Kribbella amoyensis sp. nov., isolated from rhizosphere soil of a pharmaceutical plant, *Typhonium giganteum* Engl.

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An actinomycete, designated XMU 198^T, was isolated from the rhizosphere soil of a pharmaceutical plant, *Typhonium giganteum* Engl., collected in Xiamen City, China. 16S rRNA gene sequence analysis showed that the isolate exhibited highest sequence similarities with *Kribbella flavida* KACC 20148^T, *K. karoensis* Q41^T and *K. alba* YIM 31975^T (98.7, 98.4 and 98.2 %, respectively). The chemotaxonomic characteristics further supported the assignment of strain XMU 198^T to the genus *Kribbella*: LL-diaminopimelic acid in the cell-wall peptidoglycan; glucose and galactose with minor amounts of ribose as the whole-cell sugars; polar lipids comprising phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and unidentified phospholipids; a fatty acid profile characterized by the predominance of iso-C_{16:0}, iso-C_{14:0} and anteiso-C_{15:0}; and MK-9(H₄) as the main menaquinone. Gyrase subunit B gene (*gyrB*) sequence analysis showed that the genetic distances between strain XMU 198^T and all other members of the genus *Kribbella* were greater than 0.014, the value used as the threshold for species delineation within this genus. A wide range of genotypic and phenotypic characteristics, as well as DNA–DNA relatedness between strain XMU 198^T and *K. flavida* DSM 17836^T (41.18 %), *K. karoensis* Q41^T (38.02 %) and *K. alba* DSM 15500^T (50.58 %), distinguished the isolate from its closest phylogenetic neighbours. On the basis of the above data, a novel species of the genus *Kribbella*, *Kribbella amoyensis* sp. nov., is proposed. The type strain is XMU 198^T (=DSM 24683^T=NBRC 107914^T).

The genus *Kribbella*, of the family *Nocardioideae*, was established by the reclassification of two strains in the genus *Nocardioides* (Park *et al.*, 1999). To date, most *Kribbella* species have been isolated from soil (Trujillo *et al.*, 2006). Members of this genus are aerobic, Gram-positive and non-acid-fast actinobacteria. The whole-cell hydrolysates contain LL-diaminopimelic acid and the predominant menaquinone is MK-9(H₄) (Carlsohn *et al.*, 2007a).

Strain XMU 198^T was isolated from the rhizosphere soil of a pharmaceutical plant, *Typhonium giganteum* Engl., collected in Xiamen City, China, after incubation at 28 °C for 4 weeks on modified poly(L-lactide) agar (PLA; containing 2.0 g PLA powder, 18 g agar, 1 l base medium; Jarerat *et al.*, 2002). The pure culture was preserved on International *Streptomyces* Project medium 2 (ISP 2; Shirling & Gottlieb, 1966) at 4 °C and in 20 % (v/v) glycerol at –80 °C.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of strain XMU 198^T are HM368615 and JF520392, respectively.

Two supplementary figures and three supplementary tables are available with the online version of this paper.

For chemotaxonomic and molecular systematic investigations, the isolate was cultured at 28 °C for 3–5 days in liquid YIM 38 (Jiang *et al.*, 2007), ISP 2 and trypticase soy broth (TSB; Becton Dickinson). For morphological and cultural studies, the isolate was cultured on ISP 2, 3, 4 and 5 (Shirling & Gottlieb, 1966), Czapek agar and potato agar (Stackebrandt, 1988) at 28 °C for up to 21 days. Morphology was observed by scanning electron microscopy (JSM 5600LV; JEOL) after 14 days at 28 °C. The colours of substrate and aerial mycelia were compared with colour chips from the ISCC–NBS colour charts (standard sample no. 2106; Kelly, 1964). Growth with 0–15 % (w/v) NaCl and at pH 4.0–10.0 was determined in shake flasks of liquid YIM38[#] after 21 days at 28 °C, with the pH of the medium adjusted using sterilized 1 M HCl or 20 % (w/v) Na₂CO₃. Physiological tests, including the determination of enzyme activities, were performed as described by Groth *et al.* (2003). The reference strains used for comparison were *Kribbella flavida* DSM 17836^T, *Kribbella karoensis* Q41^T and *Kribbella alba* DSM 15500^T.

The isolate showed lichenous growth on all tested media. Fig. S1 (available in IJSEM Online) shows the cell morphology after 14 days on ISP 2 at 28 °C. On ISP 3, the substrate and aerial mycelium fragmented into irregular,

Table 1. Physiological properties that serve to distinguish strain XMU 198^T from its closest phylogenetic neighbours

Strains: 1, *Kribbella amoyensis* sp. nov. XMU 198^T; 2, *K. flavida* DSM 17836^T; 3, *K. karoonensis* Q41^T; 4, *K. alba* DSM 15500^T. Data were taken from this study. All strains are positive for melanin production, casein decomposition, esterase (C4) and growth at 10 °C, at pH 7 and with 3% (w/v) NaCl. All strains are negative for growth at 45 °C, at pH 5 and with 7% (w/v) NaCl. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4
Gelatin liquefaction	+	–	–	+
Starch hydrolysis	+	+*	–	+
Nitrate reduction	+	+	+	–
Sodium citrate	+	+	+	–
Urea decomposition	–	+	+	+
Oxidase	–	+	+	–
Sole carbon sources				
L-Arabinose	–	–	–	+
Lactose	–	+†	+	+
Mannose	+	–	+	+
Xylose	+	–	+	+
Melibiose	+	+	–	+
Raffinose	–	+	+	+
L-Rhamnose	+	–	+	+
D-Galactose	+	+†	w	+
Maltose	–	+	+	+
D-Sorbitol	+	+†	–	+
Growth at/with:				
37 °C	+	–	+	–
pH 9	–	+	–†	–
5% (w/v) NaCl	+	–	–	–

*Result differs from Li *et al.* (2004).

†Result differs from Kirby *et al.* (2006).

elongated rod-shaped or coccoid elements. The colour of the substrate mycelium was light yellow to yellow. The aerial mycelium was white on ISP 2, 4 and 5 and yellow on ISP 3, Czapek agar and potato agar. Diffusible pigments were not observed on any of the media tested. The physiological characteristics of strain XMU 198^T are presented in Table 1. Although the isolate shared some physiological properties with its closest phylogenetic neighbours, it could be readily distinguished on the basis of the characteristics listed in the table.

The amino acid and sugar analysis of whole-cell hydrolysates followed the procedures described by Stanek & Roberts (1974), using micro-cellulose plates made with cellulose type 20 (Sigmacell). Cells for chemotaxonomic analysis were obtained from TSB after 5 days at 28 °C. Analysis of menaquinones, polar lipids and fatty acids was carried out by the Identification Service and Dr Brian Tindall of the DSMZ (Braunschweig, Germany). Menaquinones and polar lipids were extracted from 100 mg freeze-dried cells using the two-stage method described by

Tindall (1990a, b). Menaquinones were separated by HPLC (Tindall *et al.*, 2007). Polar lipids were examined by two-dimensional TLC and identified using published procedures (Tindall *et al.*, 2007). Fatty acids were obtained according to the methods of Miller (1982) and Kuykendall *et al.* (1988) and determined according to Kämpfer & Kroppenstedt (1996) using the Sherlock Microbial Identification System (Microbial ID) and the Hewlett Packard MIDI database. The GC parameters were: carrier gas, ultra-high-purity hydrogen; column head pressure 60 kPa; injection volume 2 µl; column split ratio, 100:1; septum purge 5 ml min^{–1}; column temperature, from 170 to 270 °C at 5 °C min^{–1}; injection port temperature, 240 °C; and detector temperature, 300 °C.

The chemotaxonomic characteristics of strain XMU 198^T were consistent with the genus *Kribbella*. The whole-cell hydrolysates contained L-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan, which corresponds to wall chemotype I (Lechevalier & Lechevalier, 1970). The whole-cell sugars comprised glucose and galactose and minor amounts of ribose. The predominant menaquinone was MK-9(H₄) (77%); minor amounts of MK-8(H₄) (10%), MK-7(H₄) (7%) and MK-9(H₆) (3%) and traces of MK-9, MK-9(H₂) and MK-9(H₈) were also detected. The phospholipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and unidentified phospholipids (phospholipid pattern III; Lechevalier *et al.*, 1977). These chemotaxonomic characteristics served to distinguish the isolate from its phylogenetic neighbours. The predominant fatty acids were iso-C_{16:0} (34.41%), iso-C_{14:0} (21.37%), anteiso-C_{15:0} (17.86%), iso-C_{15:0} (6.46%), C_{17:1}ω8c (4.11%) and iso-C_{16:1} H (2.37%), which matches the fatty acid profile of *K. flavida* in the TSBA 6.10 library. Strain XMU 198^T differed from *K. flavida* DSM 17836^T mainly by the presence of remarkable amounts of iso-C_{14:0}, C_{17:1}ω8c and iso-C_{16:1}.

Chromosomal DNA of the isolate was extracted as described by Wu *et al.* (2009) and the 16S rRNA gene was amplified as described by Carlsohn *et al.* (2007b). Amplification products were purified using an EZNA Gel Extraction kit (Omega Bio-Tek), cloned into the pMD18-T vector (Takara) and sequenced by Invitrogen (Shanghai). 16S rRNA gene sequence similarities were determined using the EzTaxon server (<http://www.eztaxon.org>; Chun *et al.*, 2007). After multiple alignment using CLUSTAL X (Thompson *et al.*, 1997), phylogenetic analysis was performed using MEGA version 4.0 (Tamura *et al.*, 2007). Genetic distances were calculated according to the Kimura two-parameter model (Kimura, 1980) and clustering was performed using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis based on 1000 resamplings was used to evaluate the topology of the neighbour-joining tree (Li *et al.*, 2006).

An almost-complete 16S rRNA gene sequence (1420 nt) was obtained for the isolate. 16S rRNA gene sequence similarities between strain XMU 198^T and its closest relatives, *K. flavida*

KACC 20148^T, *K. karoonsensis* Q41^T and *K. alba* YIM 31975^T, were 98.7, 98.4 and 98.2 %, respectively; sequence similarities between strain XMU 198^T and the other type strains of species in the genus *Kribbella* were <98.1 %. In the neighbour-joining dendrogram (Fig. 1), strain XMU 198^T formed a distinct lineage that was supported with a bootstrap value of 100 %.

To supplement the 16S rRNA gene sequence analysis, we also used *gyrB* sequence analysis. *gyrB* sequences were amplified using the primers KgyrB-F953 (5'-CSGTGCACACBTT-CGCGAACG-3') and KgyrB-R1892 (5'-CCSAGGCCCTT-GWAGCGCTGG-3') (Kirby *et al.*, 2010). Amplification products of about 939 bp were obtained. Purification and sequencing were performed as for the 16S rRNA gene. The *gyrB*-based genetic distances were calculated according to Kirby *et al.* (2010).

The *gyrB* genetic distances ((based on 390 bp) between strain XMU 198^T and the type strains of species of the genus *Kribbella* ranged from 0.07 to 0.107 (Table S1, available in IJSEM Online), which were much higher than the threshold (0.014) for species delineation within the genus *Kribbella* (Kirby *et al.*, 2010). This result suggested that strain XMU 198^T represents a novel species of the genus *Kribbella* (Table S2). The *gyrB* phylogenetic tree showed that strain XMU 198^T formed a distinct lineage within the genus (Fig. S2).

Although Kirby *et al.* (2010) considered that a *gyrB*-based genetic distance of 0.04 could be used as a cut-off point to determine whether DNA–DNA hybridization is required, DNA–DNA relatedness between strain XMU 198^T and the three reference strains was also determined, using the

fluorescent micro-well method of Christensen *et al.* (2000). Photobiotin-labelled DNA from each reference strain was hybridized with single-stranded unlabelled DNA of strain XMU 198^T covalently bound to micro-wells. Hybridization was carried out in 50 % formamide at 50 °C according to Xu *et al.* (2006). Rates of hybridization were determined according to Christensen *et al.* (2000) using a FLUOstar OPTIMA microplate reader (BMG LABTECH) and wavelengths of 360 nm for excitation and 460 nm for emission (He *et al.*, 2005). Each hybridization was performed in four independent experiments with three replicates in each experiment and DNA–DNA relatedness was calculated by taking the average of the four experiments. DNA G+C content was determined using the HPLC method of Tamaoka & Komagata (1984).

The isolate exhibited low DNA–DNA relatedness with *K. alba* DSM 15500^T (50.58 ± 6.00 %), *K. flavida* DSM 17386^T (41.18 ± 1.72 %) and *K. karoonsensis* Q41^T (38.02 ± 1.78 %) (Table S3). Given these low values, together with findings of the 16S rRNA gene and *gyrB* sequence analysis and the high *gyrB*-based genetic distances, further DNA–DNA hybridization tests were not performed.

On the basis of phenotypic, chemotaxonomic and phylogenetic analysis, we propose that strain XMU 198^T represents a novel species, *Kribbella amoyensis* sp. nov.

Description of *Kribbella amoyensis* sp. nov.

Kribbella amoyensis [a.moy.en'sis. N.L. fem. adj. *amoyensis* pertaining to Amoy (Xiamen), a city of China, in which the type strain was collected].

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1215 bp), showing the positions of strain XMU 198^T and members of the genus *Kribbella*. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. *Streptosporangium roseum* DSM 43021^T was used as an outgroup. Bar, 1 % difference in nucleotide sequence.

Gram-positive, aerobic and non-motile actinomycete. Cells are extensively branched, forming light yellow to yellow substrate mycelium and white or yellow aerial mycelium. The substrate and aerial hyphae fragment into short or elongated rod-shaped elements. Diffusible pigments are not produced. Grows at 10–37 °C (optimum 28 °C), but not at 4 or 45 °C, and at pH 6.0–8.0 (optimum pH 7.0), but not at pH 5.0 or 9.0. Grows with up to 5 % (w/v) NaCl, but not with 7 % (w/v) NaCl. Positive for casein hydrolysis and esterase (C4), but negative for oxidase and urease. Utilizes most tested carbon sources, such as glucose, sucrose, mannose, xylose, rhamnose, inositol, mannitol, sorbitol, trisodium citrate and sodium oxalate, but negative for lactose, L-arabinose, maltose and raffinose. Positive for gelatin liquefaction, nitrate reduction, milk coagulation and peptonization, melanin production and starch hydrolysis. Additional phenotypic properties are shown in Table 1. The cell wall contains LL-diaminopimelic acid, glucose, galactose and ribose. The predominant menaquinone is MK-9(H₄). The phospholipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and unidentified phospholipids. The major fatty acids are iso-C_{16:0}, iso-C_{14:0}, anteiso-C_{15:0}, iso-C_{15:0}, C_{17:1}ω8c and iso-C_{16:1} H.

The type strain is XMU 198^T (=DSM 24683^T=NBRC 107914^T), isolated from the rhizosphere soil of a pharmaceutical plant, *Typhonium giganteum* Engl., collected in Xiamen City, China. The DNA G+C content of the type strain is 69.3 mol%.

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References

- Carlsohn, M. R., Groth, I., Spröer, C., Schütze, B., Saluz, H. P., Munder, T. & Stackebrandt, E. (2007a). *Kribbella aluminosa* sp. nov., isolated from a medieval alum slate mine. *Int J Syst Evol Microbiol* 57, 1943–1947.
- Carlsohn, M. R., Groth, I., Tan, G. Y., Schütze, B., Saluz, H.-P., Munder, T., Yang, J., Wink, J. & Goodfellow, M. (2007b). *Amycolatopsis saalfeldensis* sp. nov., a novel actinomycete isolated from a medieval alum slate mine. *Int J Syst Evol Microbiol* 57, 1640–1646.
- Christensen, H., Angen, O., Mutters, R., Olsen, J. E. & Bisgaard, M. (2000). DNA–DNA hybridization determined in micro-wells using covalent attachment of DNA. *Int J Syst Evol Microbiol* 50, 1095–1102.
- 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.
- Groth, I., Schütze, B., Boettcher, T., Pullen, C. B., Rodriguez, C., Leistner, E. & Goodfellow, M. (2003). *Kitasatospora putterlickiae* sp. nov., isolated from rhizosphere soil, transfer of *Streptomyces kifunensis* to the genus *Kitasatospora* as *Kitasatospora kifunensis* comb. nov., and emended description of *Streptomyces aureofaciens* Duggar 1948. *Int J Syst Evol Microbiol* 53, 2033–2040.
- He, L., Li, W., Huang, Y., Wang, L., Liu, Z., Lanoot, B., Vancanneyt, M. & Swings, J. (2005). *Streptomyces jietaisiensis* sp. nov., isolated from soil in northern China. *Int J Syst Evol Microbiol* 55, 1939–1944.
- Jararat, A., Pranamuda, H. & Tokiwa, Y. (2002). Poly(L-lactide)-degrading activity in various actinomycetes. *Macromol Biosci* 9, 420–428.
- Jiang, Y., Tang, S. K., Wiese, J., Xu, L.-H., Imhoff, J. F. & Jiang, C.-L. (2007). *Streptomyces hainanensis* sp. nov., a novel member of the genus *Streptomyces*. *Int J Syst Evol Microbiol* 57, 2694–2698.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* 42, 989–1005.
- Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color-Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.
- 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.
- Kirby, B. M., Le Roes, M. & Meyers, P. R. (2006). *Kribbella karoensis* sp. nov. and *Kribbella swartbergensis* sp. nov., isolated from soil from the Western Cape, South Africa. *Int J Syst Evol Microbiol* 56, 1097–1101.
- Kirby, B. M., Everest, G. J. & Meyers, P. R. (2010). Phylogenetic analysis of the genus *Kribbella* based on the *gyrB* gene: proposal of a *gyrB*-sequence threshold for species delineation in the genus *Kribbella*. *Antonie van Leeuwenhoek* 97, 131–142.
- Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 38, 358–361.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 20, 435–443.
- Lechevalier, M. P., De Bievre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* 5, 249–260.
- Li, W. J., Wang, D., Zhang, Y. Q., Schumann, P., Stackebrandt, E., Xu, L. H. & Jiang, C. L. (2004). *Kribbella antibiotica* sp. nov., a novel nocardioform actinomycete strain isolated from soil in Yunnan, China. *Syst Appl Microbiol* 27, 160–165.
- Li, W. J., Wang, D., Zhang, Y. Q., Xu, L. H. & Jiang, C. L. (2006). *Kribbella yunnanensis* sp. nov., *Kribbella alba* sp. nov., two novel species of genus *Kribbella* isolated from soils in Yunnan, China. *Syst Appl Microbiol* 29, 29–35.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 16, 584–586.
- Park, Y. H., Yoon, J. H., Shin, Y. K., Suzuki, K., Kudo, T., Seino, A., Kim, H. J., Lee, J. S. & Lee, S. T. (1999). Classification of '*Nocardioides fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 in *Kribbella* gen. nov., as *Kribbella flavida* sp. nov. and *Kribbella sandramycinii* sp. nov. *Int J Syst Bacteriol* 49, 743–752.
- 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.
- Stackebrandt, E. (1988). Phylogenetic relationships vs. phenotypic diversity: how to achieve a phylogenetic classification system of the eubacteria. *Can J Microbiol* 34, 552–556.

- Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- 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.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990a).** A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b).** Lipid composition of *Halobacterium lacus-profundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tindall, B. J., Sikorski, J., Smibert, R. M. & Krieg, N. R. (2007).** Phenotypic characterization and the principles of comparative systematics. In *Methods for General and Molecular Microbiology*, 3rd edn, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. Marzluf, T. M. Schmidt & L. R. Snyder. Washington, DC: American Society for Microbiology.
- Trujillo, M. E., Kroppenstedt, R. M., Schumann, P. & Martínez-Molina, E. (2006).** *Kribbella lupini* sp. nov., isolated from the roots of *Lupinus angustifolius*. *Int J Syst Evol Microbiol* **56**, 407–411.
- Wu, Y., Lu, C., Qian, X., Huang, Y. & Shen, Y. (2009).** Diversities within genotypes, bioactivity and biosynthetic genes of endophytic actinomycetes isolated from three pharmaceutical plants. *Curr Microbiol* **59**, 475–482.
- Xu, C., Wang, L., Cui, Q., Huang, Y., Liu, Z., Zheng, G. & Goodfellow, M. (2006).** Neutrotolerant acidophilic *Streptomyces* species isolated from acidic soils in China: *Streptomyces guanduensis* sp. nov., *Streptomyces paucisporeus* sp. nov., *Streptomyces rubidus* sp. nov. and *Streptomyces yanglinensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 1109–1115.