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ORIGINAL PAPER

Kribbella soli sp. nov., isolated from soil

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Abstract A novel actinobacterial strain, designated FMN22^T, was isolated from soil and characterised using a polyphasic approach. Strain FMN22^T showed high 16S rRNA gene sequence similarity to *Kribbella karoonensis* Q41^T (99.3%), *Kribbella shirazensis* UTMC 693^T (99.0%), *Kribbella aluminosa* HKI 0478^T (98.9%) and *Kribbella hippodromi* S1.4^T (98.6%). Phylogenetic analysis using the 16S rRNA and concatenated gene (*gyr*B, *rpo*B, *rel*A, *rec*A and *atp*D) sequences showed that strain FMN22^T is closely related to the type strains of *K. karoonensis* DSM 17344^T, *K. shirazensis* UTMC 693^T, *K. aluminosa* HKI 0478^T, *K. hippodromi* S1.4^T, *Kribbella jejuensis* HD9^T and *Kribbella solani* DSA1^T. Based on concatenated gene genetic distances analysis, strain FMN22^T is distinct from all *Kribbella* type strains. DNA–DNA hybridization experiments with closely related type strains, were found to be 59.2 ± 2.4, 54.8 ± 2.1, 16.4 ± 2.3 and 38.6 ± 2.5%, relatedness to *K. karoonensis* DSM 17344^T, *K. shirazensis* DSM 45490^T, *K. aluminosa* DSM 18824^T and *K. jejuensis* DSM 17305^T, respectively. The cell wall peptidoglycan contained LL-diaminopimelic acid, and whole cell sugars were glucose, mannose and ribose. The predominant menaquinone was MK-9(H_4). The major phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol. Major fatty acids are *anteiso*-$C_{15:0}$ and *iso*-$C_{16:0}$. These chemotaxonomic traits are in good agreement with those known for representatives of the genus *Kribbella*. A

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gyr*B, *rpo*B, *rel*A, *rec*A and *atp*D gene sequences of *Kribbella soli* FMN22^T (=DSM 27132^T = KCTC 29219^T) are JN896613, KX348033, KX348034, KX348037, KX348036 and KX348035, respectively.

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combination of DNA–DNA hybridization results and phenotypic properties demonstrated that strain FMN22^T can be clearly distinguished from all close phylogenetic relatives. Therefore, strain FMN22^T (=DSM 27132^T = KCTC 29219^T) is considered to be the type strain of a novel species of the genus *Kribbella,* for which the name *Kribbella soli* is proposed.

Keywords *Actinobacteria* · *Kribbella soli* · *Nocardioidaceae* · Polyphasic taxonomy

Introduction

The genus *Kribbella* comprises strains mainly isolated from soil and habitats such as patina and biodeteriogenic biofilms from catacombs; from a medieval alum slate mine and from various parts of plant tissues. The most recently described species, *Kribbella pittospori*, is from an Australian apricot native tree (Kaewkla and Franco 2016). The genus *Kribbella* is a member of the family *Nocardioidaceae*, established by Park et al. (1999) as a result of the reclassification of the two strains, '*Nocardioides fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419, as the type strains of *Kribbella flavida* and *Kribbella sandramycini*, respectively. The type species of the genus *Hongia* (*Hongia koreensis;* Lee et al. 2000) was also subsequently transferred to the genus *Kribbella*, and the description of the genus *Kribbella* was also refined (Sohn et al. 2003). The description of the genus *Kribbella* has been emended recently by Everest et al. (2013), on the basis of polar lipid patterns, whole-cell sugars, the DNA G+C content, in addition to housekeeping gene analysis by multilocus sequences analysis (MLSA, Curtis and Meyers 2012; Everest et al. 2015). At the time of writing, the genus *Kribbella* contains 22 species with validly published names (Euzéby 2012).

Members of the genus *Kribbella* are Gram-positive or Gram-variable, non-motile, nocardioform actinomycetes that form an extensively branched vegetative mycelium and aerial hyphae that fragment into short elongated rod-like or coccoid elements. The members of the genus are designated by the presence of LL-diaminopimelic acid in the peptidoglycan (wall chemotype I sensu, Lechevalier and Lechevalier 1970), with *anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$ and *iso*-$C_{14:0}$ as predominant fatty acids, MK-9(H_4) as the main menaquinone and phosphatidylcholine as a diagnostic polar lipid (Park et al. 1999; Yoon and Park 2006). A congruence of these chemotaxonomic markers is adequate to distinguish members of the genus *Kribbella* from members of the other genera within the family of *Nocardioidaceae*.

During our investigations of novel actinomycetes from diverse habitats, a number of strains were found to have morphological properties typical of *Kribbella*. The subject of the present study was to establish the taxonomic position of the *Kribbella*-like strain FMN22^T using a polyphasic taxonomic approach. Based on phenotypic and genotypic evidence, it was established that strain FMN22^T represents a novel species of the genus *Kribbella*, for which the name *Kribbella soli* sp. nov. is proposed.

Materials and methods

Isolation and maintenance of the organism

Strain FMN22^T was isolated from soil samples collected from Southwest Troparevsky Forest Park, Moscow, Russia, using tryptone-yeast extract-glucose agar (TYGA) (0.3% tryptone, 0.5% yeast extract, 0.5% glucose, 1.5% agar) supplemented with filter sterilized cycloheximide (50 µg ml^{-1}), nalidixic acid (10 µg ml^{-1}) and rifampicin (0.5 µg ml^{-1}) after 21 days incubation at 28 °C. The strain was maintained on yeast extract-malt extract agar slants (ISP2; International *Streptomyces* Project Medium No. 2) (Shirling and Gottlieb 1966) and held in glycerol suspensions (20%, v/v) at −20 °C.

Morphological, cultural and physiological characteristics

Morphological characteristics were observed by light microscopy (Zeiss Axio Lab A1) and scanning electron microscopy (JEOL JSM 6060 instrument) using a culture grown on ISP 2 (Shirling and Gottlieb 1966) agar at 28 °C for 28 days. Growth at different temperatures (4, 10, 20, 28, 37, 45, 50 and 55 °C) was determined on ISP 2 medium (Shirling and Gottlieb 1966) after incubation for 14 days. Growth tests for pH range (4.0–12.0; at intervals of 1.0 pH unit) and NaCl tolerance (0–10% NaCl, w/v; at intervals of 1% unit) was determined on ISP 2 medium at 28 °C for

14 days. Established methods were followed to determine whether the strain degraded Tweens 20, 40 and 80 (Nash and Krent 1991); whilst the remaining degradation tests were examined using methods described by Williams et al. (1983). Carbon-source utilization was examined using ISP 9 (pH 9) medium (Shirling and Gottlieb 1966) supplemented with a final concentration of 1% (w/v) of the carbon sources, and of 0.1% (w/v) of succinic acid. Utilization of amino acids as sole nitrogen sources was determined according to Williams et al. (1983) using a final concentration of 0.1% (w/v) of each nitrogen source. In addition, cultural characteristics were determined on Czapek's agar (Waksman 1967), ISP media 2–7 (Shirling and Gottlieb 1966), modified Bennett's agar (MBA; Jones 1949), nutrient agar (Waksman 1961) and tryptic soy agar (TSA; Difco) following incubation at 28 °C for 14 days. The degree of growth, aerial mycelium and pigmentation were recorded. The ISCC-NBS colour charts were used to determine colony colours (Kelly 1964). The type strains *Kribbella karoonensis* DSM 17344^{T} (Kirby et al. 2006), *Kribbella shirazensis* DSM 45490^{T} (Mohammadipanah et al. 2013), *Kribbella aluminosa* DSM 18824^{T} (Carlsohn et al. 2007), *Kribbella jejuensis* DSM 17305^{T} (Song et al. 2004), *Kribbella hippodromi* DSM 19227^{T} (Everest and Meyers 2008) and *Kribbella solani* DSM 17294^{T} (Song et al. 2004) were also included for comparison in all tests.

Chemotaxonomic characterization

Biomass for chemotaxonomic studies was obtained by growing strain FMN22^{T} under aerobic conditions in flasks containing ISP 2 broth on a rotary shaker at 160 rpm for 14 days at 28 °C. Biomass was harvested by centrifugation, washed twice in distilled water and re-centrifuged and freeze-dried. Diaminopimelic acid in whole cell hydrolysates and whole cell sugars were obtained according to Lechevalier and Lechevalier (1970) and analysed by thin layer chromatography (Staneck and Roberts 1974). Polar lipids were extracted and analysed by the method of Minnikin et al. (1984), as modified by Kroppenstedt and Goodfellow (2006). The isoprenoid quinones were extracted and purified using the method of Collins et al. (1977) and analysed by HPLC (Kroppenstedt 1982). For the extraction of whole-cell fatty acids, cells were grown in 20 ml of Trypticase Soy Broth (TSB) at 28 °C with a shaking of 150 rpm. After five days of incubation, 5 ml of seed culture was inoculated into 50 ml of TSB. The inoculated flask was incubated as before for five days. After harvesting by cellulose membrane filtration (0.45 μm), wet cells (200 mg) were placed in an extraction tube. Cellular fatty acids were extracted, methylated and separated by gas chromatography using an Agilent Technologies 6890N instrument which was fitted with an auto sampler, in addition to a 6783 injector, according to the Standard Protocol of the Sherlock microbial identification (MIDI) System (Sasser 1990; Kämpfer and Kroppenstedt 1996). The fatty acid methyl ester peaks were then identified and quantified using TSBA 5.0 software. The DNA G + C content of the isolate was determined following the procedure of Gonzalez and Saiz-Jimenez (2005).

DNA preparation, amplification and determination of 16S rRNA, *gyr*B, *rpo*B, *rel*A, *rec*A and *atp*D gene sequence

Genomic DNA of the strain FMN22^{T} was extracted as described by Everest and Meyers (2008). The 16S rRNA gene amplification and purification of PCR product were carried out following Chun and Goodfellow (1995). The PCR amplification of the *gyr*B (Kirby et al. 2010) and the *atp*D, *rel*A, *rec*A and *rpo*B genes sequences were obtained using standard procedure of Curtis and Meyers (2012). The almost complete gene sequence of 16S rRNA (1515 bp), *gyr*B gene sequence (1115 bp), *rpo*B gene sequence (951 bp), *rel*A gene sequence (1018 bp), *rec*A gene sequence (532 bp) and *atp*D gene sequence (601 bp) of strain FMN22^{T} were determined using an ABI PRISM 3730 XL automatic sequencer. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al. 2012). Multiple alignments with sequences from closely related strains were performed by using CLUSTAL W in MEGA version 6.0 software (Tamura et al. 2013). Phylogenetic trees were constructed with the neighbour-joining (Saitou and Nei 1987), maximum parsimony (Kluge and Farris 1969) and maximum-likelihood (Felsenstein 1981) algorithms in MEGA 6.0. Evolutionary distances were calculated using the model of Jukes and Cantor (1969). Topologies of the resultant

trees were evaluated by bootstrap analysis (Felsenstein 1985) based on 1000 resamplings.

Phylogenetic relationships of the strain FMN22^T were confirmed using sequences for five individual housekeeping genes (*gyr*B, *rpo*B, *rel*A, *rec*A and *atp*D). The sequences of each locus were aligned using MEGA 6.0 sofware (Tamura et al. 2013) and trimmed manually at the same position before being used for further analysis and deposited in GenBank (www.ncbi.nlm.nih.gov/genbank). The data of the sequences were exported as a concatenated five-gene alignment for subsequent analysis using MEGA 6.0 with the model of Jukes and Cantor (1969), using the neighbour-joining (Saitou and Nei 1987) algorithm based on 1000 bootstrap replication.

DNA–DNA hybridization

DNA–DNA relatedness tests between strain FMN22^T and the type strains of the related species *K. karoonensis* DSM 17344^T, *K. shirazensis* DSM 45490^T, *K. jejuensis* DSM 17305^T and *K. aluminosa* DSM 18824^T were performed. Cells were disrupted by using a Constant Systems TS 0.75 KW (IUL Instruments, Germany) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization (DDH) was carried out as described by De Ley et al. (1970) under the consideration of the modifications described by Huss et al. (1983) using a model Cary 100 Bio UV/visual spectrophotometer equipped with a Peltier-thermostated 666 multi cell changer and a temperature controller with in situ temperature probe (Varian).

Results and discussion

Morphological observation after 14 days of culture of strain FMN22^T grown on ISP 2 medium revealed that it had the typical characteristics of the genus *Kribbella*. Strain FMN22^T produced extensively branched substrate mycelium and aerial mycelium consisting of hyphae that fragmented into irregular rod-shaped elements (Fig. S1). The strain grew well on ISP 2, 4, 5, 6 and 7 media, modified Bennett's, Czapek's, nutrient and tryptic soy agar (TSA), but there was no growth on ISP 3 (Table S1). No diffusible pigment was detected on any of the tested media. Melanoid pigments were not produced on ISP 6 or ISP 7 agars. Strain FMN22^T was found to grow well between pH 5.0–9.0, with an optimum pH of 7.0. Growth occurs at 20 and 28 °C. Growth was observed in the presence of 0–3% (w/v) NaCl. Additional phenotypic properties are given in the species description and Table 1.

Chemotaxonomic analysis of strain FMN22^T showed that it had chemotaxonomic characteristics that are coherent with type species of the genus *Kribbella*. LL-diaminopimelic acid was found as the diagnostic diamino acid in the peptidoglycan. Whole cell sugars included glucose, mannose and ribose. The polar lipids of strain FMN22^T were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI) (Fig. S2). The major menaquinones found were identified as MK-9(H_4) (77.1%); MK-9(H_2) (2.8%), MK-8(H_4) (2.0%) and MK-9(H_6) (1.5%) were also detected. The major cellular fatty acids were *anteiso*-$C_{15:0}$ (31.7%) and *iso*-$C_{16:0}$ (10.7%). Comparative cellular fatty acid compositions of strain FMN22^T, *K. karoonensis* DSM 17344^T, *K. shirazensis* DSM 45490^T, *K. aluminosa* DSM 18824^T, *K. hippodromi* DSM 19227^T, *K. jejuensis* DSM 17305^T and *K. solani* DSM 17294^T are shown in Table S2. The G + C content of the DNA was 69.3 mol%.

The almost-complete 16S rRNA gene sequence (1515 bp) of strain FMN22^T determined in this study was compared with the corresponding sequences of members of the genus *Kribbella*. The neighbour-joining tree (Fig. 1), based on the 16S rRNA gene sequence, forms a clade with *K. karoonensis* DSM 17344^T (99.29%; 10 nt differences at 1417 locations), *K. shirazensis* UTMC 693^T (99.02%; 14 nt differences at 1422 locations), *K. aluminosa* HKI 0478^T (98.94%; 15 nt differences at 1421 locations), *K. hippodromi* S1.4^T (98.56%; 20 nt differences at 1389 locations), *K. jejuensis* HD9^T (98.31%; 25 nt differences at 1481 locations) and *K. solani* DSA1^T (98.17%; 27 nt differences at 1479 locations) with high bootstrap values. Lower sequence similarities (<98.4%) were found with the type strains of all other species of the genus *Kribbella*. Topologies of phylogenetic trees built using the maximum-likelihood algorithm (Supplementary Fig. S3) were similar to the tree reconstructed by neighbour-joining analysis. In the phylogenetic tree built using the maximum-parsimony algorithm (Supplementary Fig. S4) strain FMN22

Table 1 Phenotypic properties of strain FMN22^T and the type strains of closely related species

Characteristics	1	2	3	4	5	6	7
Biochemical tests							
Allantoin hydrolysis	+	+	+	−	−	−	−
Nitrate reduction	+	+	+	+	+	−	+
Urea hydrolysis	+	+	+	+	−	+	−
pH tolerence							
5	+	+	+	+	+	−	−
9	+	+	+	−	+	+	−
Temperature							
37	−	+	+	+	+	+	−
NaCl (%, w/v)							
4.0	−	+	+	−	+	−	+
5.0	−	−	−	−	+	−	+
Degradation of (%, w/v)							
Adenine (0.5%)	+	+	+	+	+	−	−
Casein (1%)	−	+	−	+	+	−	−
Elastin (0.3%)	−	+	−	−	+	−	−
Hypoxanthine (0.4%)	+	+	+	+	+	−	−
Starch (1%)	+	+	+	+	+	−	−
Tween 20 (1%)	−	+	+	+	+	+	+
Tween 80 (1%)	−	−	+	+	+	−	+
Xanthine (0.4%)	−	+	+	+	−	−	+
Xylan (0.4%)	−	+	−	+	−	−	−
Carbon source utilization (1.0%, w/v)							
Adonitol	+	+	+	+	+	+	−
D-Galactose	+	+	−	+	+	+	−
D-Melibiose	+	−	+	+	+	+	+
Inulin	+	−	+	+	+	+	−
Mannitol	+	+	+	+	+	+	−
Myo-Inositol	+	+	+	+	+	−	−
Succinic acid (0.1%)	+	+	+	−	−	−	−
Xylose	+	+	+	+	−	+	+
Nitrogen source utilization (0.1%, w/v)							
L-Alanine	+	+	−	+	+	+	+
L-Arginine	+	+	−	+	+	+	+
L-Histidine	+	+	−	+	+	+	+
L-Isoleucine	+	+	+	+	−	+	+
L-Lysine	+	+	−	+	+	−	−
L-Phenylalanin	+	+	+	−	−	+	−
L-Tyrosine	+	+	−	+	+	+	+
L-Valine	+	+	+	+	−	−	−

Strains: 1, FMN22^T; 2, *K. karoonensis* DSM 17344^T; 3, *K. shirazensis* DSM 45490^T; 4, *K. aluminosa* DSM 18824^T; 5, *K. hippodromi* DSM 19227^T; 6, *K. jejuensis* DSM 17305^T; 7, *K. solani* DSM 17294^T. +, positive; −, negative. All the data are from this study

forms a clade with *K. karoonensis* DSM 17344^T and *K. shirazensis* DSM 45490^T. Mohammadipanah et al. (2013) determined that 16S rRNA gene sequences of AY995146 and AY995147 were flawed and used DSM strains [*K. karoonensis* DSM 17344^T (FN643222) and *K. swartbergensis* DSM 17345^T (FN643223)] for phylogenetic trees, but the sequences have now been corrected (AY995146.2 and AY995147.2) and are identical to the DSM sequences. So, we used these new sequences for generating phylogenetic trees.

The housekeeping genes of five protein-coding loci, namely *gyr*B, *rpo*B, *rel*A, *rec*A and *atp*D concatenated and aligned sequences contained 4250 bp for the strain FMN22^T. Sequence similarity of the five housekeeping genes together with 16S rRNA gene for the strain FMN22^T are summarised in Table 2. Accession numbers for the *Kribbella* sequences used in generating the concatenated-sequence tree are given in Supplementary Table S3. The neighbour-joining tree based on the five concatenated genes had a similar topology to the 16S rRNA gene tree, and strain FMN22^T forms a clade with *K. karoonensis* DSM 17344^T, *K. shirazensis* DSM 45490^T, *K. aluminosa* DSM 18824^T, *K. hippodromi* S1.4^T, *K. jejuensis* CIP 108509^T and *K. solani* CIP 108508^T with a very high bootstrap value (Fig. 2). This high bootstrap value is also supported by maximum-likelihood and maximum-parsimony algorithms (99% bootstrap values for each algorithms).

Genetic distance values were calculated between strain FMN22^T and all *Kribbella* type strains based on *gyr*B, *rpo*B, *rel*A, *rec*A and *atp*D genes to define the likelihood of this strain representing a novel genomic species. The *gyr*B-based genetic distance values ranged from 0.051 to 0.120. These values are 0.071, 0.062, 0.077, 0.077, 0.074, 0.086 and 0.051 between strain FMN22^T with the type strains of *K. karoonensis* DSM 17344^T, *K. shirazensis* DSM 45490^T, *K. aluminosa* DSM 18824^T, *K. hippodromi* S1.4^T, *K. jejuensis* CIP 108509^T, *K. solani* CIP 108508^T and *Kribbella swartbergensis* DSM 17345^T, respectively. The *gyr*B genetic distance values are all above the 0.04 *gyr*B genetic distance threshold proposed to represent novel species in the genus (Kirby et al. 2010). The *gyr*B-*rpo*B-*rel*A-*rec*A-*atp*D based genetic distances values ranged from 0.047 to 0.091. These values between strain FMN22^T with the type strains of *K. karoonensis* DSM 17344^T, *K. shirazensis* DSM 45490^T, *K. aluminosa* DSM 18824^T, *K. hippodromi* S1.4^T, *K. jejuensis*

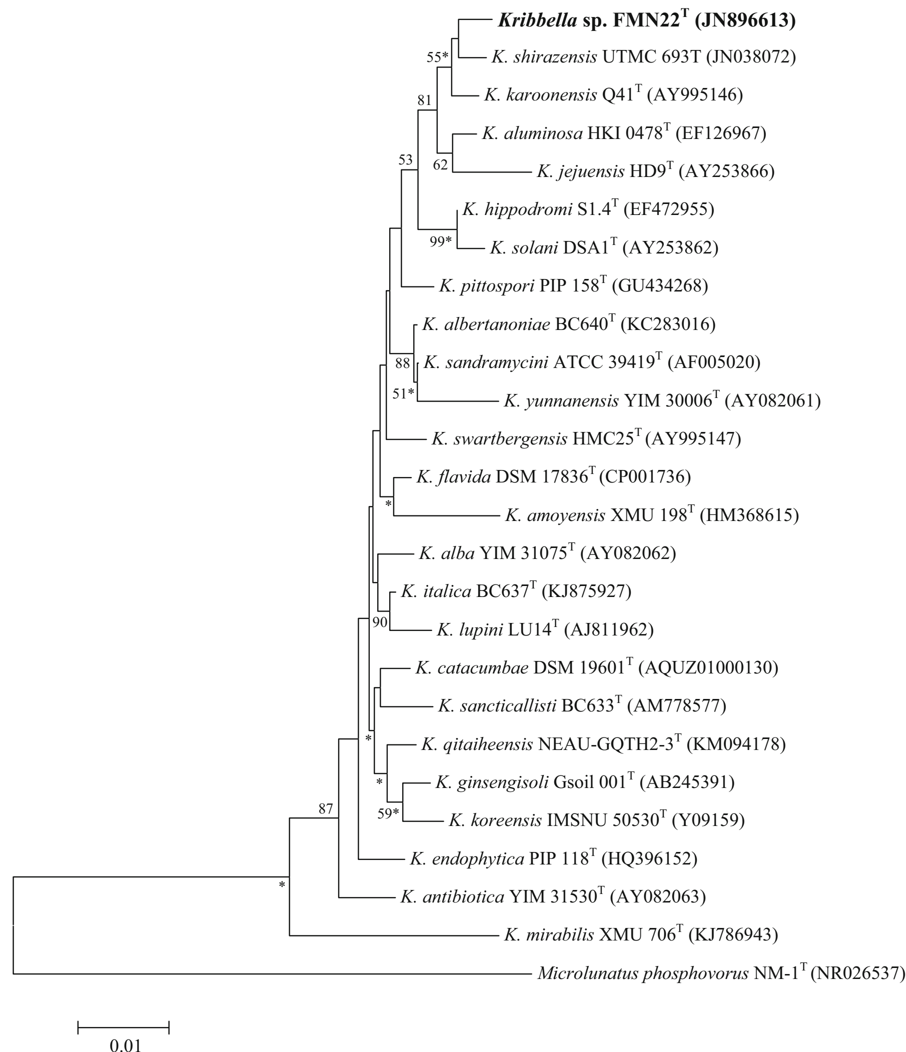

Fig. 1 Phylogenetic tree of strain FMN22^T and other type strains of the genus *Kribbella* inferred from 16S rRNA gene based on 1486 bp of sequences under the neighbour-joining criterion. Bootstrap percentages based on 1000 replicates are shown; values ≥50% are shown. *Bar*, 0.01 substitutions per nucleotide position. *Asterisks* indicate clades that were conserved in the neighbour-joining, maximum-likelihood and maximum-parsimony trees. Accession numbers are indicated in parentheses. *Microlunatus phosphovorus* NM-1^T was used as an outgroup

Table 2 Sequence similarities (%) for the 16S rRNA, *gyr*B, *rec*A, *rel*A, *rpo*B and *atp*D genes between FMN22^T and closely related type species

Strain	Similarity (%) with strain FMN22^T					
	16S rRNA	*gyr*B	*rpo*B	*rel*A	*rec*A	*atp*D
K. karoonensis DSM 17344^T	99.3	93.6	97.5	94.7	92.1	94.8
K. shirazensis DSM 45490^T	99.0	94.0	97.2	95.5	95.3	95.6
K. aluminosa DSM 18824^T	98.9	93.0	97.8	93.8	95.3	95.8
K. hippodromi S1.4^T	98.6	93.0	96.9	93.3	95.1	95.0
K. jejuensis CIP 108509^T	98.3	93.2	96.5	94.3	94.6	94.3
K. solani CIP 108508^T	98.2	92.3	96.4	94.1	94.7	94.8

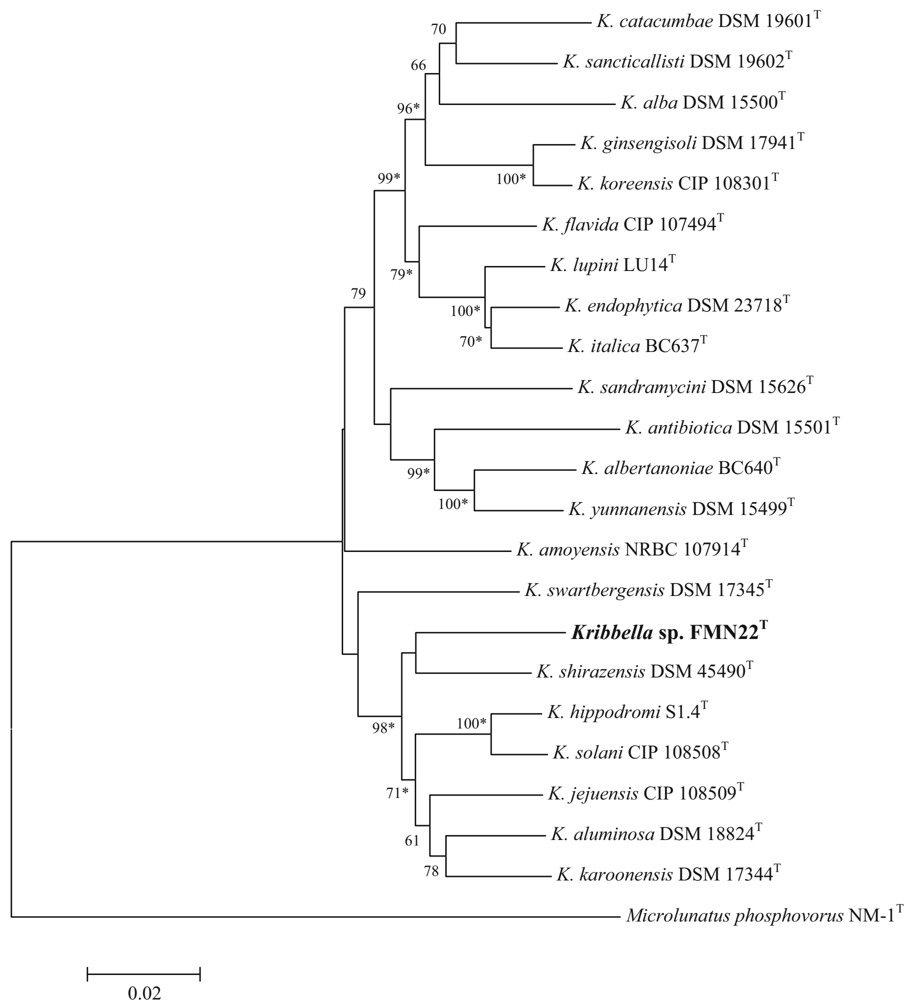

Fig. 2 *gyr*B-*rpo*B-*rel*A-recA-atpD phylogenetic tree showing the position of strain FMN22^T within the genus Kribbella. The tree was reconstructed using the neighbour-joining method based on 4250 bp of sequence. Bootstrap percentages based on 1000 replicates are shown; values $\geq$50% are shown. Bar, 0.02 substitutions per nucleotide position. *Asterisks* indicate clades that were conserved in the neighbour-joining, maximum-likelihood and maximum-parsimony trees. Accession numbers of all the gene sequences used are listed in Supplementary Table S3. Microlunatus phosphovorus NM-1^T was used as an outgroup

CIP 108509^T, *K. solani* CIP 108508^T and *K. swartbergensis* DSM 17345^T were 0.057, 0.047, 0.052, 0.058, 0.057, 0.059 and 0.061, respectively. The values are also all above 0.04 *gyr*B-*rpo*B-*rel*A-*rec*A-*atp*D genetic distance threshold proposed by Curtis and Meyers (2012).

DNA–DNA relatedness values between strain FMN22^T and the type strains of *K. karoonensis* DSM 17344^T, *K. shirazensis* DSM 45490^T, *K. aluminosa* DSM 18824^T and *K. jejuensis* DSM 17305^T were found to be 59.2 ± 2.4, 54.8 ± 2.1, 16.4 ± 2.3 and 38.6 ± 2.5%, respectively. These values are well below the 70% cut-off point recommended for recognition of genomic species (Wayne et al. 1987).

Based on a combination of chemical, morphological, molecular and physiological data strain FMN22^T is considered to represent a novel species of the genus *Kribbella*, for which the name *Kribbella soli* sp. nov. is proposed.

Description of *Kribbella soli* sp. nov

Kribbella soli (so'li. N.L. neut. gen. n. *soli* of soil)

Aerobic, Gram-positive, non-motile actinomycete that forms an extensively branched substrate and aerial mycelium that fragment into rod-shaped elements. Vegetative mycelium appears cream to orange colour-series. Good growth on ISP 2, ISP 4, ISP 5, ISP 6, ISP 7, TSA, modified Bennett's, Czapek's and nutrient agar media, but no growth on ISP 3 medium. No diffusible pigment is produced on ISP 5. Melanoid pigments are not produced on ISP 6 or ISP 7 agars. Growth occurs at pH 5.0–9.0, but not at pH 4.0 or 10.0. Growth occurs at 20 and 28 °C, but no growth at 10 or 37 °C. Optimal growth occurs at 28 °C and pH 7.0. Growth is observed in the presence of 0–3% (w/v) NaCl. Aesculin, allantoin, arbutin and urea hydrolysis and nitrate reduction are positive. Adenine, gelatine, hypoxanthine, starch and Tween 40 are degraded, but casein, elastin, guanine, Tweens 20 and 80, xanthine and xylan are not. Adonitol, dextrin, D-arabinose, D-cellobiose, D-galactose, D-glucose, D-fructose, D-mannitol, D-mannose, D-melezitose, D-melibiose, D-ribose, D-raffinose, D-trehalose, inulin, lactose, L-arabinose, L-rhamnose, maltose, myo-inositol, succinic acid, sucrose and xylose are utilized as sole carbon and energy sources, but not dextran, D-sorbitol or xylitol. DL-phenylalanine, glycine, L-alanine, L-arginine, L-asparagine, L-cysteine, L-histidine, L-hydroxyproline, L-isoleucine, L-lysine, L-methionine, L-phenylalanin, L-proline, L-serine, L-threonine, L-tyrosine and L-valine are utilized as sole nitrogen sources. MK-9(H_4) is the major menaquinone. The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol. Major fatty acids (present as >10% of total fatty acids) are *anteiso* $C_{15:0}$, and *iso* $C_{16:0}$. The G+C content of the genomic DNA of the type strain is 69.3 mol%.

The type strain, FMN22^T (=DSM 27132^T = KCTC 29219^T) was isolated from soil in Yugo-Zapadnaya Southwest Troparevsky Forest Park, Moscow, Russia.

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References

Carlsohn MR, Groth I, Spröer C, Schütze B, Saluz HP, Munder T, Stackebrandt E (2007) *Kribbella aluminosa* sp. nov., isolated from a medieval alum slate mine. Int J Syst Evol Microbiol 57:1943–1947

Cashion P, Holder-Franklin MA, Mc Cully J, Franklin M (1977) A rapid method for the base ratio determination of bacterial DNA. Anal Biochem 81:461–466

Chun J, Goodfellow M (1995) A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. Int J Syst Bacteriol 45:240–245

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221–230

Curtis SM, Meyers PR (2012) Multilocus sequence analysis of the actinobacterial genus Kribbella. Syst Appl Microbiol 35:441–446

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:143–153

Euzéby JP (2012) Genus Kribbella. List of Prokaryotic Names with Standing in Nomenclature. http://www.bacterio.net/ijk/kribbella.html

Everest GJ, Meyers PR (2008) *Kribbella hippodromi* sp. nov., isolated from soil from a racecourse in South Africa. Int J Syst Evol Microbiol 58:443–446

Everest GJ, Curtis SM, De Leo F, Urzì C, Meyers PR (2013) *Kribbella albertanoniae* sp. nov., isolated from a Roman catacomb, and emended description of the genus *Kribbella*. Int J Syst Evol Microbiol 63:3591–3596

Everest GJ, Curtis SM, De Leo F, Urzì C, Meyers PR (2015) Description of *Kribbella italica* sp. nov., isolated from a Roman catacomb. Int J Syst Evol Microbiol 65:491–496

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

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

Gonzalez JM, Saiz-Jimenez C (2005) A simple fluorimetric method for the estimation of DNA–DNA relatedness between closely related microorganisms by thermal denaturation temperatures. Extremophiles 9:75–79

Huss VAR, Festl H, Schleifer KH (1983) Studies on the spectrometric determination of DNA hybridisation from renaturation rates. Syst Appl Microbiol 4:184–192

Jones KL (1949) Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. J Bacteriol 57:141–145

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism, vol 3. Academic Press, New York, pp 21–132

Kaewkla O, Franco CMM (2016) *Kribbella pittospori* sp. nov., an endophytic actinobacterium isolated from the surface-sterilized stem of an Australian native apricot tree, *Pittosporum angustifolium*. Int J Syst Evol Microbiol 66:2284–2290

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

Kelly KL (1964) Inter-society color council-national bureau of standards color-name charts illustrated with centroid colors. US Government Printing Office, Washington, DC

Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M, Na H, Park S-C, Jeon YS, Lee JH, Yi H, Won S, Chun J (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

Kirby BM, Le Roes M, Meyers PR (2006) *Kribbella karoonensis* 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 BM, Everest GJ, Meyers PR (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

Kluge AG, Farris FS (1969) Quantitative phyletics and the evolution of anurans. Syst Zool 18:1–32

Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger. J Liq Chromatogr 5:2359–2387

Kroppenstedt RM, Goodfellow M (2006) The Family *Thermomonosporaceae*: *Actinocorallia*, *Actinomadura*, *Spirillispora* and *Thermomonospora*. In: Dworkin M, Falkow S, Schleifer KH, Stackebrandt E (eds) The prokaryotes. *Archaea* and *Bacteria*: Firmicutes, Actinomycetes, vol 3, 3rd edn. Springer, New York, pp 682–724

Lechevalier MP, Lechevalier HA (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20:435–443

Lee SD, Kang SO, Hah YC (2000) Hongia gen. nov., a new genus of the order Actinomycetales. Int J Syst Evol Microbiol 50:191–199

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal K, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Mohammadipanah F, Hamedi J, Göker M, Fiebig A, Pukall R, Spröer C, Klenk H-P (2013) *Kribbella shirazensis* sp. nov., isolated from Iranian soil. Int J Syst Evol Microbiol 63:3369–3374

Nash P, Krent MM (1991) Culture media. In: Ballows A, Hauser WJ, Herrmann KL, Isenberg HD, Shadomy HJ (eds) Manual of clinical microbiology, 3rd edn. American Society for Microbiology, Washington DC, pp 1268–1270

Park YH, Yoon JH, Shin YK, Suzuki K, Kudo T, Seino A, Kim HJ, Lee JS, Lee ST (1999) Classification of ''*Nocardioides fulvus*'' IFO 14399 and *Nocardioides* sp. ATCC 39419 in *Kribbella* gen. nov., as *Kribbella flavida* sp. nov. and *Kribbella sandramycini* 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

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. Technical Note 101. MIDI Inc, Newark

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

Sohn K, Hong SG, Bae KS, Chun J et al (2003) Transfer of *Hongia koreensis* Lee et al. 2000 to the genus Kribbella Park et al. 1999 as *Kribbella koreensis* comb. nov. Int J Syst Evol Microbiol 53:1005–1007

Song J, Kim BY, Hong SB, Cho HS, Sohn K, Chun J, Suh JW (2004) *Kribbella solani* sp. nov. and *Kribbella jejuensis* sp. nov., isolated from potato tuber and soil in Jeju. Korea. Int J Syst Evol Microbiol 54:1345–1348

Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol 28:226–231

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Waksman SA (1961) The actinomycetes, classification, identification and description of genera and species, vol 2. Williams & Wilkins, Baltimore

Waksman SA (1967) The actinomycetes. A summary of current knowledge. Ronald Press, New York

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ (1983) Numerical classification of *Streptomyces* and related genera. J Gen Microbiol 129:1743–1813

Yoon JH, Park YH (2006) The genus Nocardioides. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes, vol 3. Springer, New York, pp 1099–1113