# *Actinoallomurus acaciae* sp. nov., an endophytic actinomycete isolated from *Acacia auriculiformis* A. Cunn. ex Benth.

Arinthip Thamchaipenet,[1] Chantra Indananda,[1]† Chakrit Bunyoo,[1] Kannika Duangmal,[2] Atsuko Matsumoto[3] and Yoko Takahashi[3]

Correspondence
Arinthip Thamchaipenet
arinthip.t@ku.ac.th

[1]Department of Genetics, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand

[2]Department of Microbiology, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand

[3]Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

A novel endophytic actinomycete, strain GMKU 931$^T$, was isolated from the root of a wattle tree, *Acacia auriculiformis* A. Cunn. ex Benth., collected at Kasetsart University, Bangkok, Thailand. Strain GMKU 931$^T$ produced short spiral chains of smooth-surfaced spores on the aerial mycelium. Lysine and *meso*-diaminopimelic acid were present in the cell-wall peptidoglycan. Whole-cell hydrolysates contained galactose, madurose and mannose. The predominant menaquinones were MK-9($H_6$) and MK-9($H_8$). The major fatty acids were iso-$C_{16:0}$ and iso-$C_{16:1}$. The major phospholipids were phosphatidylinositol and phosphatidylglycerol. A phylogenetic analysis based on 16S rRNA gene sequences suggested that strain GMKU 931$^T$ forms a distinct phyletic line within the recently proposed genus *Actinoallomurus*. The significant differences in phenotypic and genotypic data indicate that strain GMKU 931$^T$ represents a novel species of the genus *Actinoallomurus*, for which the name *Actinoallomurus acaciae* sp. nov. is proposed. The type strain is GMKU 931$^T$ (=BCC 28622$^T$ =NBRC 104354$^T$ =NRRL B-24610$^T$).

Endophytic actinomycetes have recently attracted a lot of attention. Their mutual association with plants may play important roles in protecting the plant from pathogenic infection, promoting plant growth or assisting plant survival during environmental stress (Kunoh, 2002; Hasegawa *et al.*, 2006). The isolation of novel endophytic actinomycetes is expected to lead to the identification of novel bioactive compounds and/or growth-regulating agents as well as novel actinomycete genera and species. Thailand is a natural-resource-rich country and there is a wide range of plant diversity. Therefore, we have established a programme for the isolation and identification of endophytic actinomycetes from plants, including those of important agricultural and medicinal species. In this work, endophytic actinobacteria were isolated from the wattle tree (*Acacia auriculiformis* A. Cunn. ex Benth.), of which one is described here.

†Present address: Department of Biology, Faculty of Science, Burapha University, Chonburi 20131, Thailand.

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GMKU 931$^T$ is EU429322.

The genus *Actinoallomurus* (Tamura *et al.*, 2009) is the most recently described genus in the family *Thermomonosporaceae* (Kroppenstedt & Goodfellow, 1991; Zhang *et al.*, 1998, 2001), which includes *Actinocorallia*, *Actinomadura* (Nonomura & Ohara, 1971), *Excellospora*, *Spirillospora* and *Thermomonospora*. Members of the genus *Actinoallomurus* were found to be phylogenetically related to *Actinomadura spadix*, which exhibited low 16S rRNA gene sequence similarity with other *Actinomadura* species (Tamura *et al.*, 2009). Polyphasic investigation also revealed that *Actinomadura spadix* could be clearly distinguished at the genus level from other *Actinomadura* species and, therefore, the species was assigned to the genus *Actinoallomurus* as *Actinoallomurus spadix* comb. nov. (Tamura *et al.*, 2009). At the same time, other isolates from soil and dung samples, collected from various places in Japan, were chemotaxonomically and phylogenetically characterized and eight more novel species belonging to the genus *Actinoallomurus* were proposed (Tamura *et al.*, 2009). *Actinoallomurus spadix* was designated the type species of the genus.

Members of the genus *Actinoallomurus* contain *meso*-diaminopimelic acid in the cell wall and madurose as a

characteristic sugar in whole-cell hydrolysates, which is similar to the closely related genus *Actinomadura* (Lechevalier & Lechevalier, 1970; Goodfellow, 1989). Additionally, the cell-wall peptidoglycan encloses D- and L-lysine, which is an important characteristic to differentiate *Actinoallomurus* species from *Actinomadura* species (Tamura *et al.*, 2009). The acyl type of the muramic acid is *N*-acetyl. The fatty acid profiles include iso-hexadecanoic acid (iso-$C_{16:0}$) as the major component and the phospholipid patterns contain phosphatidylinositol mannoside. The main menaquinones are MK-9($H_6$) and MK-9($H_8$).

Strain GMKU 931$^T$ was isolated from a root of *Acacia auriculiformis* A. Cunn. ex Benth. collected at Kasetsart University, Bangkok, Thailand. The root was surface sterilized with 95 % ethanol and 1 % sodium hypochlorite before being ground and spread onto starch-casein agar (Küster & Williams, 1964) supplemented with ($ml^{-1}$) 100 μg ampicillin, 2.5 U penicillin G and 20 μg ketoconazole. After incubation at 30 °C for 21 days, abundant small, white colonies were observed. The strain was isolated and purified on mannitol soya (MS) agar (Hobbs *et al.*, 1989). The pure culture was maintained as a suspension in 20 % glycerol at –80 °C and as lyophilized cells for long-term preservation.

Strain GMKU 931$^T$ grew well on International *Streptomyces* Project (ISP) medium 2 (Shirling & Gottlieb, 1966) and MS agar at 30 °C and it started to produce whitish spores after 14 days. Moderate growth was observed on ISP 3, oatmeal-nitrate agar ($l^{-1}$: 3.0 g Quaker white oat, 0.2 g $KNO_3$, 0.5 g $K_2HPO_4$, 0.2 g $MgSO_4 . 7H_2O$, 15.0 g agar; pH 7.0) and 1/10 yeast extract-starch agar, poor growth was observed on ISP 5 and there was no growth on ISP 4. No soluble pigment was produced on any of the media tested. No production of melanin pigment was observed on ISP 1 or ISP 7. Morphological characteristics were examined by subculturing the strain on oatmeal-nitrate agar and observing under a light microscope and a scanning electron microscope (JSM 5600 LV; JEOL). Strain GMKU 931$^T$ formed short spiral chains of smooth-surfaced spores (Fig. 1). Growth of strain GMKU 931$^T$ was determined over the temperature range 5–50 °C in a temperature-gradient incubator over 14 days on ISP 2. Growth was observed at 12–41 °C, with the optimal temperature for good growth being 28–30 °C. The strain was able to grow at pH 5.0–8.0, with optimal growth at pH 6.0–7.0. On ISP 2, the strain was able to tolerate NaCl up to 3 % (w/v); no growth was observed at 4 % NaCl.

Urease activity was determined by a colour change in urea broth (Gordon *et al.*, 1974). Hydrolysis of casein and gelatin was evaluated using the media of Gordon *et al.* (1974). Reduction of nitrate was determined using ISP 8 by the method of the ISP (Shirling & Gottlieb, 1966). Catalase and oxidase activities were determined with 3 % (v/v) hydrogen peroxide solution and 1 % tetramethyl-*p*-phenylenediamine solution, respectively. Strain GMKU 931$^T$ showed activity for catalase and urease but not for oxidase. Degradation of gelatin and casein and reduction of nitrate were negative.

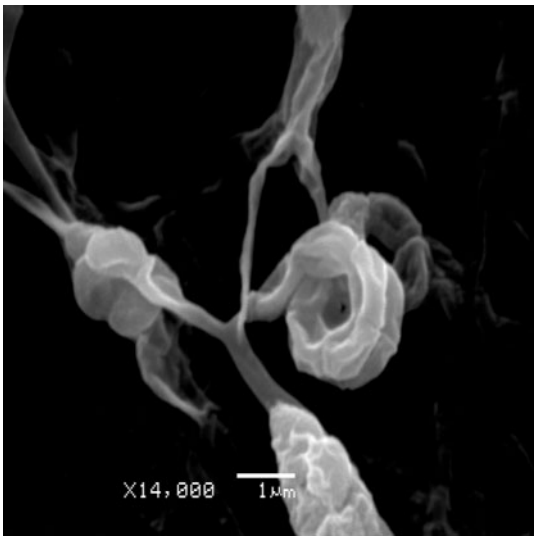


**Fig. 1.** Scanning electron micrograph of short spiral spore chains of strain GMKU 931$^T$ grown on oatmeal-nitrate agar at 27 °C for 5 weeks. Bar, 1 μm.

Genomic DNA of strain GMKU 931$^T$ was extracted from mycelium material scraped from a well-grown culture on ISP 2 according to the protocol described by Kieser *et al.* (2000). The 16S rRNA gene sequence was amplified using primers described by Tajima *et al.* (2001). Amplification was carried out in a thermal cycler (TaKaRa), with an initial incubation step at 94 °C for 1 min, 30 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1–5 min and a final extension step at 72 °C for 2 min. The PCR product was purified using a QIAquick Gel Extraction kit (Qiagen) and was sequenced directly on an ABI model 3130 automatic DNA sequencer using a BigDye Terminator cycle sequencing kit (Applied Biosystems). An almost-complete 16S rRNA gene sequence of strain GMKU 931$^T$ (1468 bp) was preliminarily compared with 16S rRNA gene sequences in the GenBank database, which indicated a close relationship with members of the genus *Actinoallomurus* (Tamura *et al.*, 2009). Multiple alignment of the sequences obtained from strain GMKU 931$^T$ and the type strains of the nine *Actinoallomurus* species with validly published names, using *Actinomadura madurae* NBRC 14623$^T$ as an outgroup, was performed using CLUSTAL X version 2 (Larkin *et al.*, 2007). A phylogenetic tree was constructed with MEGA version 4.0 (Tamura *et al.*, 2007) using the neighbour-joining method (Saitou & Nei, 1987) and the reliability of the tree topology was evaluated by bootstrap analysis with 1000 resamplings (Felsenstein, 1985). The result of the phylogenetic analysis indicated that strain GMKU 931$^T$ formed a distinct clade within the genus *Actinoallomurus* (Fig. 2). The closest phylogenetic

neighbours were *Actinoallomurus caesius* NBRC 103678$^T$, *Actinoallomurus amamiensis* NBRC 103682$^T$ and *Actinoallomurus fulvus* NBRC 103680$^T$, with 16S rRNA gene sequence similarity values of 99.30, 99.20 and 99.11 %, respectively.

Strain GMKU 931$^T$ was analysed chemically using the methodology for the genus *Actinoallomurus* (Tamura *et al.*, 2009). The biomass was obtained after incubation at 27 °C for 5 days in ISP 2 broth. Whole-cell amino acids and sugars were analysed using the method of Hasegawa *et al.* (1983) and Becker *et al.* (1965), respectively. The acyl type of the cell wall was analysed according to the method of Uchida & Aida (1984). Phospholipids were extracted and determined by the method of Minnikin *et al.* (1984). Menaquinones were extracted and purified by using the method of Collins *et al.* (1977) and isoprene units were analysed by HPLC using a Jasco 802-SC chromatograph equipped with a Shiseido CAPCELL PAK C18 column as described by Tamaoka *et al.* (1983). Analysis of the fatty acids was performed according to the procedures for the Sherlock Microbial Identification System (Microbial ID). Mycolic acids were analysed by TLC according to the method of Tomiyasu (1982). The G+C content (mol%) of DNA, which was isolated according to the method of Marmur (1961), was determined by HPLC according to the method of Tamaoka & Komagata (1984). The diagnostic amino acids of the peptidoglycan layer of strain GMKU 931$^T$ were *meso*-diaminopimelic acid, lysine, alanine and glutamic acid. The sugars presented in whole-cell hydrolysates were galactose, glucose, madurose, mannose and ribose. Madurose was the characteristic sugar, indicating type-B whole-cell sugars (Lechevalier & Lechevalier, 1970). The *N*-acyl group of the muramic acid in peptidoglycan was of the acetyl type. Phosphatidylinositol and phosphatidylglycerol were detected as the major phospholipids. The major menaquinones were MK-9($H_6$) and MK-9($H_8$), while a small amount of MK-9($H_4$) was also detected. The predominant fatty acids were iso-$C_{16:0}$ (40.9 %) and iso-$C_{16:1}$ (16 %). The minor fatty acids were $C_{16:0}$ (4.3 %), 10-methyl $C_{16:0}$ (3.4 %), $C_{17:0}$ (3.8 %), anteiso-$C_{17:0}$ (5 %), 10-methyl $C_{17:0}$ (6.9 %), $C_{18:0}$ (4.6 %) and 10-methyl $C_{18:0}$ (tuberculostearic acid; 3.3 %). No mycolic acids were detected. The G+C content of the DNA of strain GMKU 931$^T$ was 70.6 mol%.

There are a number of phenotypic differences between strain GMKU 931$^T$ and its closest phylogenetic neighbours, *Actinoallomurus caesius* NBRC 103678$^T$, *Actinoallomurus amamiensis* NBRC 103682$^T$ and *Actinoallomurus fulvus* NBRC 103680$^T$, including differences in morphological characteristics, optimal temperature for growth, utilization of sole carbon sources, degradation abilities and enzymic activities (Table 1). The significant characteristics that distinguish strain GMKU 931$^T$ from the other three strains are that strain GMKU 931$^T$ does not produce diffusible pigment on ISP 2, ISP 3 or yeast-starch agar, does not grow on ISP 4, grows well at 28–30 °C, does not utilize maltose, raffinose or gelatin, hydrolyses urea and exhibits α-glucosidase activity when examined with the API ZYM enzyme assay.

To examine the finer taxonomic relationships between strain GMKU 931$^T$ and its three closest phylogenetic neighbours, DNA–DNA hybridization relatedness values (means of duplicate measurements) were determined fluorometrically by the method of Ezaki *et al.* (1989). The results supported the phenotypic and genotypic data and confirmed that strain GMKU 931$^T$ belongs to a different species: low DNA–DNA relatedness values were found between strain GMKU 931$^T$ and *Actinoallomurus caesius* NBRC 103678$^T$ (44 %), *Actinoallomurus amamiensis* NBRC 103682$^T$ (43 %) and *Actinoallomurus fulvus* NBRC 103680$^T$ (43 %).

On the basis of the data presented in this study, it is evident that strain GMKU 931$^T$ represents a distinct novel genomic species belonging to the genus *Actinoallomurus*. Strain GMKU 931$^T$ is readily distinguished from its closest phylogenetic neighbours *Actinoallomurus caesius*, *Actinoallomurus amamiensis* and *Actinoallomurus fulvus* on the basis of distinct phyletic lines, differences in phenotypic data and low levels of DNA–DNA relatedness. The name *Actinoallomurus acaciae* sp. nov. is proposed.

## Description of *Actinoallomurus acaciae* sp. nov.

*Actinoallomurus acaciae* (a.ca.ci′ae. L. n. *acacia* the acacia tree and also the name of a botanical genus; L. gen. n. *acaciae* of *Acacia*, referring to the isolation of the type strain from a root of *Acacia auriculiformis* A. Cunn. ex Benth.).

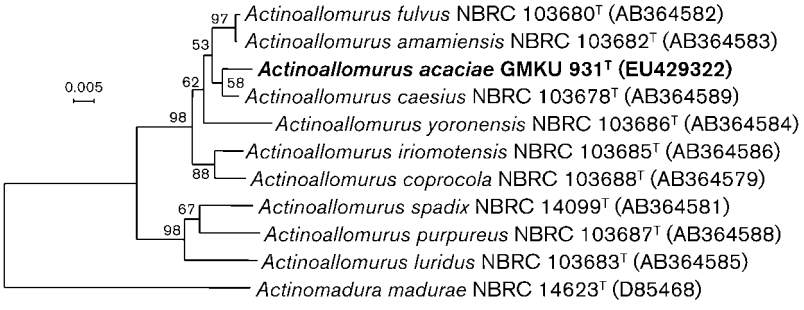


**Fig. 2.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relative position of strain GMKU 931$^T$ and the nine type strains of the genus *Actinoallomurus*. *Actinomadura madurae* NBRC 14623$^T$ was used as an outgroup. Bootstrap values (>50 %) based on 1000 resampled datasets are shown at branch nodes. Bar, 0.005 substitutions per nucleotide position.

**Table 1.** Phenotypic characteristics that differentiate strain GMKU 931$^T$ from the type strains of the most closely phylogenetically related *Actinoallomurus* species

Strains: 1, *Actinoallomurus acaciae* sp. nov. GMKU 931$^T$; 2, *Actinoallomurus caesius* NBRC 103678$^T$; 3, *Actinoallomurus amamiensis* NBRC 103682$^T$; 4, *Actinoallomurus fulvus* NBRC 103680$^T$. Data for reference strains were taken from Tamura *et al.* (2009). +, Positive; W, weakly positive; V, variable; −, negative; NA, not applicable.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Growth on ISP 2 | | | | |
| Growth | Good | Good | Good | Good |
| Aerial mycelium | Pale yellow brown | Moderate brown | Light yellowish brown to dark reddish brown | Dark reddish brown |
| Soluble pigment | None | Moderate yellowish brown | Strong brown | Light greyish brown |
| Growth on ISP 3 | | | | |
| Growth | Moderate | Moderate | Good | Moderate |
| Aerial mycelium | White | Light yellow | Pale yellow | Moderate yellow |
| Soluble pigment | None | None | Pale yellow | None |
| Growth on ISP 4 | | | | |
| Growth | Absent | Moderate | Moderate | Moderate |
| Aerial mycelium | NA | Pale yellow to yellowish pink | Pale yellow to moderate orange | Moderate to strong yellow |
| Soluble pigment | NA | None | None | None |
| Growth on yeast-starch agar | | | | |
| Growth | Moderate | Moderate | Moderate | Moderate |
| Aerial mycelium | White | Pale yellow | Pale yellow to light olive | Pale yellow |
| Soluble pigment | None | None | None | Pale yellow |
| Optimum growth temperature (°C) | 28–30 | 20–37 | 20–37 | 20–37 |
| Utilization of: | | | | |
| L-Arabinose | − | − | + | + |
| Dulcitol | − | − | + | + |
| D-Fructose | − | − | − | + |
| D-Galactose | − | + | + | + |
| D-Glucose | − | + | + | + |
| *myo*-Inositol | + | − | + | + |
| Maltose | − | W | + | + |
| D-Mannitol | − | + | + | − |
| Raffinose | − | + | + | + |
| D-Sorbitol | − | − | + | + |
| Sucrose | − | − | + | + |
| Hydrolysis of: | | | | |
| Gelatin | − | + | + | + |
| Starch | W | − | + | + |
| Urea | + | − | − | − |
| Enzyme activities | | | | |
| *N*-Acetyl-$\beta$-glucosaminidase | + | V | V | + |
| Alkaline phosphatase | + | + | − | + |
| $\beta$-Glucuronidase | + | V | − | V |
| $\alpha$-Glucosidase | + | − | − | − |
| $\beta$-Glucosidase | + | − | − | V |

Aerobic and Gram-positive. Cells grow well on ISP 2 and MS agar and show moderate growth on ISP 3, oatmeal-nitrate agar and 1/10 yeast-starch agar, forming a well-developed white aerial mycelium that differentiates into short spiral spore chains with smooth surfaces. Neither diffusible pigment nor melanin is produced on any of the media tested. The optimal temperature for growth is 28–30 °C and optimal pH is pH 6.0–7.0. Tolerates up to 3 % (w/v) NaCl. Catalase- and urease-positive, oxidase-negative. Nitrate reduction is negative. Hydrolysis of casein, milk and gelatin is negative and degradation of starch is weakly positive. D-Mannose, L-rhamnose and trehalose are utilized as sole carbon sources but L-arabinose, dulcitol, D-fructose, D-galactose, D-glucose, $\beta$-lactose, maltose, D-mannitol, raffinose, D-sorbitol, sucrose and D-xylose are not utilized. With the API ZYM enzyme assay,

acid phosphatase, *N*-acetyl-$\beta$-glucosaminidase, alkaline phosphatase, esterase (C4), $\alpha$- and $\beta$-glucosidases, $\beta$-glucuronidase, leucine aminopeptidase, lipase (C8), $\alpha$-mannosidase and phosphoamidase are detected; chymotrypsin, cystine aminopeptidase, $\alpha$-fucosidase, $\alpha$- and $\beta$-galactosidases, lipase (C14), trypsin and valine aminopeptidase are not detected. The diagnostic diamino acids of the peptidoglycan are *meso*-diaminopimelic acid, lysine, alanine and glutamic acid. Whole-cell sugars include galactose, madurose and mannose. The glycan moiety of the murein is acetylated. The predominant menaquinones are MK-9($H_6$) and MK-9($H_8$), with MK-9($H_4$) as a minor component. The major fatty acids are iso-$C_{16:0}$ and iso-$C_{16:1}$ and the minor fatty acids are $C_{16:0}$, 10-methyl $C_{16:0}$, $C_{17:0}$, anteiso-$C_{17:0}$, 10-methyl $C_{17:0}$, $C_{18:0}$ and 10-methyl $C_{18:0}$. The phospholipid pattern comprises phosphatidylinositol and phosphatidylglycerol. The G+C content of the DNA of the type strain is 70.6 mol%.

The type strain, GMKU 931$^T$ (=BCC 28622$^T$ =NBRC 104354$^T$ =NRRL B-24610$^T$), was isolated from a root of *Acacia auriculiformis* A. Cunn. ex Benth. collected in Kasetsart University, Bangkok, Thailand.

## Acknowledgements

We would like to thank Dr J. P. Euzéby for kind advice on naming the species. C. I. was awarded a PhD scholarship from the Commission on Higher Education (CHE), Ministry of Education, Thailand. C. B. was granted an MS scholarship from co-funding of the Thailand Research Fund (TRF) and the Faculty of Science, Kasetsart University. This work was supported by co-funding of TRF, CHE and the Kasetsart University Research and Development Institute.

## References

**Becker, B., Lechevalier, M. P. & Lechevalier, H. A. (1965).** Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Appl Microbiol* **13**, 236–243.

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

**Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989).** Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution 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. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

**Goodfellow, M. (1989).** Maduromycetes. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2509–2551. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

**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.

**Hasegawa, T., Takizawa, M. & Tanida, S. (1983).** A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.

**Hasegawa, S., Meguro, A., Shimizu, M., Nishimura, T. & Kunoh, H. (2006).** Endophytic actinomycetes and their interactions with host plants. *Actinomycetologica* **20**, 72–81.

**Hobbs, G., Frazer, C. M., Gardner, D. C. J., Cullum, J. A. & Oliver, S. G. (1989).** Dispersed growth of *Streptomyces* in liquid culture. *Appl Microbiol Biotechnol* **31**, 272–277.

**Kieser, Y., Bibb, M. J., Buttner, M. J., Chater, K. F. & Hopwood, D. A. (2000).** *Practical Streptomyces Genetics*. Norwich: The John Innes Foundation.

**Kroppenstedt, R. M. & Goodfellow, M. (1992).** 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.

**Kunoh, H. (2002).** Endophytic actinomycetes: attractive biocontrol agents. *J Gen Plant Pathol* **68**, 249–252.

**Küster, E. & Williams, S. T. (1964).** Media for the isolation of streptomycetes: starch casein medium. *Nature* **202**, 928–929.

**Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. & other authors (2007).** CLUSTAL W and CLUSTAL X version 2.0. *Bioinformatics* **23**, 2947–2948.

**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.

**Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

**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.

**Nonomura, H. & Ohara, Y. (1971).** Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier et al. *J Ferment Technol* **49**, 904–912.

**Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

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

**Tajima, K., Takahashi, Y., Seino, A., Iwai, Y. & Ōmura, S. (2001).** Description of two novel species of the genus *Kitasatospora* Ōmura *et al.* 1982, *Kitasatospora cineracea* sp. nov. and *Kitasatospora niigatensis* sp. nov. *Int J Syst Evol Microbiol* **51**, 1765–1771.

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

**Tamaoka, J., Katayara-Fujimura, Y. & Kuraishi, H. (1983).** Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.

**Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

**Tamura, T., Ishida, Y., Nozawa, Y., Otogturo, M. & Suzuki, K. (2009).** Transfer of *Actinomadura spadix* Nonomura and Ohara 1971 to *Actinoallomurus spadix* gen. nov., comb. nov., and description of *Actinoallomurus amamiensis* sp. nov., *Actinoallomurus caesius* sp. nov., *Actinoallomurus coprocola* sp. nov., *Actinoallomurus fulvus* sp. nov., *Actinoallomurus iriomotensis* sp. nov., *Actinoallomurus luridus* sp. nov., *Actinoallomurus purpureus* sp. nov. and *Actinoallomurus yoronensis* sp. nov. *Int J Syst Evol Microbiol* **59**, 1867–1874.

**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 acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.

**Zhang, Z., Wang, Y. & Ruan, J. (1998).** Reclassification of *Thermomonospora* and *Microtetraspora*. *Int J Syst Bacteriol* **48**, 411–422.

**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.