not for aesculin or urea hydrolysis. Degrades casein, elastin, starch, Tweens 20, 40 and 80 and testosterone, but not adenine, chitin, gelatin, guanine, hypoxanthine, xanthine or xylan. Utilizes adonitol, (+)-L-arabinose, cellobiose, (–)-D-fructose, (–)-D-sorbitol, (+)-D-galactose, melezitose, melibiose, D-mannitol, dextrin, dextran, inulin, (–)-L-sorbose, (+)-L-rhamnose, lactose, maltose, myo-inositol, sucrose, xylose and succinic acid as sole carbon and energy sources, but not (+)-D-mannose, L-glutamate or xylitol as sole carbon sources. Utilizes glycine, L-alanine, L-cysteine, leucine, L-proline and L-valine as sole nitrogen sources, but not α-isoleucine, D-phenylalanine, L-phenylalanine, L-arginine, L-histidine, L-hydroxyproline, L-methionine, L-serine, L-threonine or L-tyrosine. The quinone system consists of MK-9(H₆), MK-9(H₄), MK-9(H₈) and MK-9(H₂). The polar lipid profile contains diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The major fatty acids are iso-C_{16:0}, C_{17:1}cis9, C_{16:0}, C_{15:0} and 10-methyl C_{17:0}.

The type strain, A8036^T (=DSM 45508^T=KCTC 19868^T), was isolated from soil in Canakkale, Turkey. The G+C content of the genomic DNA of the type strain is 70.8 mol%.

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References

- Ara, I., Matsumoto, A., Bakir, M. A., Kudo, T., Omura, S. & Takahashi, Y. (2008). *Actinomadura bangladeshensis* sp. nov. and *Actinomadura chokoriensis* sp. nov. *Int J Syst Evol Microbiol* **58**, 1653–1659.
- Athalye, M., Goodfellow, M., Lacey, J. & White, R. P. (1985). Numerical classification of *Actinomadura* and *Nocardopsis*. *Int J Syst Bacteriol* **35**, 86–98.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Cook, A. E., le Roes, M. & Meyers, P. R. (2005). *Actinomadura napierensis* sp. nov., isolated from soil in South Africa. *Int J Syst Evol Microbiol* **55**, 703–706.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gauze, G. F., Preobrazhenskaya, T. P., Kudrina, E. S., Blinov, N. O., Ryabova, I. D. & Sveshnikova, M. A. (1957). *Problems of Classification of Actinomycetes-Antagonists*. Moscow: Government Publishing House of Medical Literature.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2005). A simple fluorimetric method for the estimation of DNA-DNA relatedness between closely related microorganisms by thermal denaturation temperatures. *Extremophiles* **9**, 75–79.
- Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628–636.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–145.
- 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.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.
- Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* **5**, 2359–2367.
- Kroppenstedt, R. M. & Goodfellow, M. (1991). The family *Thermomonosporaceae*. In *The Prokaryotes*, 2nd edn, pp. 1085–1114. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Kroppenstedt, R. M. & Goodfellow, M. (2006). The family *Thermomonosporaceae*: *Actinocorallia*, *Actinomadura*, *Spirillispora* and *Thermomonospora*. In *The Prokaryotes, Archaea and Bacteria: Firmicutes, Actinomycetes*, 3rd edn, vol. 3, pp. 682–724. Edited by M. Dworkin, S. Falkow, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- Kroppenstedt, R. M., Stackebrandt, E. & Goodfellow, M. (1990). Taxonomic revision of the actinomycete genera *Actinomadura* and *Microtetrastora*. *Syst Appl Microbiol* **13**, 148–160.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- le Roes, M. & Meyers, P. R. (2007). *Actinomadura rudentiformis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 45–50.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). Phospholipids in the taxonomy of actinomycetes. *Zentralbl Bakteriol Hyg Abt 1 (Suppl. 11)*, 111–116.
- Lee, S. D. & Jeong, H. S. (2006). *Actinomadura hallensis* sp. nov., a novel actinomycete isolated from Mt. Halla in Korea. *Int J Syst Evol Microbiol* **56**, 259–264.
- 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.
- Miyadoh, S. & Miyara, T. (2001). Family *Thermomonosporaceae*. In *Identification Manual of Actinomycetes*, pp. 281–291. Edited by The Society for Actinomycetes, Japan. Tokyo: Business Center for Academic Societies.
- Promnuan, Y., Kudo, T. & Chantawannakul, P. (2009). Actinomycetes isolated from beehives in Thailand. *World J Microbiol Biotechnol* **25**, 1685–1689.
- Qin, S., Zhao, G. Z., Li, J., Zhu, W. Y., Xu, L. H. & Li, W. J. (2009). *Actinomadura flavalba* sp. nov., an endophytic actinomycete isolated from leaves of *Maytenus austroyunnanensis*. *Int J Syst Evol Microbiol* **59**, 2453–2457.
- Quintana, E. T., Trujillo, M. E. & Goodfellow, M. (2003). *Actinomadura mexicana* sp. nov. and *Actinomadura meyerii* sp. nov., two novel soil sporoactinomycetes. *Syst Appl Microbiol* **26**, 511–517.
- 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.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Sierra, G. (1957).** A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek* **23**, 15–22.
- Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Trujillo, M. E. & Goodfellow, M. (1997).** Polyphasic taxonomic study of clinically significant actinomadurae including the description of *Actinomadura latina* sp. nov. *Zentralbl Bakteriol* **285**, 212–233.
- Trujillo, M. E. & Goodfellow, M. (2003).** Numerical phenetic classification of clinically significant aerobic sporoactinomycetes and related organisms. *Antonie van Leeuwenhoek* **84**, 39–68.
- Wang, Y. X., Zhi, X. Y., Chen, H. H., Zhang, Y. Q., Tang, S. K., Jiang, C. L., Xu, L. H. & Li, W. J. (2007).** *Actinomadura alba* sp. nov., isolated from soil in Yunnan, China. *Int J Syst Evol Microbiol* **57**, 1735–1739.
- 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.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983).** Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Yassin, A. F., Spröer, C., Siering, C. & Klenk, H.-P. (2010).** *Actinomadura sputi* sp. nov., isolated from the sputum of a patient with pulmonary infection. *Int J Syst Evol Microbiol* **60**, 149–153.
- Zhang, Z., Kudo, T., Nakajima, Y. & Wang, Y. (2001).** Clarification of the relationship between the members of the family *Thermomonosporaceae* on the basis of 16S rDNA, 16S–23S rRNA internal transcribed spacer and 23S rDNA sequences and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 373–383.