Streptomyces qinglanensis sp. nov., isolated from mangrove sediment

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A *Streptomyces*-like strain, 172205^T, was obtained from mangrove soil collected at Qinglan Harbour, Wenchang, Hainan, China. The strain was characterized by white aerial mycelium and long spore chains. Comparison of 16S rRNA gene sequences indicated that the strain represents a novel member of the genus *Streptomyces*, exhibiting highest levels of similarity (<98.29%) to the type strains of members of the genus *Streptomyces*. However, DNA–DNA relatedness and phenotypic data readily distinguished strain 172205^T from phylogenetically related type strains. The predominant menaquinones were MK-9(H₈) and MK-9(H₉). The major fatty acids were iso-C_{15:0} (10.31%), anteiso-C_{15:0} (35.19%), iso-C_{16:0} (20.24%) and anteiso-C_{17:0} (10.05%). The diagnostic phospholipid was phosphatidylethanolamine. The cell wall contained L-diaminopimelic acid and meso-diaminopimelic acid and whole-cell hydrolysates contained ribose, galactose and glucose. The results of DNA–DNA hybridization, physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strain 172205^T from phylogenetically related type strains. Therefore, strain 172205^T is considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces qinglanensis* sp. nov. is proposed. The type strain is 172205^T (=CGMCC 4.6825^T =DSM 42035^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and, at the time of writing, encompasses over 500 recognized species (Euzéby, 2010; <http://www.bacterio.cict.fr/s/streptomyces.html>). Species of the genus *Streptomyces* are well known as a rich source of antibiotics and bioactive molecules and they are thus considered to be a rich biotechnological resource (Chun *et al.*, 1997; Labeda *et al.*, 1997; Iwai & Takahashi, 1992). The ability of streptomycetes to produce antibiotics and other industrially significant secondary metabolites remains unsurpassed (Sanglier *et al.*, 1996; Ōmura *et al.*, 2001; Watve *et al.*, 2001; Bentley *et al.*, 2002; Ikeda *et al.*, 2003; Ward & Goodfellow, 2004; Bérdy, 2005). One environment from which novel species of streptomycetes have been isolated is mangroves (Xu *et al.*, 2009; Xiao *et al.*, 2009; Sui *et al.*, 2011).

A soil sample from Qinglan Harbour mangrove was pretreated by the dispersion and differential centrifugation

technique (Hopkins *et al.*, 1991) and strain 172205^T was isolated on Gauze No. 1 medium (Gauze *et al.*, 1983). Strong activity against the human immortalized myelogenous leukaemia cell line K562 was discovered from crude extracts of strain 172205^T fermentation broth (Hong *et al.*, 2009). The purified strain was maintained on yeast extract-malt extract agar (International *Streptomyces* Project medium no. 2, ISP 2) (Shirling & Gottlieb, 1966) slopes at 4 °C for short-term preservation and as glycerol suspensions (20%, v/v) at –80 °C for long-term preservation (Wellington & Williams, 1978).

Morphology was observed by light microscopy (80i; Nikon) and scanning electron microscopy (S-3000N; Hitachi) of ISP 3 agar cultures incubated at 28 °C for 15 days. Cultural characteristics were determined after 2 weeks at 28 °C according to the methods proposed by Shirling & Gottlieb (1966). Colony colour was determined by reference to ISCC-NBS colour charts (Kelly, 1964). The physiological and biochemical characteristics of strain 172205^T and its phylogenetically closest neighbours were tested under the same conditions, by using the well-established procedures of Williams *et al.* (1983) and Kämpfer *et al.* (1991). The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 172205^T is HQ660227.

One supplementary table and a supplementary figure are available with the online version of this paper.

utilization of sole carbon and nitrogen sources was analysed according to the methods described by Shirling & Gottlieb (1966). Growth at 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C was tested on modified Bennett's agar (Jones, 1949) plates incubated for 7–14 days. NaCl tolerance (0, 1, 3, 5, 7, 9, 10, 15 and 25 %, w/v) and the pH range for growth (pH 4, 5, 7, 9, 10 and 11) were determined in liquid modified Bennett's medium (1.0 g yeast extract, 0.8 g beef extract, 2.0 g Casitone, 10.0 g glycerol, 18.0 g agar, pH 7.3) incubated at 28 °C for 7–14 days.

Biomass for chemical and molecular systematic studies was obtained after incubation at 28 °C for 3–6 days in shaken flasks (about 180 r.p.m.) of trypticase soy broth (TSB). Amino acids and sugars in whole-cell hydrolysates were analysed according to the procedures of Lechevalier & Lechevalier (1980) by TLC. Cellular fatty acids were extracted according to the method of Sasser (1990) and the composition was determined by GC (Oliver & Colwell, 1973). Menaquinones were extracted from freeze-dried biomass, purified according to Minnikin *et al.* (1984) and analysed by HPLC with an ODS-BP C₁₈ column (4.6 × 250 mm). Phospholipid analysis was carried out by TLC by using the technique described by Minnikin *et al.* (1984).

Preparation of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out by using the procedures described by Goodfellow *et al.* (2007). The 16S rRNA gene sequence of strain 172205^T was multiply

aligned with representative sequences of related streptomycetes obtained from the GenBank/EMBL/DDBJ databases by using CLUSTAL X software. The alignment was manually verified and adjusted prior to the reconstruction of a phylogenetic tree. The unrooted phylogenetic tree was generated via the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) tree-making algorithms in MEGA, version 4.0 (Tamura *et al.*, 2007); evolutionary distances for the neighbour-joining algorithm were calculated with the Kimura two-parameter model (Kimura, 1980). The topologies of the resultant trees were evaluated in a bootstrap analysis (Felsenstein, 1985) of 1000 replicates. Levels of pairwise 16S rRNA gene sequence similarity between the closest strains were determined by using the EzTaxon server (<http://www.Eztaxon.org>) (Chun *et al.*, 2007). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option).

Chromosomal DNA was extracted as described by Pospiech & Neumann (1995). The DNA G+C content of strain 172205^T was determined by the HPLC method (Mesbah *et al.*, 1989). Levels of DNA–DNA relatedness were measured on nylon membranes according to the method described by Wang *et al.* (2011).

Morphological features were observed on ISP 1–7 (Shirling & Gottlieb, 1966), modified Bennett's agar (Jones, 1949), Czapek's agar (Wiese *et al.*, 2008) and nutrient agar. Results of the physiological characterization are given in the species

Fig. 1. Neighbour-joining phylogenetic tree, based on nearly complete 16S rRNA gene sequences (1455 nt), showing the relationships between strain 172205^T and related representative species of the genus *Streptomyces*. Numbers at branch points indicate bootstrap percentages (based on 1000 replications); only values >50 % are shown. Asterisks indicate branches of the tree that were also recovered by using the maximum-parsimony tree-making algorithm. Bar, 0.002 substitutions per nucleotide position.

Table 1. Physiological and biochemical properties of strain 172205^T that differentiate it from its closest phylogenetic neighbours in the genus *Streptomyces*

Strains: 1, 172205^T; 2, *S. flocculus* NBRC 13041^T; 3, *S. ferralitis* SFOp68^T; 4, *S. haliclonae* DSM 41970^T. ++, Strongly positive; +, positive; ±, weakly positive; –, negative. The data presented are from this study unless indicated otherwise.

Characteristic	1	2	3	4
Morphology				
Colour of colony on ISP 1	White	White	None	White
Reverse side of colony on ISP 1	Green–beige	Sulfur yellow	Beige	Beige
Growth on ISP 1	Good	Good	Fair	Fair
Colour of colony on Czapek's agar	White	None	None	White
Reverse side of colony on Czapek's agar	Oyster white	Ivory	Beige	Dull white
Growth on Czapek's agar	Fair	Sparse	Sparse	Fair
Growth at/with:				
40 °C	–	+	+	–
45 °C	–	+	–	–
7 % (w/v) NaCl	+	+	–	–*
9, 10 % (w/v) NaCl	+	+	–	–
pH 5	–	+	+	+
Carbon utilization				
Raffinose	±	+	–	+
L-Arabinose	++	+	–	–
Sodium citrate	–	+	–	–
Cellobiose	++	++	–	+
D-(+)-Galactose	++	++	–†	++
Melibiose	±	–	–	++
L-(+)-Rhamnose	–	–	–	+*
Nitrogen utilization				
L-Valine	–	+	+	–
L-Histidine	–	+	+	–
D-Arginine	+	+	–	+
L-Glutamine	+	+	–	+
L-Methionine	–	–	+	–
Hydroxy-L-proline	–	–	+	–
L-Phenylalanine	–	–	+	–
L-Proline	–	+	+	–
L-Ornithine	–	–	+	–
L-Asparagine	–	+	+	+
Glycine	–	+	+	–
L-Serine	–	+	+	+
Degradation of:				
CM-cellulose	+	–	±	–
Starch	+	–	–	+*
Gelatin	–	+	–	+
Urea	+	+	–†	+*
Aesculin	–	+	–	+
Tweens 20, 40, 80	+	+	–	+
DNA G + C content (mol%)	70.33	71	73.8	72‡
Menaquinones	MK-9(H ₆), MK-9(H ₈)	MK-9(H ₆), MK-9(H ₈)	MK-9(H ₆), MK-9(H ₈)	MK-9(H ₆), MK-9(H ₈)
Phospholipids§	PE, ALs, PLs	PE, DPG, ALs, PLs	PE, DPG, PLs	PE, DPG, ALs, 5 PLs

*Data from Khan *et al.* (2010) are contradictory to the result obtained in this study.

†Data from Saintpierre-Bonaccio *et al.* (2004) are contradictory to the result obtained in this study.

‡Data from Khan *et al.* (2010).

§AL, unknown aminolipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PL, unknown phospholipid.

description below. The almost-complete 16S rRNA gene sequence of strain 172205^T (1487 nt) was obtained and compared with those sequences available on the EzTaxon server (Chun *et al.*, 2007). Strain 172205^T showed highest 16S rRNA gene sequence similarity of 98.29 % with *Streptomyces flocculus* NBRC 13041^T, corresponding to 25 nt differences at 1463 sites. A neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain 172205^T and its closest phylogenetic relatives is presented in Fig. 1. Strain 172205^T formed an independent subclade, supported by maximum-parsimony analysis, but was associated with *S. flocculus* NBRC 13041^T (98.29 % 16S rRNA gene sequence similarity), *Streptomyces ferralitis* SFOP68^T (98.14 %) and *Streptomyces haliclonae* DSM 41970^T (98.08 %). Levels of DNA–DNA relatedness between strain 172205^T and *S. flocculus* NBRC 13041^T, *S. ferralitis* SFOP68^T and *S. haliclonae* DSM 41970^T were 50.28 ± 4, 49.15 ± 11 and 37.56 ± 11 % (mean ± SD of 6 determinations), respectively, values below the 70 % threshold proposed by Wayne *et al.* (1987) for distinguishing genomic species.

The quinone system supported the affiliation of strain 172205^T to the genus *Streptomyces*, where the major menaquinones were MK-9(H₆) and MK-9(H₈) (Collins *et al.*, 1977; Minnikin *et al.*, 1984). The diagnostic phospholipid of strain 172205^T was phosphatidylethanolamine. The main fatty acids of strain 172205^T were iso-C_{12:0} (6.68 %), iso-C_{14:0} (9.35 %), iso-C_{15:0} (10.31 %), anteiso-C_{15:0} (35.19 %), C_{14:0} 3-OH (8.14 %), iso-C_{16:0} (20.24 %) and anteiso-C_{17:0} (10.05 %). This profile differs from those of its three closest phylogenetic relatives (see Supplementary Table S1 available in IJSEM Online).

Differences in various characteristics show that strain 172205^T is phenotypically distinct from its closest phylogenetic relatives, *S. flocculus* NBRC 13041^T, *S. ferralitis* SFOP68^T and *S. haliclonae* DSM 41970^T (Table 1). Therefore, it is evident that strain 172205^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces qinglanensis* sp. nov. is proposed.

Description of *Streptomyces qinglanensis* sp. nov.

Streptomyces qinglanensis (qing.lan.en'sis. N.L. masc. adj. *qinglanensis* pertaining to Qinglan Harbour, Wenchang, Hainan, from where the type strain was isolated).

Good growth occurs on ISP 1, 2, 3, 6 and 7, modified Bennett's agar, nutrient agar and Czapek's agar, while weak growth is observed on ISP 4 and 5 agar media. White aerial mycelium is formed on ISP 1, 2, 3, 4 and 6, modified Bennett's agar, Czapek's agar and nutrient agar. No diffusible pigment or melanin is detected on any of the tested media. After 15 days of growth on ISP 3 agar at 28 °C, both aerial and vegetative hyphae are abundant, well developed and show typical characteristics of the genus *Streptomyces*. Spore chains are long and *Rectiflexibiles* in nature, consisting of about 16 spores. Spores are elliptical or short rod-shaped (0.3–0.7 × 0.5–1.0 µm). The spore surfaces

are smooth (see Supplementary Fig. S1). Positive in tests for CM-cellulose degradation, catalase production, starch hydrolysis, urease production, and degradation of Tweens 20, 40 and 80; negative for gelatin liquefaction, aesculin degradation and nitrate reduction (Table 1). Growth occurs at pH 7.0–10.0 (optimum, pH 7.0), at 15–37 °C (optimum, 28 °C) and with 0–25 % (w/v) NaCl [optimum, 1–3 % (w/v) NaCl]. Utilizes raffinose (weakly), inositol, L-arabinose, sucrose, D-glucose, D-mannitol, D-arabinose, cellobiose, D-(+)-galactose, melibiose (weakly), trehalose, D-(+)-xylose, D-fructose and D-(+)-mannose as sole carbon and energy sources, but not sodium citrate, D-sorbitol or L-(+)-rhamnose. Metabolizes L-alanine, D-arginine and L-glutamine as sole nitrogen sources, but not L-valine, L-cysteine, L-histidine, L-methionine, hydroxy-L-proline, L-phenylalanine, L-proline, L-ornithine, L-asparagine, glycine or L-serine. Possesses both LL-diaminopimelic acid and meso-diaminopimelic acid and whole-cell hydrolysates contain ribose, galactose and glucose. The polar lipid profile comprises phosphatidylethanolamine, an unknown amino-lipid and five unknown phospholipids. Major menaquinones are MK-9(H₆) and MK-9(H₈). The major fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. The G + C content of the genomic DNA of the type strain is 70.33 mol%.

The type strain, 172205^T (=CGMCC 4.6825^T =DSM 42035^T), which exhibits cytotoxic activity against human immortalized myelogenous leukaemia cell line K562, was isolated from a composite mangrove soil sample collected in Qinglan Harbour, Wenchang, Hainan Province, China.

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References

- Bentley, S. D., Chater, K. F., Cerdeño-Tárraga, A. M., Challis, G. L., Thomson, N. R., James, K. D., Harris, D. E., Quail, M. A., Kieser, H. & other authors (2002). Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**, 141–147.
- Bérdy, J. (2005). Bioactive microbial metabolites. *J Antibiot (Tokyo)* **58**, 1–26.
- Chun, J., Youn, H. D., Yim, Y.-I., Lee, H., Kim, M. Y., Hah, Y. C. & Kang, S.-O. (1997). *Streptomyces seoulensis* sp. nov. *Int J Syst Bacteriol* **47**, 492–498.
- 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.
- Euzéby, J. (2010). List of prokaryotic names with standing in nomenclature. <http://www.bacterio.cict.fr/streptomycesa.html>

- Felsenstein, J. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- 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., Sveshnikova, M. A., Terekhova, L. P. & Maksimova, T. S. (1983).** *Opredelitel' aktinomitse-tov (Manual of Actinomycetes)*. Moscow: Nauka.
- Goodfellow, M., Kumar, Y., Labeda, D. P. & Sembaliing, L. (2007).** The *Streptomyces violaceusniger* clade: a home for streptomycetes with rugose ornamented spores. *Antonie van Leeuwenhoek* **92**, 173–199.
- Hong, K., Gao, A. H., Xie, Q. Y., Gao, H., Zhuang, L., Lin, H. P., Yu, H. P., Li, J., Yao, X. S. & other authors (2009).** Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. *Mar Drugs* **7**, 24–44.
- Hopkins, D. W., Macnaughton, S. J. & O'Donnell, A. G. (1991).** A dispersion and differential centrifugation technique for representatively sampling microorganisms from soil. *Soil Biol Biochem* **23**, 217–225.
- Ikeda, H., Ishikawa, J., Hanamoto, A., Shinose, M., Kikuchi, H., Shiba, T., Sakaki, Y., Hattori, M. & Ōmura, S. (2003).** Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nat Biotechnol* **21**, 526–531.
- Iwai, H. & Takahashi, Y. (1992).** Selection of microbial sources of bioactive compounds. In *The Search for Bioactive Compounds from Microorganisms*, pp. 281–302. Edited by S. Ōmura. New York: Springer.
- 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.
- Kämpfer, P., Kroppenstedt, R. M. & Dott, W. (1991).** A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. *J Gen Microbiol* **137**, 1831–1891.
- 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.
- Khan, T. K., Tamura, T., Takagi, M. & Shin-ya, K. (2010).** *Streptomyces tateyamensis* sp. nov., *Streptomyces marinus* sp. nov. and *Streptomyces haliclona* sp. nov., isolated from the marine sponge *Haliclona* sp. *Int J Syst Evol Microbiol* **60**, 2775–2779.
- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Labeda, D. P., Lechevalier, M. P. & Testa, R. T. (1997).** *Streptomyces stramineus* sp. nov., a new species of the verticillate streptomycetes. *Int J Syst Bacteriol* **47**, 747–753.
- Lechevalier, M. P. & Lechevalier, H. A. (1980).** The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy*, pp. 22–291. Edited by A. Dietz & D. W. Thayer. Arlington, VA: Society for Industrial Microbiology.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. K. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Oliver, J. D. & Colwell, R. R. (1973).** Extractable lipids of gram-negative marine bacteria: fatty-acid composition. *Int J Syst Bacteriol* **23**, 442–458.
- Ōmura, S., Ikeda, H., Ishikawa, J., Hanamoto, A., Takahashi, C., Shinose, M., Takahashi, Y., Horikawa, H., Nakazawa, H. & other authors (2001).** Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. *Proc Natl Acad Sci U S A* **98**, 12215–12220.
- Pospiech, A. & Neumann, B. (1995).** A versatile quick-prep of genomic DNA from gram-positive bacteria. *Trends Genet* **11**, 217–218.
- Saintpierre-Bonaccio, D., Amir, H., Pineau, R., Lemriss, S. & Goodfellow, M. (2004).** *Streptomyces ferralitis* sp. nov., a novel streptomycete isolated from a New-Caledonian ultramafic soil. *Int J Syst Evol Microbiol* **54**, 2061–2065.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sanglier, J. J., Haag, H., Huck, T. A. & Fehr, T. (1996).** Review of actinomycetes compounds 1990–1995. *Expert Opin Investig Drugs* **5**, 207–223.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, Technical Note 101. Newark, DE: Microbial ID.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Sui, J. L., Xu, X. X., Qu, Z., Lin, H. P., Ruan, J. S. & Hong, K. (2011).** *Streptomyces sanyensis* sp. nov., isolated from mangrove sediment. *Int J Syst Evol Microbiol* **61**, 1632–1637.
- 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.
- Waksman, S. A. & Henrici, A. T. (1943).** The nomenclature and classification of the actinomycetes. *J Bacteriol* **46**, 337–341.
- Wang, C., Xu, X. X., Qu, Z., Wang, H. L., Lin, H. P., Xie, Q. Y., Ruan, J. S. & Hong, K. (2011).** *Micromonospora rhizosphaerae* sp. nov., isolated from mangrove rhizosphere soil. *Int J Syst Evol Microbiol* **61**, 320–324.
- Ward, A. C. & Goodfellow, M. (2004).** Phylogeny and functionality: taxonomy as a roadmap to genes. In *Microbial Diversity and Bioprospecting*, pp. 288–313. Edited by A. T. Bull. Washington, DC: American Society for Microbiology.
- Watve, M. G., Tickoo, R., Jog, M. M. & Bhole, B. D. (2001).** How many antibiotics are produced by the genus *Streptomyces*? *Arch Microbiol* **176**, 386–390.
- 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.
- Wellington, E. M. H. & Williams, S. T. (1978).** Preservation of actinomycete inoculum in frozen glycerol. *Microbiol Lett* **6**, 151–157.
- Wiese, J., Jiang, Y., Tang, S. K., Thiel, V., Schmaljohann, R., Xu, L. H., Jiang, C. L. & Imhoff, J. F. (2008).** A new member of the family *Micromonosporaceae*, *Planosporangium flavigriseum* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **58**, 1324–1331.
- 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.
- Xiao, J., Wang, Y., Luo, Y. X., Xie, S. J., Ruan, J. S. & Xu, J. (2009).** *Streptomyces avicenniae* sp. nov., a novel actinomycete isolated from the rhizosphere of the mangrove plant *Avicennia mariana*. *Int J Syst Evol Microbiol* **59**, 2624–2628.
- Xu, J., Wang, Y., Xu, J., Xie, S. J., Xiao, J. & Ruan, J. S. (2009).** *Streptomyces xiamensis* sp. nov., isolated from mangrove sediment. *Int J Syst Evol Microbiol* **59**, 472–476.