INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Kuncharoen *et al.*, *Int J Syst Evol Microbiol*
DOI 10.1099/ijsem.0.002625

MICROBIOLOGY SOCIETY

Micromonospora globbae sp. nov., an endophytic actinomycete isolated from roots of *Globba winitii* C. H. Wright

Nattakorn Kuncharoen,[1] Pattama Pittayakhajonwut[2] and Somboon Tanasupawat[1,*]

Abstract

A novel endophytic actinomycete, strain WPS1-2^T, isolated from a root of *Globba winitii* C. H. Wright, was characterized taxonomically by using a polyphasic approach. Strain WPS1-2^T exhibited identical characteristics to the members of the genus *Micromonospora*. Single spores were observed directly on substrate mycelia. The cell-wall peptidoglycan of the strain contained *meso*-diaminopimelic acid and 3-OH-*meso*-diaminopimelic acid. Whole-cell hydrolysates contained glucose, ribose, arabinose and xylose. The predominant menaquinones were MK-10(H_8) and MK-10(H_{10}). The major cellular fatty acids consisted of iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{15:0}$. According to the 16S rRNA gene sequence of the strain, WPS1-2^T showed highest similarity to *Micromonospora costi* CS1-12^T (99.02 %). Phylogenetic analysis of the gyrase subunit B (*gyrB*) gene indicated that the strain was related to *M. costi* CS1-12^T. The DNA G+C content was 73.7 mol%. The strain could be distinguished from closely related type strains by using a combination of morphological, chemotaxonomic, physiological and biochemical data together with DNA–DNA relatedness values. Based on these observations, strain WPS1-2^T is considered to represent a novel species of the genus *Micromonospora*, for which the name *Micromonospora globbae* sp. nov. is proposed. The type strain is WPS1-2^T (=KCTC 39787^T=NBRC 112325^T=TISTR 2405^T).

The genus *Micromonospora*, containing aerobic Gram-stain-positive, filamentous bacteria which bear single, non-motile spores directly on the substrate mycelium, was first described by Ørskov [1]. Species of the genus *Micromonospora* are widely distributed in different environments: soils [2], sea sands [3], near-shore sediment [4], marine sponge [5], root nodules [6], roots of rice [7] and leaves of a medicinal plant [8]. To date, the genus *Micromonospora* consists of 79 species with validly published names (http://www.bacterio.net/micromonospora.html). Strains representing species of the genus *Micromonospora* have been recognized as an important source of bioactive secondary metabolites: gentamicin, netamicin, lupinacidins A-C and tetrocracin A [9, 10]. In this paper, we report on the isolation and taxonomy of a novel strain representing the genus *Micromonospora* WPS1-2^T.

The roots of *Globba winitii* C. H. Wright, which were used for the isolation of strain WPS1-2^T, were collected from the botanical garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. The excised roots were washed with running tap water and surface-sterilized by using the modified methods of Coombs and Franco [11] and Qin *et al.* [12]. The root samples were cut to 2 cm in length, then soaked in 75 % (v/v) ethanol for 3 min and subsequently immersed in 3 % (v/v) NaOCl for 10 min. Surface-sterilized roots were washed three times with sterile distilled water. The roots were soaked in 10 % (w/v) $NaHCO_3$ for 10 min to disrupt root tissues and delay the growth of fungi. The roots were crushed with 4 % (w/v) sucrose solution in a sterile motar. The crushed roots suspension was incubated at 60 °C in a water bath for 10 min. The suspension (0.1 ml) was spread onto starch casein gellan gum medium (SCG) [13] supplemented with 25 µg nalidixic acid ml^{-1} and 50 µg cycloheximide ml^{-1} and incubated at 30 °C for 21 days. Colonies of strain WPS1-2^T were purified on ISP2 medium [14]. The pure culture was maintained in 15 % (v/v) glycerol solution at −80 °C and lyophilized for long-term preservation.

Cultural characteristics of strain WPS1-2^T were determined following the standard method of the International *Streptomyces* Project [14]. The morphology of spores was observed by scanning electron microscopy (JEOL, JSM-6610LV) after the strain was grown on 10-fold-diluted yeast extract-malt extract agar medium [0.2 % (w/v) yeast extract, 0.4 % (w/v)

Author affiliations: [1]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand; [2]National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathumthani 12120, Thailand.
***Correspondence:** Somboon Tanasupawat, Somboon.T@chula.ac.th
Keywords: *Micromonosporaceae*; endophytic actinomycete; *Micromonospora globbae*; *Globba winitii*.
Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and *gyrB* gene sequence of strain WPS1-2^T are LC177396 and LC191306, respectively.
Six supplementary figures and one supplementary table are available with the online version of this article.

malt extract, 0.2 % (w/v) dextrose, and 1.5 % (w/v) agar] at 30 °C for 21 days. The colour of the colony and diffusible pigments was examined by using the ISCC-NBS colour system [15]. Biochemical and physiological properties were determined according to the standard procedures of Arai [16], and Williams and Cross [17]. The activities of enzymes were determined using API ZYM (bioMérieux). The effects of temperature (20–55 °C), pH (4-9) and NaCl (1–7 %, w/v) tolerance on growth were examined on ISP2 medium. The utilization of various carbon sources was determined by standard methods [14].

The freeze-dried cells of strain WPS1-2^T, which were used for chemotaxonomic studies, were obtained from culture grown in yeast extract-dextrose broth [4] in an incubator shaker (180 r.p.m.) at 30 °C for 7 days. The isomers of diaminopimelic acid in the cell wall and whole-cell sugars composition were determined following the method of Staneck and Roberts [18]. The *N*-acyl type of muramic acid in peptidoglycan was determined by the method of Uchida and Aida [19]. The presence of mycolic acid was monitored by TLC following the procedure of Tomiyasu [20]. Phospholipids in cells were extracted and identified by using two-dimensional TLC as previously described by Minnikin *et al.* [21]. Fatty acid methyl esters were extracted using the method of Sasser [22] and were analysed by gas chromatography (MIDI, Sherlock Microbial Identification System, TSBA6 Sherlock version 6.2B). Menaquinones were extracted and purified following the method of Collins *et al.* [23] and were analysed by using HPLC with a μBondapak C18 column (Waters).

Genomic DNA of strain WPS1-2^T for PCR amplification of 16S rRNA and gyrase subunit B (*gyrB*) genes was extracted by the method of Raeder and Broda [24]. Amplification of the 16S rRNA gene was carried out as previously described by Suriyachadkun *et al.* [25], and the gene was sequenced (Macrogen, Korea) using universal primers [26]. The 16S rRNA gene sequence was analysed using BioEdit software [27]. The sequence similarity values between strain WPS1-2^T and related type strains were determined using BLAST analysis and the EzTaxon-e server [28]. The PCR amplification and sequencing of the *gyrB* gene were carried out following the method of Garcia *et al.* [29]. Phylogenetic distances were calculated with the Kimura-2-parameter model [30], and tree topologies were inferred by using the neighbour-joining (NJ) [31], maximum-parsimony (MP) [32] and maximum-likelihood (ML) [33] methods, with trees reconstructed using the MEGA 6.0 program [34]. The confidence values of the branch nodes were evaluated by using the bootstrap resampling method with 1000 replications [35]. The DNA G+C content was determined using HPLC [36]. DNA–DNA hybridization was performed as previously described by Ezaki *et al.* [37].

Strain WPS1-2^T produced substrate mycelium without fragmentation but did not produce aerial mycelium on various agar media tested. Spores were borne singly on the tips of the substrate mycelium (1.1–1.2 μm in size). Spore surface was smooth (Fig. S1, available in the online version of this article). Colonies were vivid orange–yellow on ISP2 agar plates. The cultural characteristics of strain WPS1-2^{T}are described in Table S1. The strain grew well on ISP2, ISP6 and nutrient agar, moderately on ISP3 and ISP4, and poorly on ISP5 and ISP7. The strain grew well at pH 5.0–9.0, and weakly at pH 4.0. The temperature range for growth of strain WPS1-2^T was 20–45 °C (optimum at 30–37 °C). No growth was observed at 50 °C after incubation for 1 week. Other physiological and biochemical characteristics of strain WPS1-2^{T}were compared with those of the closely related species of the genus *Micromonospora* and revealed differences from *Micromonospora costi* CS1-12^T, *Micromonospora fulviviridis* NBRC 14026^T and *Micromonospora krabiensis* MA-2^T (Table 1).

Chemotaxonomic properties of strain WPS1-2^T revealed typical characteristics of those members of the *genus Micromonospora*. It contained *meso*-diaminopimelic acid and 3-OH-*meso*-diaminopimelic acid in cell-wall peptidoglycan and contained arabinose, glucose, ribose and xylose in whole-cell hydrolysates. According to the classification of Lechevalier and Lechevalier [38], the presence of xylose and arabinose as diagnostic sugars should be classified as sugar pattern D. The

Table 1. Differential phenotypic characteristics of strain WPS1-2^T and closely related type strains

Strains: 1, WPS1-2^T; 2, *M. costi* CS1-12^T; 3, *M. fulviviridis* NBRC 14026^T; 4, *M. krabiensis* MA-2^T. +, Positive; w, weakly positive; −, negative. All data are from this study.

Characteristic	1	2	3	4
Spore morphology				
Shape	Oval	Global	Global	Oval
Spore surface	Smooth	Rough and nodulated	Rough	Rough
Maximum NaCl tolerance (%, w/v)	3	4	2	3
Growth at 45 °C	+	+	w	−
Utilization of:				
Glycerol	w	−	−	−
myo-Inositol	+	−	−	w
Raffinose	+	−	+	−
D-Salicin	+	−	−	−
Sucrose	+	−	+	+
API ZYM				
Acid phosphatase	w	+	+	−
Alkaline phosphatase	w	+	+	+
α-Chymotrypsin	+	+	+	−
Cystine arylamidase	−	−	+	+
Esterase (C4)	w	+	+	+
Esterase lipase (C8)	w	w	+	+
α-Galactosidase	+	+	+	−
β-Galactosidase	+	+	+	w
β-Glucosidase	w	w	+	−
Naphthol-AS-BI-phosphohydrolase	w	+	+	−
Valine arylamidase	w	w	+	+

N-acyl type of muramic acid was glycolyl. Mycolic acids were absent. The polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol, three unidentified phospholipids, aminophospholipids, unidentified aminolipids and two unidentified glycolipids (Fig. S2). The major fatty acids (>10 %) were iso-$C_{15:0}$ (27.6 %), iso-$C_{16:0}$ (14.1 %) and anteiso-$C_{15:0}$ (10.3 %). Strain WPS1-2^T showed a fatty acid profile similar to those of *M. costi* CS1-12^T and *M. krabiensis* MA-2^T; nonetheless, the amounts of each fatty acid were different, as shown in Table 2. The major menaquinones were MK-10(H_8) (59.9 %) and MK-10(H_{10}) (40.1 %). The DNA G+C content was 73.7 mol%.

The almost-complete 16S rRNA gene sequence (1457 nt) of strain WPS1-2^T showed the highest similarity to that of *M. costi* CS1-12^T (99.02 %; 14 nt difference at 1438). A subset of the closest phylogenetic relatives of strain WPS1-2^T based on the NJ method are presented in Fig. 1. The phylogenetic tree that includes all species of the genus *Micromonospora* with validly published names is available as Fig. S3. Although the clade containing strain WPS1-2^{T}in the NJ tree could be recovered in the tree reconstructed by the ML method (Fig. S4), it could not be recovered in the MP phylogenetic tree (Fig. S5). To clarify the affiliation of strain WPS1-2^T to its closest phylogenetic neighbours, the *gyrB* gene sequence (1117 nt) of strain WPS1-2^T and representatives of closely related species in the genus *Micromonospora* were compared. The level of *gyrB* gene sequence similarity of strain WPS1-2^T and all available sequences belonging to the genus *Micromonospora* ranged from 91.0 to 95.0 %. The phylogenetic tree of all available species of the genus *Micromonospora* based on *gyrB* gene sequence (Fig. S6) revealed that strain WPS1-2^T was recovered in a cluster with *M. costi* CS1-12^T, supported by a 97 % bootstrap value. The result demonstrated that strain WPS1-2^T was closely related to *M. costi* CS1-12^T. Based on the highest 16S rRNA gene sequence similarity and phylogenetic tree analysis based on the *gyrB* gene, *M. costi* CS1-12^T, *M. fulviviridis* DSM 43906^T and *M. krabiensis* MA-2^T were selected for comparative purposes.

By the integration of morphological and chemotaxonomic characteristics including 16S rRNA gene and *gyrB* gene analysis as mentioned above, this novel strain could be classified in the genus *Micromonospora*. In comparison with the closely related species of the genus *Micromonospora*, the strain WPS1-2^T could be distinguished from the type strains *M. costi* CS1-12^T, *M. fulviviridis* NBRC 14026^T and *M. krabiensis* MA-2^T by the morphology of spores, the whole-cell sugars pattern and several phenotypic properties, especially the utilization of carbon sources, enzyme activity and NaCl tolerance (Table 1). Moreover, strain WPS1-2^T showed DNA–DNA relatedness values with *M. costi* CS1-12^T (60.0 ±2.1 %), *M. fulviviridis* NBRC 14026^T (19.0±0.3 %) and *M. krabiensis* MA-2^T (43.1±0.3 %) which were lower than 70 %, the threshold value for assigning strains to the same species [39].

Table 2. Cellular fatty acid contents (percentages) of strain WPS1-2^T and closely related type strains

Strains: 1, WPS1-2^T; 2, *M. costi* CS1-12^T; 3, *M. fulviviridis* NBRC 14026^T; 4, *M. krabiensis* MA-2^T. All data were analysed in this study. Fatty acids amounting to less than 0.5 % in all strains were omitted.

Fatty acid	1	2	3	4
Saturated fatty acids				
$C_{14:0}$	–	0.6	–	0.7
$C_{16:0}$	1.8	3.3	1.4	5.9
$C_{17:0}$	5.5	6.3	2.1	5.4
$C_{18:0}$	1.1	2.0	3.9	6.6
$C_{19:0}$	–	0.5	–	–
Unsaturated fatty acids				
$C_{16:1}$ 2-OH	0.5	–	–	0.5
$C_{15:1}\omega6c$	0.6	–	–	–
$C_{17:1}\omega8c$	5.6	2.2	–	0.8
$C_{18:1}\omega9c$	1.0	0.7	1.8	0.9
Unsaturated branched fatty acids				
iso-$C_{15:1}$ G	3.4	1.0	–	2.4
anteiso-$C_{15:1}$ A	–	–	–	0.5
iso-$C_{16:1}$ G	2.3	0.4	–	3.8
iso-$C_{17:1}\omega5c$	–	1.4	–	–
anteiso-$C_{17:1}\omega9c$	1.1	0.6	2.2	1.3
Branched fatty acids				
iso-$C_{14:0}$	1.3	0.9	1.7	2.0
iso-$C_{15:0}$	27.6	30.0	10.9	17.0
anteiso-$C_{15:0}$	10.3	15.0	2.6	14.6
iso-$C_{16:0}$	14.1	10.1	35.8	18.4
iso-$C_{17:0}$	6.1	6.3	5.2	4.6
anteiso-$C_{17:0}$	9.9	15.0	11.4	9.5
iso-$C_{18:0}$	–	–	–	0.7
10-Methyl fatty acids				
10-Methyl $C_{17:0}$	1.5	1.3	4.7	0.7
Summed feature 3*	1.3	1.2	0.6	0.6
Summed feature 9*	1.4	–	7.2	0.9

*Summed feature 3 comprised $C_{16:1}\omega7c$ or $C_{16:1}\omega6c$; summed feature 9 comprised 10-methyl $C_{16:0}$ or iso-$C_{17:1}\omega9c$.

On the basis of data from this taxonomic study using phenotypic characteristics, chemotaxonomic characteristics and genotypic characteristics together with DNA–DNA relatedness, strain WPS1-2 should be classified as a representative of a novel species of the genus *Micromonospora*, for which the name *Micromonospora globbae* sp. nov. is proposed.

DESCRIPTION OF *MICROMONOSPORA GLOBBAE* SP. NOV.

Micromonospora globbae (glob′bae. N.L. gen. n. *globbae* of *Globba*, a botanical genus).

Aerobic, Gram-stain-positive, mesophilic actinomycete which forms single spores (1.1–1.2 µm) directly on substrate mycelium. Substrate mycelium is well developed on ISP2

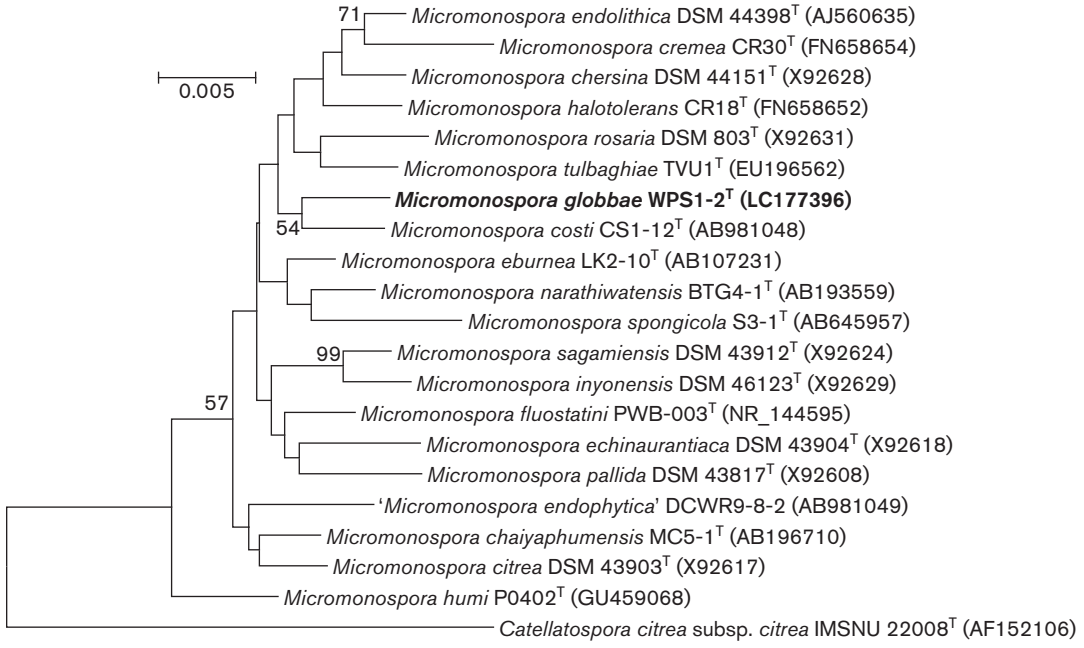

Fig. 1. Phylogenetic tree, based on NJ analysis of 16S rRNA gene sequences of strain WPS1-2^T and the closely related members of the genus *Micromonospora*. Numbers at branch nodes indicate bootstrap percentages derived from 1000 replications (only values >50 % are shown at nodes). Bar, 0.005 substitutions per nucleotide position. An extended version of this tree containing all species of the genus *Micromonospora* is available as Fig. S3.

medium, and the colony turns to vivid orange–yellow at 30 °C after incubation for 14 days. No soluble pigment is produced in any culture media. Optimum temperature for growth is 30–37 °C. Growth occurs at pH 5.0–9.0. The maximum NaCl concentration for growth is 3 % (w/v). Hydrolysis of starch and peptonization and coagulation of skimmed milk are positive. Gelatin liquefaction is negative. Nitrate is not reduced to nitrite. Utilizes glucose, glycerol, *myo*-inositol, D-mannitol, mannose, raffinose, D-salicin and sucrose as sole carbon sources, but L-arabinose, cellobiose, D-fructose, galactose, rhamnose, ribose, sorbose and D-xylose are not utilized. Shows activities of α-chymotrypsin, α-galatosidase, α-glucosidase, β-galactosidase, *N*-acetyl-β-glucosamidase, leucine arylamidase and trypsin, but shows weakly positive activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase, and shows no activity of lipase (C14), cystine arylamidase, β-glucuronidase, α-mannosidase or α-fucosidase. Whole-cell hydrolysates contain ribose, xylose, arabinose and glucose. Cell-wall peptidoglycan contains *meso*-diaminopimelic and 3-OH-*meso*-diamonopimelic acid. The acyl type of the cell wall is glycolyl. The phospholipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylglycerol. The predominant menaquinones are MK-10 (H_8) and MK-10(H_{10}). Major cellular fatty acids are composed of iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{15:0}$.

The type strain, WPS1-2^T(=KCTC 39787^T=NBRC 112325^T =TISTR 2405^T), was isolated from roots of *Globba winitii* C. H. Wright collected from the botanical garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. The DNA G+C content of the type strain is 73.7 mol%.

Funding information
This study was supported by the Thailand Research Fund for a 2015 Royal Golden Jubilee PhD Program as a scholarship to N. K.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. **Ørskov J.** *Investigations into the Morphology of the Ray Fungi*. Copenhagen: Levin and Munksgaard; 1923.
2. **Genilloud O.** Genus I *Micromonospora* Ørskov 1923, 156^{AL}. In: Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki K *et al.* (editors). *Bergey's Manual of Systematic Bacteriology*, vol. 5. New-York: Springer; 2012. pp. 1039–1057.
3. **Tanasupawat S, Jongrungruangchok S, Kudo T.** *Micromonospora marina* sp. nov., isolated from sea sand. *Int J Syst Evol Microbiol* 2010;60:648–652.
4. **Phongsopitanun W, Kudo T, Mori M, Shiomi K, Pittayakhajonwut P *et al.*** *Micromonospora fluostatini* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 2015;65:4417–4423.
5. **Supong K, Suriyachadkun C, Pittayakhajonwut P, Suwanborirux K, Thawai C.** *Micromonospora spongicola* sp. nov., an actinomycete isolated from a marine sponge in the Gulf of Thailand. *J Antibiot* 2013;66:505–509.

6. **Trujillo ME, Kroppenstedt RM, Schumann P, Carro L, Martínez-Molina E.** *Micromonospora coriariae* sp. nov., isolated from root nodules of *Coriaria myrtifolia*. *Int J Syst Evol Microbiol* 2006;56: 2381–2385.
7. **Kittiwongwattana C, Thanaboripat D, Laosinwattana C, Koohakan P, Parinthawong N *et al.*** *Micromonospora oryzae* sp. nov., isolated from roots of upland rice. *Int J Syst Evol Microbiol* 2015;65:3818–3823.
8. **Thawai C.** *Micromonospora costi* sp. nov., isolated from a leaf of Costus speciosus. *Int J Syst Evol Microbiol* 2015;65:1456–1461.
9. **Bérdy J.** Bioactive microbial metabolites. *J Antibiot* 2005;58:1–26.
10. **Zhao J, Guo L, Liu C, Zhang Y, Guan X *et al.*** *Micromonospora lycii* sp. nov., a novel endophytic actinomycete isolated from wolfberry root (*Lycium chinense* Mill). *J Antibiot* 2016;69:153–158.
11. **Coombs JT, Franco CM.** Visualization of an endophytic *Streptomyces* species in wheat seed. *Appl Environ Microbiol* 2003;69:4260–4262.
12. **Qin S, Li J, Chen HH, Zhao GZ, Zhu WY *et al.*** Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Appl Environ Microbiol* 2009;75:6176–6186.
13. **Küster E, Williams ST.** Selection of media for the isolation of *Streptomycetes*. *Nature* 1964;202:928–929.
14. **Shirling EB, Gottlieb D.** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 1966;16:313–340.
15. **Kelly KL.** *Inter-Society Color Council - National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office; 1964.
16. **Arai T.** *Culture Media for Actinomycetes*. Tokyo: The Society for Actinomycetes Japan; 1975.
17. **Williams ST, Cross T.** Chapter XI Actinomycetes. *Methods Microbiol* 1971;4:295–334.
18. **Staneck JL, Roberts GD.** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 1974;28:226–231.
19. **Uchida K, Aida KO.** An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* 1984;30:131–134.
20. **Tomiyasu I.** Mycolic acid composition and thermally adaptative changes in *Nocardia* . *J Bacteriol* 1982;151:828–837.
21. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.*** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2: 233–241.
22. **Sasser M.** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
23. **Collins MD, Pirouz T, Goodfellow M, Minnikin DE.** Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
24. **Raeder U, Broda P.** Rapid preparation of DNA from filamentous fungi. *Lett Appl Microbiol* 1985;1:17–20.
25. **Suriyachadkun C, Chunhametha S, Thawai C, Tamura T, Potacharoen W *et al.*** *Planotetraspora thailandica* sp. nov., isolated from soil in Thailand. *Int J Syst Evol Microbiol* 2009;59: 992–997.
26. **Lane DJ.** 16S/23S rRNA sequencing. In: Stackebrandt E and Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. Chichester: Wiley; 1991. pp. 115–148.
27. **Hall TA.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98 NT. *Nucleic acids Symp Ser* 1999;41:95–98.
28. **Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.*** Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
29. **Garcia LC, Martínez-Molina E, Trujillo ME.** *Micromonospora pisi* sp. nov., isolated from root nodules of *Pisum sativum*. *Int J Syst Evol Microbiol* 2010;60:331–337.
30. **Kimura M.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
31. **Saitou N, Nei M.** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
32. **Fitch WM.** Toward defining the course of evolution: minimum change for specific tree topology. *Syst Zool* 1971;20:406–416.
33. **Felsenstein J.** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
34. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
35. **Felsenstein J.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
36. **Tamaoka J, Komagata K.** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 1984;25:125–128.
37. **Ezaki T, Hashimoto Y, Yabuuchi E.** Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 1989;39:224–229.
38. **Lechevalier MP, Lechevalier H.** Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 1970;20:435–443.
39. **Wayne LG, Moore WEC, Stackebrandt E, Kandler O, Colwell RR *et al.*** Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.