Actinoplanes subglobosus sp. nov., isolated from mixed deciduous forest soil

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A novel filamentous bacterial strain, A-T 5400^T, which developed subglobose sporangia at the end of sporangiophores on substrate mycelia, was isolated from mixed deciduous forest soil collected in Thailand. The taxonomic position of this micro-organism was described using a polyphasic approach. The 16S rRNA gene sequence and phylogenetic analysis indicated that strain A-T 5400^T belonged to the genus *Actinoplanes* and was most closely related to '*Actinoplanes hulinensis*' NEAU-M9 (98.82 % 16S rRNA gene sequence similarity) and *Actinoplanes philippinensis* NBRC 13878^T (98.75 %). The DNA–DNA relatedness values that distinguished the novel strain from the closest species were below 70 %. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid. The whole-cell sugars were ribose, galactose, glucose and xylose. The predominant menaquinone was MK-9(H_4). The diagnostic phospholipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The predominant cellular fatty acids were unsaturated fatty acids $C_{16:1}$, branched fatty acids iso-$C_{16:0}$ and iso-$C_{15:0}$. The G+C content of the genomic DNA was 71 mol%. Following evidence from phenotypic, chemotaxonomic and genotypic studies, the new isolate is proposed to represent a novel species of the genus *Actinoplanes* named *Actinoplanes subglobosus* sp. nov. The type strain is A-T 5400^T (=BCC 42734^T=TBRC 5832^T=NBRC 109645^T).

The genus *Actinoplanes* was first described by Couch (1950) with *Actinoplanes philippinensis* as the type species. Members of this genus are characterized by the presence of globose, subglobose, cylindrical, lobate, flask-shaped, bell-shaped, or irregular sporangia that contain motile sporangiospores with a clump of polar or peritrichous flagella at the tip of sporangiophores on substrate mycelia (Goodfellow *et al.*, 1990). Aerial mycelia are scant. Cells are Gram-positive, non-acid-fast and aerobic. The peptidoglycan of this genus contains *meso*-diaminopimelic acid, but hydroxyl-diaminopimelic acid is also present. The whole-cell sugars contain xylose as a marker. MK-9(H_4) is the major isoprenoid quinone. The phospholipid pattern is of type II and usually includes phosphatidylethanolamine as the diagnostic phospholipid. Predominant cellular fatty acids are often present as iso-/anteiso-branched and monounsaturated fatty acids and/or cis-9, 10-octadecanoic acid (Tamura & Hatano, 2001). The DNA G+C content is 67–76 mol% (Sazak *et al.*, 2012). Many species of this genus produce bioactive compounds, such as friulimicins, a novel lipopeptide antibiotic from *Actinoplanes friuliensis* (Aretz *et al.*, 2000), and candiplanecin, a new antifungal from *Actinoplanes regularis* (Itoh *et al.*, 1981).

During a study on the diversity of actinomycetes in Thailand, strain A-T 5400^T was isolated from mixed deciduous forest soil collected in Lamphun Province, Northern Thailand. The soil sample was taken from the organic layer on the soil surface and kept at 4 °C. The sample was dried at room temperature for 10 days then heat-dried at 100 °C for 1 h. The dried sample was treated using a method of Suzuki (2001) and plated on soil extract agar (Suriyachadkun *et al.*, 2009) supplemented with 25 mg l^{-1}nalidixic acid, 50 mg l^{-1} cycloheximide and 1 mg l^{-1} terbinafine, then incubated at 28 °C for 21 days. The isolate was purified and maintained on ISP medium no. 2 as a working culture. The pure culture was preserved in glycerol (10 %, v/v) at −80 °C and in liquid-dried form for long-term storage. Strains of the two closest species, '*Actinoplanes hulinensis*' NEAU-M9 and *A.*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A-T 5400^T is KM396265.

Two supplementary figures are available with the online Supplementary Material.

philippinensis NBRC 13878^T were used in this study for comparison of physiological, morphological and chemotaxonomic properties and DNA–DNA hybridization.

Genomic DNA for determination of G+C content analysis and 16S rRNA gene sequence analysis was prepared according to the method of Saito & Miura (1963) from a culture grown in yeast extract-glucose broth on a rotary shaker at 200 r.p.m., 30 °C for 7 days. The G+C content of the genomic DNA of strain A-T 11038^T was determined using the method of Tamaoka & Komagata (1984) after treatment with P1 nuclease and alkaline phosphatase. HPLC was carried out using a Shimadzu LC-6AD apparatus equipped with a Cosmosil 5C_{18}-AR column (4.6×150 mm; Nacalai Tesque). The phylogenetic position of strain A-T 5400^T was determined by 16S rRNA gene sequence analysis. The 16S rRNA gene was amplified by PCR as described previously (Katsura *et al.*, 2001; Yamada *et al.*, 2000). The PCR product for the 16S rRNA gene was sequenced by Macrogen Inc. using universal primers. BLAST analysis was used to compare the nearly complete 16S rRNA gene sequences of strain A-T 5400^T with sequences of representatives of the genus *Actinoplanes* retrieved from the nucleotide databases (EzTaxon-e server; Kim *et al.,* 2012). The 16S rRNA gene sequence similarity was calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.,* 2012). Multiple alignments were carried out with CLUSTAL W (Thompson *et al.*, 1994) of the software BioEdit Sequence Alignment Editor (version 7.0.0). Phylogenetic trees were generated by neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1983) and maximum-likelihood (Felsenstein, 1981) methods using the MEGA 6 software package. The evolutionary distance matrix using the Tamura–Nei model was calculated for neighbour-joining and maximum-likelihood method. The maximum-parsimony search method used Subtree-Pruning-Regrafting (SPR). The robustness for individual branches was estimated by bootstrapping with 1000 replications (Felsenstein, 1985). DNA–DNA hybridization was carried out using a photobiotin-labelling method in microdilution wells as described by Ezaki *et al.* (1989). DNA–DNA relatedness (%) was determined using the colourimetric method (Verlander, 1992).

Pairwise sequence similarities of strain A-T 5400^T were calculated using the 16S rRNA gene sequence of 1443 bases. This strain exhibited the highest 16S rRNA gene sequence similarities of 98.82 % and 98.75 % with '*A. hulinensis*' NEAU-M9 and *A. philippinensis* NBRC 13878^T, respectively. Phylogenetic trees reconstructed by neighbour-joining (Fig. 1), maximum parsimony (Fig. S1a, available in the online Supplementary Material) and maximum likelihood (Fig. S1b) methods showed similar tree topologies. The phylogenetic relationship between strain A-T 5400^T and members of the genus *Actinoplanes* revealed that the novel strain formed a cluster with *A. philippinensis* NBRC 13878^T. The G+C content of genomic DNA from strain A-T 5400^T was 71 mol%. DNA–DNA relatedness levels of strain A-T 5400^T were 34–44 % compared with '*A. hulinensis*' NEAU-M9 and 33–45 % compared with *A. philippinensis* NBRC 13878^T, which are significantly below 70 %, the cut-off point recommended by Wayne *et al.* (1987) for the delineation of separate bacterial species. Results of 16S rRNA gene sequence analysis and DNA–DNA relatedness were sufficient to categorize strain A-T 5400^T as a distinct species from previously described species of the genus *Actinoplanes*.

Morphological characteristics of strain A-T 5400^T were observed using a light microscope (model CX 31; Olympus) with a 40× long working distance objective lens (model LUCPLFLN40XRC; Olympus) and a scanning electron microscope (model JSM-5410 LV; JEOL) after cultivation on soil extract agar (Suriyachadkun *et al.*, 2009) at 30 °C for 14–21 days. Cultures for scanning electron microscopy were prepared as described previously (Itoh *et al.*, 1989). For observations of spore motility, the strain was grown on soil extract agar (30 °C, 30 days), sporangia flooded with 0.1 M potassium phosphate buffer (pH 7) at 30 °C for 30 min and observed with a light microscope. Cultural characteristics of strain A-T 5400^T compared with '*A. hulinensis*' NEAU-M9 and *A. philippinensis* NBRC 13878^T were determined on various media described by Shirling & Gottlieb (1966), ISP2, ISP3, ISP4, ISP5, ISP7 and yeast extract-starch agar, following incubation at 28 °C for 14 and 21 days. The colony colour was determined using ISCC-NBS colour charts (Kelly, 1964). Physiological characteristics were examined using several standard methods. Temperature, pH and NaCl tolerances were determined using ISP2 as a basal medium after incubation for 2 weeks. The temperature range for growth was determined at 20, 25, 30, 37 and 45 °C. The pH range for growth was determined at pH 4.0–11.0 (intervals of 1.0 pH unit) at 30 °C. NaCl tolerance was determined with 0, 1, 2, 3, 4 and 5 % (w/v) NaCl at 30 °C. Utilization of carbohydrates as sole carbon sources was tested using ISP9 medium as a basal medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the carbon sources. Hydrolysis of various compounds was examined using a basal medium recommended by Gordon *et al.* (1974). Tests in the API ZYM commercial system (bioMérieux) were performed according to the manufacturer's instructions. Gelatin liquefaction, peptonization of milk, nitrate reduction, and starch hydrolysis were determined through cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Catalase and oxidase activities were determined using the method of Greenwood & Pickett (1979).

Freeze-dried cells for chemotaxonomic analysis were obtained from a culture growing in glucose-yeast extract broth (Tamura *et al.*, 1994) on a rotary shaker at 200 r.p.m., 30 °C for 7 days. The isomer of diaminopimelic acid in the cell wall was determined using the method of Staneck & Roberts (1974). The acyl type of peptidoglycan was determined using the method of Uchida & Aida (1984). The whole-cell sugars compositions were analysed using TLC cellulose according to the method of Komagata & Suzuki (1987). Polar lipids in whole cells were extracted and analysed by two-dimensional TLC according to the method of Minnikin *et al.* (1984). Cellular fatty acid methyl esters were

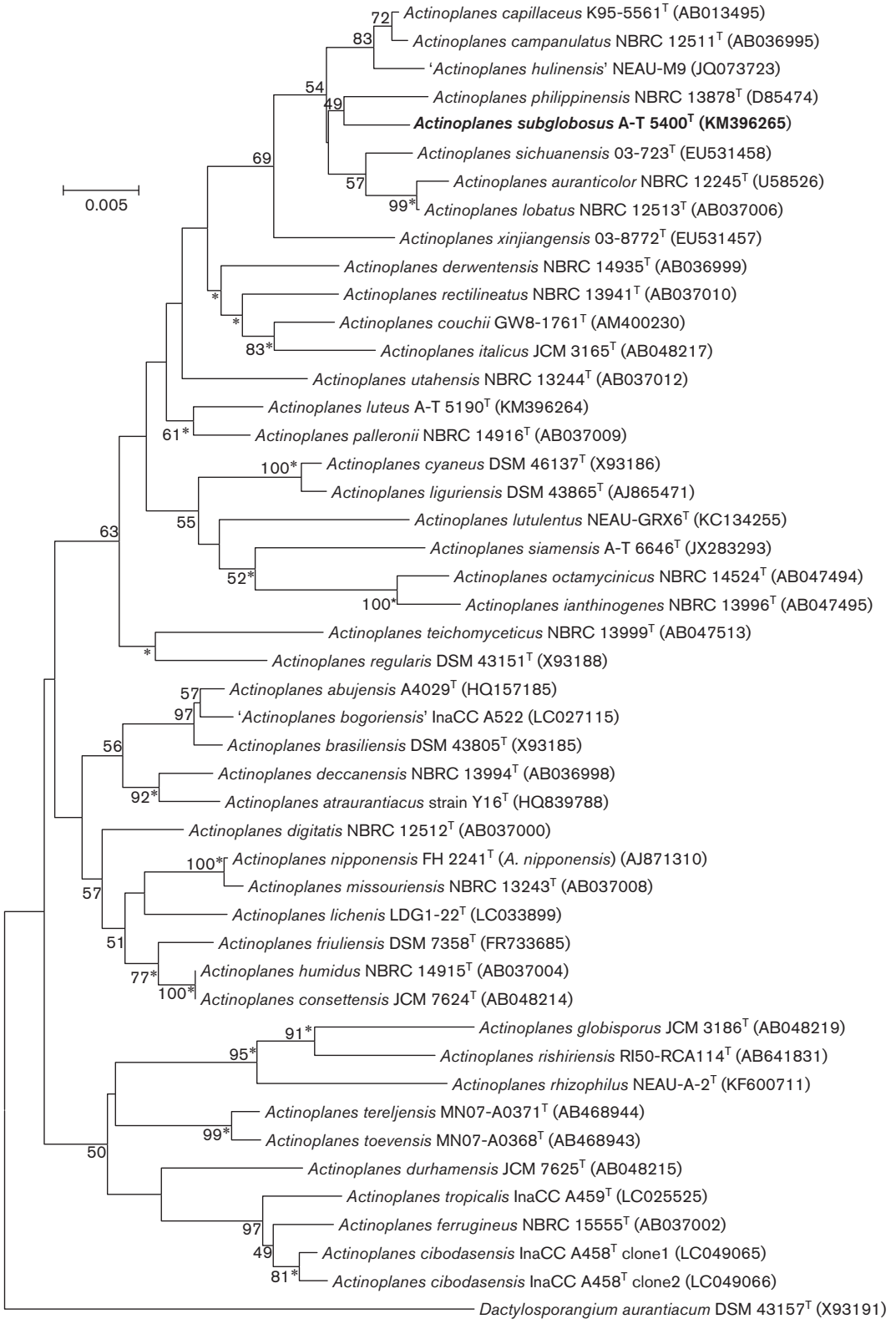

Fig. 1. Phylogenetic relationships derived from 16S rRNA gene sequences between strain A-T 5400^T and other species of the genus *Actinoplanes*. The phylogenetic tree was reconstructed by using the neighbour-joining method. *Dactylosporangium aurantiacum* DSM 43157^T was used as an out-group. Asterisks represent clades that were also recovered with the maximum-

likelihood and maximum-parsimony algorithms. Numbers at nodes indicate bootstrap percentages derived from 1000 replications; only values ≥49 % are indicated. Bar, 0.005 substitutions per nucleotide position.

prepared and analysed following the instructions of TSBA6 method of the Microbial Identification System (MIDI, Sherlock version 6.2B) (Kämpfer & Kroppenstedt, 1996; Sasser, 1990). The presence of mycolic acids in the cell wall was analysed by TLC according to the method of Tomiyasu (1982). Isoprenoid quinones were extracted and purified using the method of Collins *et al.* (1977) and determined using reverse-phase HPLC [Cosmosil $5C_{18}$ column (4.6×150 mm); Nacalai Tesque] with a mixture of methanol and 2-propanol (2 : 1, v/v) as elution solvent (Wu *et al.*, 1989).

Strain A-T 5400^T had characteristics typical of the genus *Actinoplanes*. It developed large subglobose sporangia (10–15 μm in diameter) containing motile sporangiospores at the tip of sporangiophores on substrate mycelia (Fig. 2). The spores were motile. Fragmentation of substrate mycelium was not observed. Phenotypic characteristics of strain A-T 5400^T compared with '*A. hulinensis*' NEAU-M9 and *A. philippinensis* NBRC 13878^{T}are indicated in detail in Table 1 and the species description. Strain A-T 5400^T showed good growth on ISP medium no. 2, 4 and 5, and yeast extract-starch agar. Moderate growth was observed on ISP3. Substrate mycelium was pale yellow to strong orange yellow. Soluble pigment was not observed on the media used.

The isomer of diaminopimelic acid of strain A-T 5400^T was *meso*-diaminopimelic acid. The acyl type of the cell-wall muramic acid was glycolylmuramic acid. Whole-cell hydrolysates contained ribose, galactose, glucose and xylose [whole-cell sugar pattern D of Lechevalier & Lechevalier (1970)]. Menaquinones were MK-9(H_2) (3 %), MK-9(H_4) (93 %), and MK-9(H_6) (4 %). Phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unidentified glycolipids and two unidentified phospholipids were detected, while phosphatidylcholine was not detected (Fig. S2). The unsaturated fatty acids $C_{16:1}$ (15.5 %), branched fatty acids iso-$C_{16:0}$ (24.8 %), and iso-$C_{15:0}$ (10.6 %) were major fatty acids (>10 % each) and saturated fatty acids $C_{14:0}$ (6.9 %), $C_{16:0}$ (5.9 %) and unsaturated fatty acids $C_{18:1}$ (8.2 %) were found in moderate amounts. Mycolic acids were absent.

The strain A-T 5400^T developed subglobose sporangia, while the closest species, '*A. hulinensis*' NEAU-M9 and *A. philippinensis* NBRC 13878^T developed bell-shaped and globose sporangia, respectively. Strain A-T 5400^T and '*A. hulinensis*' NEAU-M9 did not produce soluble pigment, while *A. philippinensis* NBRC 13878^T produced soluble pigment (Table 1). Low DNA–DNA relatedness values were observed between strain A-T 5400^T and type strains of the closest species. The phenotypic (Table 1) and genotypic data show that strain A-T 5400^T can be distinguished from previously described species of the genus *Actinoplanes*. It is therefore concluded that this strain be classified as representative a novel species of the genus *Actinoplanes*, for which the name *Actinoplanes subglobosus* sp. nov. is proposed.

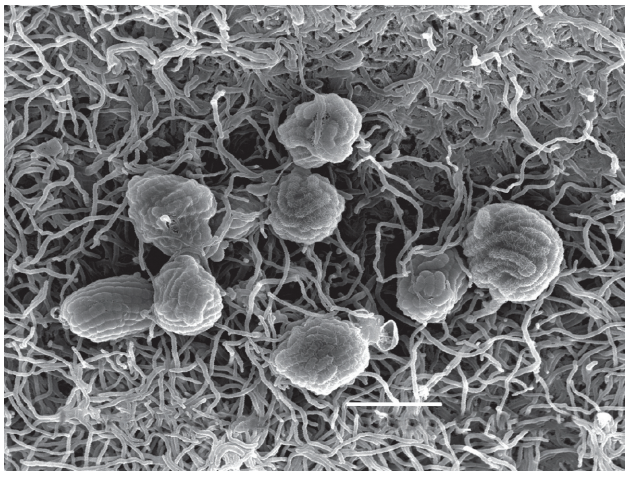

Fig. 2. Scanning electron micrograph of *Actinoplanes subglobosus* sp. nov. A-T 5400^T grown on soil extract agar at 30 °C for 3 weeks. Bar, 1 μm.

Description of *Actinoplanes subglobosus* sp. nov.

Actinoplanes subglobosus (sub.glo.bo′sus. L. pref. *sub* nearly; L. adj. *globosus* globose, spherica; N.L. masc. adj. *subglobosus* referring to the nearly globose or subglobose sporangia that are developed on substrate mycelium).

Cells are aerobic, Gram-positive and non-acid-fast. Substrate mycelium is light orange on ISP2 and ISP4 and vivid orange on ISP5. Large subglobose sporangia, 10–15 μm in diameter, containing motile sporangiospores formed at the tip of sporangiophores on substrate mycelia. Growth occurs at 20–37 °C (optimum 25–30 °C), pH 6–10 (optimum pH 8–9) and in a maximum NaCl concentration of 1 % (w/v). Utilizes L-arabinose, cellobiose, D-galactose, D-glucose, glycerol, D-fructose, inositol, lactose, D-mannose, D-mannitol, raffinose, L-rhamnose, salicin, sucrose, trehalose and D-xylose, but not D-arabitol, melibiose, sorbose, D-ribose, D-sorbitol or D-xylitol. Hydrolysis of starch, xanthine and hypoxanthine are positive. Nitrate reduction and coagulation of milk are negative. Positive for activities of alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase,

Table 1. Characteristics differentiating strain A-T 5400^T, '*A. hulinensis*' NEAU-M9 and *A. philippinensis* NBRC 13878^T

Strains: 1, *Actinoplanes subglobosus* sp. nov. A-T 5400^T; 2, '*A. hulinensis*' NEAU-M9; 3, *A. philippinensis* NBRC 13878^T. All strains were positive for utilization of L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, inositol, lactose, D-mannose, D-mannitol, L-rhamnose and sucrose, and negative for utilization of D-arabitol, melibiose, sorbose and D-xylitol. +, Positive; −, negative.

Characteristic	1	2	3
Colonial properties			
ISP2 (substrate mycelium)	Light orange	Deep reddish-orange	Light orange
ISP4 (substrate mycelium)	Light orange yellow	Deep yellowish-brown	Strong yellowish-brown
ISP5 (substrate mycelium)	Vivid orange	Dark orange yellow	Moderate orange
Soluble pigment	−	−	+
Shape of sporangia	Subglobose	Bell-shaped	Globose
Major menaquinones	MK-9(H_4)	MK-9(H_4), MK-9(H_6)	MK-9(H_4)
Coagulation of milk	−	+	+
Growth at 37 °C	+	−	+
Utilization of:			
Glycerol	+	−	+
Raffinose	+	+	−
D-Ribose	−	−	+
Salicin	+	−	+
D-sorbitol	−	+	+
D-Xylose	+	−	+
Enzyme activity			
Esterase Lipase (C 8)	−	+	+
Valine arylamidase	+	−	−
Cystine arylamidase	+	−	+
α-Chymotrypsin	+	+	−
Acid phosphatase	+	−	+
β-Galactosidase	−	+	+
N-Acetyl-β-glucosaminidase	−	+	+

α-glucosidase, β-glucosidase and catalase. Cell-wall amino acids contain *meso*-diaminopimelic acid. Acyl type of the cell-wall muramic acid is glycolylmuramic acid. The characteristic whole-cell sugars are ribose, galactose, glucose and xylose. The predominant menaquinone is MK-9(H_4). The diagnostic phospholipids are phosphatidylethanolamine and diphosphatidylglycerol as major components; lacks phosphatidylcholine and aminoglycolipids. The major fatty acids are unsaturated fatty acids $C_{16:1}$, and branched fatty acids iso-$C_{16:0}$ and iso-$C_{15:0}$.

The type strain is A-T 54090^T (=BCC 42734^T=TBRC 5832^T=NBRC 109645^T), isolated from mixed deciduous forest soil. The G+C content of the DNA of the type strain is 71 mol%.

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References

Arai, T. (1975). *Culture Media for Actinomycetes*. Tokyo: The Society for Actinomycetes Japan (in Japanese).

Aretz, W., Meiwes, J., Seibert, G., Vobis, G. & Wink, J. (2000). Friulimicins: novel lipopeptide antibiotics with peptidoglycan synthesis inhibiting activity from *Actinoplanes friuliensis* sp. nov. I. Taxonomic studies of the producing microorganism and fermentation. *J Antibiot (Tokyo)* **53**, 807–815.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Couch, J. N. (1950). *Actinoplanes*, a new genus of the *Actinomycetales*. *J Elisha Mitchell Sci Soc* **66**, 87–92.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). 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* **39**, 224–229.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1983). Parsimony in systematics: biological and statistical issues. *Annu Rev Ecol Syst* **14**, 313–333.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Goodfellow, M., Stanton, L. J., Simpson, K. E. & Minnikin, D. E. (1990). Numerical and chemical classification of *Actinoplanes* and some related actinomycetes. *J Gen Microbiol* **136**, 19–36.

Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca, Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.

Greenwood, J. R. & Pickett, M. J. (1979). Salient features of *Haemophilus vaginalis*. *J Clin Microbiol* **9**, 200–204.

Itoh, T., Kudo, T., Parenti, F. & Seino, A. (1989). Amended description of the genus *Kineosporia*, based on chemotaxonomic and morphological studies. *J Antibiot* (Tokyo) **39**, 168–173.

Itoh, Y., Enokita, R., Okazaki, T., Iwado, S., Torikata, A., Haneishi, T. & Arai, M. (1981). Candiplanecin, a new antibiotic from *Ampullariella regularis* subsp. *mannitophila* subsp. nov. I. Taxonomy of producing organism and fermentation. *J Antibiot (Tokyo)* **34**, 929–933.

Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

Katsura, K., Kawasaki, H., Potacharoen, W., Saono, S., Seki, T., Yamada, Y., Uchimura, T. & Komagata, K. (2001). *Asaia siamensis* sp. nov., an acetic acid bacterium in the alpha-proteobacteria. *Int J Syst Evol Microbiol* **51**, 559–563.

Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standard Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Komagata, K. & Suzuki, K. I. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lechevalier, M. P. & Lechevalier, H. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.

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 Meth* **2**, 233–241.

Saito, H. & Miura, K.-I. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim Biophys Acta* **72**, 619–629.

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.

Sazak, A., Sahin, N. & Camas, M. (2012). *Actinoplanes abujensis* sp. nov., isolated from Nigerian arid soil. *Int J Syst Evol Microbiol* **62**, 960–965.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Suriyachadkun, C., Chunhametha, S., Thawai, C., Tamura, T., Potacharoen, W., Kirtikara, K. & Sanglier, J. J. (2009). *Planotetraspora thailandica* sp. nov., isolated from soil in Thailand. *Int J Syst Evol Microbiol* **59**, 992–997.

Suzuki, S.-I. (2001). Establishment and use of gellan gum media for selective isolation and distribution survey of specific rare actinomycetes. *Actinomycetol* **15**, 55–60.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Tamura, T. & Hatano, K. (2001). Phylogenetic analysis of the genus *Actinoplanes* and transfer of *Actinoplanes minutisporangius* Ruan *et al.* 1986 and '*Actinoplanes aurantiacus*' to *Cryptosporangium minutisporangium* comb. nov. and *Cryptosporangium aurantiacum* sp. nov. *Int J Syst Evol Microbiol* **51**, 2119–2125.

Tamura, T., Nakagaito, Y., Nishii, T., Hasegawa, T., Stackebrandt, E. & Yokota, A. (1994). A new genus of the order *Actinomycetales, Couchioplanes* gen. nov., with descriptions of *Couchioplanes caeruleus* (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov. *Int J Syst Bacteriol* **44**, 193–203.

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.

Tomiyasu, I. (1982). Mycolic acid composition and thermally adaptative changes in *Nocardia asteroides*. *J Bacteriol* **151**, 828–837.

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.

Verlander, C. P. (1992). Detection of horseradish peroxidase by colorimetry. In *Nonisotopic DNA Probe Techniques*, pp. 185–201. Edited by L. J. Kricka. New York: Academic Press.

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. & Cross, T. (1971). Actinomycetes. *Methods Microbiol* **4**, 295–334.

Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology [English translation of Microbiology (Beijing)]* **16**, 176–178.

Yamada, Y., Katsura, K., Kawasaki, H., Widyastuti, Y., Saono, S., Seki, T., Uchimura, T. & Komagata, K. (2000). *Asaia bogorensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α-*Proteobacteria*. *Int J Syst Evol Microbiol* **50**, 823–829.