Kribbella mirabilis sp. nov., isolated from rhizosphere soil of a herbaceous plant, *Mirabilis jalapa* L.

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Strain XMU 706^T, isolated from the rhizosphere soil of a herbaceous plant, *Mirabilis jalapa* L., collected from Xiamen City, China, was characterized using a polyphasic approach to clarify its taxonomic position. Strain XMU 706^T shared the highest 16S rRNA gene sequence similarity with *Kribbella antibiotica* YIM 31530^T (97.2 %), and formed a distinct branch in the subclade of the genus *Kribbella* in the 16S rRNA gene phylogenetic tree. The genetic distances of gyrase subunit B gene (*gyrB*) sequence between strain XMU 706^T and other species of the genus *Kribbella* ranged from 0.045 to 0.116, greater than the threshold value of 0.014 for species delineation of this genus. DNA–DNA hybridization experiments gave a DNA–DNA relatedness value of 34.82±6.31 % between strain XMU 706^T and *K. antibiotica* YIM 31530^T. The chemotaxonomic properties further supported the assignment of strain XMU 706^T to the genus *Kribbella*. LL-Diaminopimelic acid was the diagnostic amino acid in the cell-wall peptidoglycan and cell hydrolysates contained ribose and glucose. The major menaquinone was MK-9(H_4). The polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and other unidentified phospholipids and lipids. The major fatty acids of the strain were anteiso-$C_{15:0}$ and iso-$C_{15:0}$, and the G+C content of the genomic DNA was 67.3 mol%. Based on the results of phylogenetic analysis, phenotypic and genotypic characterization, strain XMU 706^T represents a novel species of the genus *Kribbella*, for which the name *Kribbella mirabilis* sp. nov. is proposed. The type strain is XMU 706^T (=KCTC 29676^T=MCCC 1K00429^T).

The genus *Kribbella* is classified under the family *Nocardioidaceae* (Nesterenko *et al.*, 1985; Rainey *et al.*, 1996) and was established by Park *et al.* (1999) as a result of the reclassification of two members of the genus *Nocardioides*. Members of the genus *Kribbella* contain LL-diaminopimelic acid (wall chemotype I) as the diagnostic diamino acid (Lechevalier & Lechevalier, 1970), anteiso- and iso- branched components as the major fatty acids, with type P III phospholipids (Lechevalier *et al.*, 1977) and MK-9(H_4) as the predominant menaquinone (Carlsohn *et al.*, 2007). At the time of writing, there are 20 species of the genus *Kribbella* with validly published names according to the List of Prokaryotic names with Standing in Nomenclature website (LPSN; http://www.bacterio.net/index.html). This paper describes a novel species of the genus *Kribbella*, identified in a taxonomic study using a polyphasic approach.

Strain XMU 706^T was isolated from the rhizosphere soil of a herbaceous plant, *Mirabilis jalapa* L., collected from the Xiang'an district of Xiamen City (24.6° N 118.2° E), Fujian Province, South-east China. A 100-fold dilution of this soil suspension was prepared in sterilized distilled water and 0.1 ml was spread on modified PLA [poly(L-lactide)] agar, containing 2 g PLA powder and 18 g agar in 1 l basic medium and incubated at 28 °C for 2–3 weeks. The purified isolate was cultured on International Streptomyces Project medium 2 (ISP 2) (Shirling & Gottlieb, 1966) and preserved in 20 % (v/v) glycerol at −80 °C.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and *gyrB* sequences of strain XMU 706^T are KJ786943 and KM189813, respectively.

Seven supplementary figures and four supplementary tables are available with the online Supplementary Material.

Cultural characteristics of strain XMU 706^T were determined after incubation for 2 weeks at 28 °C on ISP 2, 3, 4 and 5 agar (Shirling & Gottlieb, 1966), potato dextrose agar, Czapek agar and nutrient agar (Stackebrandt, 1988). Colour designation of substrate mycelium and aerial hypha were compared with National Bureau of Standards (NBS) Colour Name Charts (Kelly, 1964). Morphological characteristics were observed by scanning electron microscopy (Zeiss Sigma SEM) after 21 days of growth on ISP 2 medium at 28 °C. Growth at different temperatures (4, 10, 15, 20, 28, 30, 37, 42, 45 and 55 °C) was tested with ISP 2 medium plates. Growth at different NaCl concentrations (0–15 %, at intervals of 1.0 %, w/v) and different pH [pH 4.0–10.0, at intervals of 1.0 pH unit, using the buffer system described by Xu *et al.* (2005)] was tested in shake flasks of liquid ISP 2 medium at 28 °C for 2–3 weeks. Utilization of sole carbon and nitrogen sources for energy and growth was carried out according to Shirling & Gottlieb (1966). Other physiological and biochemical tests, including nitrate reduction, milk coagulation and peptonization, H_2S production and decomposition of urea, starch and gelatin were performed as described by Smibert & Krieg (1994).

The amino acid content of the cell wall and the sugars of whole-cell hydrolysates were analysed according to the methods established by Hasegawa *et al.* (1983). Cells for chemotaxonomic analyses were obtained from 3–4-day-old cultures grown in tryptic soy broth (TSB) medium at 28 °C and harvested by centrifugation. Analyses of menaquinones and polar lipids was carried out by the Identification Service of the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Menaquinones were extracted according to Collins *et al.* (1977) and identified by HPLC (Kroppenstedt, 1985). Polar lipids were analysed by two-dimensional TLC as described by Minnikin *et al.* (1979). Cellular fatty acids were extracted, methylated and analysed by using the Sherlock Microbial Identification System (version 6.0B; MIDI database: TSBA6) according to the method of Sasser (1990). The G + C content of the genomic DNA was determined as described by Mesbah *et al.* (1989).

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were performed as described by Wu *et al.* (2009). The 16S rRNA gene sequence obtained in this study was compared with sequences from EzBioCloud (http://www.ezbiocloud.net/eztaxon; Kim *et al.*, 2012). Analysis of the *gyrB* sequence was performed as a supplement to the 16S rRNA gene sequence analysis. The *gyrB* sequence was amplified using the primers K*gyr*B-F953 (5′-CSGTGCACA-CBTTCGCGAACG-3′) and K*gyr*B-R1892 (5′-CCSAGRCC-CTTGWAGCGCTGG-3′) as described by Kirby *et al.* (2010). Purification and sequencing were performed as for the 16S rRNA gene. The calculation of *gyrB*-based genetic distances was conducted by MEGA software version 6.06 (Tamura *et al.*, 2013). DNA–DNA hybridization was determined using the fluorescent micro-well method described by Christensen *et al.* (2000). To clarify the phylogenetic position of strain XMU 706^T within the related taxa, phylogenetic trees based on 16S rRNA gene sequences were reconstructed. Phylogenetic trees were reconstructed by the software MEGA version 6.06, using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and minimum-evolution (Takahashi & Nei, 2000) methods. Genetic distances were calculated by Kimura's two-parameter model (Kimura, 1980). Bootstrap analysis based on 1000 resamplings was used to evaluate the topology of the trees (Felsenstein, 1985).

An almost complete 16S rRNA gene sequence (1488 bp) was generated from isolate XMU 706^T, and shared 96.4–97.2 % similarities to the type strains of species of the genus *Kribbella*, with the highest similarity (97.2 %) to *Kribbella antibiotica* YIM 31530^T (Li *et al.*, 2004). *K. antibiotica* YIM 31530^T was the only type strain that shared a 16S rRNA gene sequence similarity >97 % with strain XMU 706^T. Hence, *K. antibiotica* YIM 31530^T was used as a reference strain for DNA–DNA hybridization as well as physiological and biochemical studies. In the 16S rRNA gene phylogenetic tree created using the neighbour-joining method (Fig. 1), strain XMU 706^T located in the genus *Kribbella* and formed a monophyletic clade in the subclade of this genus. This result was further supported by maximum-likelihood and minimum-evolution methods (Figs S1 and S2, available in the online Supplementary Material). The DNA–DNA relatedness value between strain XMU 706^T and *K. antibiotica* YIM 31530^T was 34.82±6.31 % (Table S1), which was below the 70 % delineating limit for species determination (Wayne *et al.*, 1987).

For calculation of the genetic distances, a 946 bp DNA sequence of the *gyrB* gene of strain XMU 706^T (GenBank accession number KM189813) was amplified. The *gyrB*-based genetic distances (based on 390 bp) between strain XMU 706^T and type strains of other species of the genus *Kribbella* ranged from 0.045 to 0.116 (Tables S2 and S3), much greater than the value of 0.014 used as a threshold for species delineation within the genus *Kribbella* (Kirby *et al.*, 2010). The *gyrB*-based phylogenetic tree showed that strain XMU 706^T formed a distinct lineage within the genus (Fig. S3).

Strain XMU 706^T grew well on most of the tested medium. Colonies had lichenous shapes and irregular edges with colours ranging from cream to light yellow. No diffusible pigment was observed. A scanning electron micrograph of strain XMU 706^T is shown in Fig. S4. The substrate mycelium was extensively branched and fragmented into rods, while the aerial mycelium was fragmented into rod-like or coccoid elements. The typical morphological characteristics of strain XMU 706^T were similar to those of the type species of the genus *Kribbella* (*Kribbella flavida*) since they both had pasty colonies with lichenous shapes and irregular edges, the vegetative mycelium of both were extensively branched and often fragmented into rod- to coccoid-shaped elements, and aerial mycelium fragmented into short to elongated rod-like elements (Park *et al.*, 1999). Optimum growth occurred at 28–30 °C, pH 7.0–7.5, and in the presence of 0–2 % (w/v) NaCl. Comparison of cultural and physiological

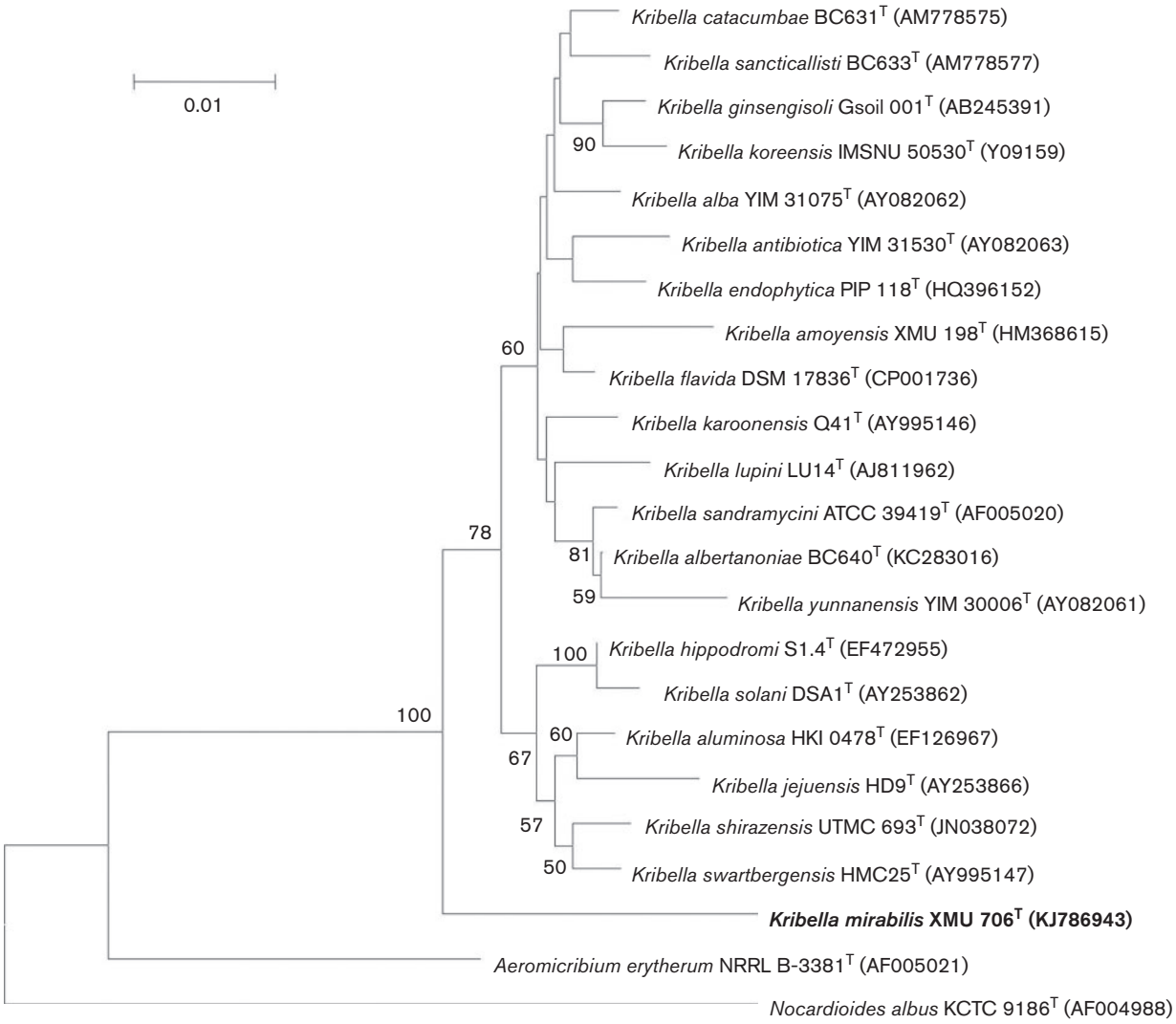

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain XMU 706^T among species of the genus *Kribbella* and its phylogenetic neighbours. Numbers at nodes are bootstrap values shown as percentages of 1000 replicates; only values >50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide.

characteristics of strain XMU 706^T and the reference strain *K. antibiotica* YIM 31530^T are shown in Table 1. Strain XMU 706^T shared some physiological and biochemical characteristics *K. antibiotica* YIM 31530^T. Both strains tested positive for gelatin liquefaction, starch hydrolysis and milk coagulation and peptonization, but tested negative for oxidase and H_2S production. Growth of both strains occurred at pH 6.0–9.0 with optimum growth at 28 °C and pH 7.0–7.5, and NaCl was tolerated up to 4 % (w/v). However, strain XMU 706^T could be differentiated from *K. antibiotica* YIM 31530^T in utilization of D-fructose, α-lactose, L-rhamnose, D-ribose, D-sorbose, L-cystine, L-histidine and L-phenylalanine, as well as in the major components of whole-cell sugars and polar lipids as listed in Table 1.

The whole-cell hydrolysates of strain XMU 706^T contained LL-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan (Fig. S5). The whole-cell sugars comprised glucose and ribose (Fig. S6). MK-9(H_4) (92 %) and MK-9(H_6) (7 %) were the predominant menaquinones. The phospholipids of the isolate consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, seven unidentified phospholipids and three unidentified lipids (Fig. S7). The predominant fatty acids were anteiso-$C_{15:0}$ (40.1 %) and iso-$C_{15:0}$ (20.8 %) (Table S4). The genomic DNA G+C content was found to be 67.3 mol%.

The phylogenetic, morphological and chemotaxonomic data showed that strain XMU 706^T belongs to the genus

Table 1. Differential characteristics between strain XMU 706^T and *Kribbella antibiotica* YIM 31530^T

Strains: 1, XMU 706^T; 2, *K. antibiotica* YIM 31530^T. All data were obtained under identical growth conditions in this study unless indicated otherwise. +, Positive; w, weakly positive; −, negative.

Characteristic	1	2
Temperature range for growth (°C)	15–30	10–30
Nitrate reduction	+	−
Tween 20 hydrolysis	+	−
Urease activity	−	+
Utilization of carbon sources		
D-Fructose	w	−
α-Lactose	w	+
L-Rhamnose	−	+
D-Ribose	+	w
D-Sorbose	−	w
Utilization of nitrogen sources		
L-Cystine	−	+
L-Histidine	w	+
L-Phenylalanine	w	+
Whole-cell sugars	Ribose, glucose	Ribose, xylose, glucose*
Polar lipids†	DPG, PG, PC	DPG, PG, PC, PI*

*Data from Li *et al.* (2004).

†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol.

Kribbella. However, the novel isolate could be differentiated from closely related species of this genus based on the DNA–DNA relatedness, *gyrB*-based genetic distance and physiological properties. On the basis of data presented above, we conclude that strain XMU 706^T represents a novel species of the genus *Kribbella*, for which the name *Kribbella mirabilis* sp. nov. is proposed.

Description of *Kribbella mirabilis* sp. nov.

Kribbella mirabilis (mi.ra′bi.lis. N.L. fem. gen. n. *mirabilis* of *Mirabilis*, pertaining to the plant *Mirabilis jalapa*).

Gram-positive, strictly aerobic actinomycete. Colonies appear cream to light yellow and show lichenous shapes with no diffusible pigment. Substrate and aerial mycelia fragment into irregular, rod-like or coccoid elements. Growth occurs at 15–30 °C and pH 6.0–9.0 with optimum growth at 28–30 °C and pH 7.0–7.5. NaCl is tolerated up to 4 % (w/v). D-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, inositol, α-lactose, maltose, D-mannose, melibiose, raffinose, D-ribose, D-sorbitol, sucrose, trisodium citrate and D-xylose can be utilized as sole carbon sources, but not L-rhamnose or L-sorbose. L-Alanine, L-asparagine, DL-asparaginic acid, L-arginine, glycine, L-histidine, L-hydroxyproline, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-tryptophan and D-valine can be used as sole nitrogen sources, but not L-cystine. Positive result in tests for gelatin liquefaction, starch hydrolysis, milk coagulation and peptonization, nitrate reduction, tween 20 hydrolysis and esterase activities, but negative result in tests for H_2S production, oxidase and urease activities. The cell wall contains LL-diaminopimelic acid, glucose and ribose. The predominant menaquinone is MK-9(H_4), with minor amounts of MK-9(H_6). The phospholipids comprise diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, several unidentified phospholipids and unknown lipids. The major fatty acids are anteiso-$C_{15:0}$ and iso-$C_{15:0}$.

The type strain, XMU 706^T (=KCTC 29676^T=MCCC 1K00429^T), was isolated from the rhizosphere soil of a herbaceous plant, *Mirabilis jalapa* L., collected from Xiamen city, Fujian province, China. The DNA G+C content of the type strain is 67.3 mol%.

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