Kribbella karoonsensis sp. nov. and *Kribbella swartbergensis* sp. nov., isolated from soil from the Western Cape, South Africa

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Two novel nocardioform actinomycetes, strains Q41^T and HMC25^T, were isolated from soil samples collected in the Western Cape province, South Africa. Rapid genus identification revealed that the isolates belonged to the genus *Kribbella* (based on single-digestion restriction analysis of the 16S rRNA gene sequences with *MboI*, *VspI*, *SphI*, *SnaBI*, *SalI* and *AgeI*). Both isolates had LL-diaminopimelic acid and glycine in their cell-wall peptidoglycan, and contained mannose and ribose as whole-cell sugars. Strain HMC25^T is able to grow at 45 °C and in the presence of NaCl (3 %), cephaloridine (10 µg ml⁻¹) and gentamicin sulphate (10 µg ml⁻¹). Strain Q41^T grows in the presence of NaCl (2 %). Neither strain was able to grow under anaerobic conditions, whereas *Kribbella flavida* KACC 20248^T, *Kribbella jejuensis* HD9^T, *Kribbella koreensis* KACC 20250^T and *Kribbella sandramycini* KACC 20249^T exhibited weak but distinct growth under anaerobic conditions. Physiological test results and 16S rRNA gene sequence analysis allowed Q41^T and HMC25^T to be distinguished from other members of the genus with validly published names. Strains HMC25^T (= NRRL B-24426^T = DSM 17345^T) and Q41^T (= NRRL B-24425^T = DSM 17344^T) therefore represent the type strains of novel species, for which the names *Kribbella swartbergensis* sp. nov. and *Kribbella karoonsensis* sp. nov., respectively, are proposed.

The genus *Kribbella* was established by Park *et al.* (1999) to accommodate nocardioform actinomycetes that contain LL-diaminopimelic acid in the cell wall. The type species of the genus *Hongia*, *Hongia koreensis*, was subsequently transferred to the genus *Kribbella* (Sohn *et al.*, 2003). With the recent addition of *Kribbella lupini* (Trujillo *et al.*, 2006), the genus *Kribbella* currently contains seven species with validly published names (<http://www.bacterio.cict.fr/ijk/kribbella.html>). Species descriptions of '*Kribbella alba*' and '*Kribbella yunnanensis*' have been effectively published (Li *et al.*, 2006).

We describe the characterization of two further members of this genus, which were isolated from soil samples collected in Western Cape province, South Africa. The soil from which Q41^T was isolated was gathered from the base of a giant quiver tree, *Aloe pillansii*, growing in the Karoo Desert National Botanical Garden, Worcester. HMC25^T was isolated from a soil sample collected from the banks of Gamka River, in Die Hel, situated in the Groot Swartberg mountain range.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains Q41^T and HMC25^T are AY995146 and AY995147, respectively.

Scanning electron micrographs of mycelium of strains Q41^T and HMC25^T are available as supplementary material in IJSEM Online.

The strains were isolated using two different soil pre-treatments. The soil sample from which HMC25^T was isolated was initially heated at 120 °C for 1 h. Approximately 0.1 g soil was then added to 1 ml sterile distilled water and vortexed for 1 min. The sample was then serially diluted in sterile distilled water and spread-plated on MC agar (Nonomura & Ohara, 1971). The soil sample from which Q41^T was isolated was dried at 28 °C for 24 h; 0.1 g soil was added to 1 ml sterile distilled water and vortexed for 1 min. The soil suspension was then serially diluted in sterile distilled water and spread-plated on PV8 agar (Tormo *et al.*, 2003). All isolation plates contained nalidixic acid (10 µg ml⁻¹) and cycloheximide (50 µg ml⁻¹) and were incubated at 28 °C (PV8 plates for 5 days and MC plates for 21 days). Following isolation, both HMC25^T and Q41^T were maintained on either yeast extract-malt extract agar (ISP 2 medium) (Shirling & Gottlieb, 1966) or Czapek's solution agar (Atlas, 1993).

Genomic DNA extraction and 16S rRNA gene sequence amplification were performed as previously described by Cook & Meyers (2003). For rapid genus identification, DNA was amplified using primers F1 and R5, and the resulting amplicons were restricted singly with *MboI* (isoschizomer of *Sau3AI*), *VspI* (isoschizomer of *AsnI*), *SphI*, *SnaBI*, *SalI* and *AgeI* and electrophoresed on a 1.5 % agarose gel. For 16S rRNA gene sequence analysis, the PCR product was purified

using a Cleanmix kit (TA050CLN; Talent) and sequenced. A neighbour-joining phylogenetic tree (Saitou & Nei, 1987) was constructed using CLUSTAL X, version 1.81 (Thompson *et al.*, 1997), and MEGA, version 2.1 (Kumar *et al.*, 2001).

Selected physiological tests were performed as described by Williams *et al.* (1989). ISP media were prepared according to the methods of Shirling & Gottlieb (1966). All physiological tests were incubated at 30 °C (unless otherwise stated) and the results were recorded after the recommended incubation periods (Shirling & Gottlieb, 1966; Williams *et al.*, 1989). *Kribbella antibiotica* YIM 31530^T was incubated at room temperature (22 °C). Utilization of carbon sources was tested according to the method of Shirling & Gottlieb (1966). All carbon sources were tested at 1 % except for sodium acetate, sodium benzoate and sodium citrate, which were tested at 0.1 % (all carbon sources were filter-sterilized). Antibiotic susceptibility was tested by incorporating the antibiotics into Bennett's medium (Atlas, 1993) at the given concentrations and incubating at 30 °C for 14 days. Salt tolerance was tested on ISP 2 (Shirling & Gottlieb, 1966). Plates were incubated at 30 °C for 14 days. The ability to grow at pH 5, 7 and 9 was determined on Bennett's medium plates incubated for 14 days at 30 °C. Degradation of adenine, hypoxanthine and xanthine was determined on modified Bennett's medium plates (Atlas, 1993) incubated at 30 °C for 21 days. The temperature range for growth was tested (at 20, 30, 37 and 45 °C) on Bennett's medium plates.

Growth under anaerobic conditions was tested on ATCC medium 172 and ISP 9 with glucose as the sole carbon source (Shirling & Gottlieb, 1966). Plates were incubated at 25 °C for 21 days in an anaerobic chamber (model 1024; Forma Scientific) in an atmosphere comprising H₂/CO₂/N₂ (5:10:85). When plates were first placed in the chamber, each one was opened for 5 s, before being sealed in a plastic bag, to ensure that all residual O₂ was removed.

Cultures for chemotaxonomic analysis were grown in ISP 2 in shake flasks for 5 days at 30 °C. Samples were prepared according to the method of Hasegawa *et al.* (1983), with the exception that freeze-dried cells were used.

Antimicrobial testing against *Citrobacter braaki* strain 90 (clinical isolate), *Enterococcus faecalis* (clinical isolate), *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* strain 66 (clinical isolate), *Mycobacterium aurum* A+, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 was performed on 10-day-old stab-inoculated cultures by using standard agar overlays. HMC25^T and Q41^T were stab-inoculated into Czapek's solution agar, Middlebrook 7H9 agar containing 10 mM glucose (Difco; ADC supplement omitted) and ISP 2 agar for antimicrobial testing. For testing against *Mycobacterium tuberculosis* H37Rv (= ATCC 27294), *Kribbella* cultures were grown in production medium (Matson & Bush, 1989) for 5 days at 30 °C. The cultures were filtered through coffee filters (size 102; House of Coffees) and the cells were washed with two culture volumes of deionized water. For each strain, 10 ml

methanol was added to the washed cells, which were stirred for 2 h at room temperature. The methanol extracts were filtered to remove the cells and dried down. Each of these crude extracts was weighed, redissolved in methanol and spotted on silica TLC plates (Merck 1.05554.0001) in a concentration range of 5–60 µg per spot. *M. tuberculosis* was grown in Middlebrook 7H9 broth containing the AD supplement (final concentrations: 0.5 % bovine serum albumin, 0.2 % glucose) and was incubated as a standing culture at 37 °C (with intermittent agitation) for 7 days. For antimicrobial testing, the TLC plates were dabbed with the *M. tuberculosis* culture, using sterile non-absorbent cotton wool, and incubated for 24 h at 37 °C in a sealed plastic container containing moist paper towel. Following incubation, the plates were dabbed with 0.25 % thiazolyl blue tetrazolium bromide (Sigma) in PBS (pH 7.3) and incubated for 24 h at 37 °C before the results were read. All experiments with *M. tuberculosis* were conducted in a Biosafety Level 3 laboratory.

The restriction fragment patterns indicated that both isolates belonged to the genus *Kribbella* (see Tables 1, 2 and 3 of Cook & Meyers, 2003). The lengths of the 16S rRNA gene sequence obtained for Q41^T and HMC25^T were 1422 and 1419 bp, respectively. A standard nucleotide–nucleotide BLAST search (Altschul *et al.*, 1997) of the GenBank database showed that HMC25^T was most similar to *Kribbella jejuensis* strain HD9^T (= KACC 20266^T) (97 % similarity over 1430 bp), while Q41^T was most similar to *Kribbella flava* KACC 20248^T and *Kribbella sandramycini* KACC 20249^T (both 98 % over 1421 bp). The 16S rRNA gene sequence similarity between HMC25^T and Q41^T was 98.4 % (determined using DNAMAN, version 4.13; Lynnon Biosoft). These values are low enough to indicate a high probability that the DNA–DNA relatedness between these pairs of strains would be below 70 % (Keswani & Whitman, 2001; Trujillo *et al.*, 2006). For example, the 16S rRNA gene sequence similarity values between *Kribbella flava* KACC 20248^T and *Kribbella solani* DSA1^T, between *Kribbella jejuensis* HD9^T and *Kribbella koreensis* KACC 20250^T and between *Kribbella antibiotica* YIM 31530^T and *Kribbella flava* KACC 20248^T are 98.6 % (over 1509 nt), 97.8 % (over 1510 nt) and 98.4 % (over 1427 nt), respectively (similarities calculated by pairwise global alignment using DNAMAN, version 4.13), yet the reported DNA relatedness values for these pairs of strains are 50.4, 20.9 and 18 %, respectively (Li *et al.*, 2004; Song *et al.*, 2004). As HMC25^T and Q41^T were phenetically (Table 1) and phylogenetically (Fig. 1) distinct from other *kribbellae*, DNA–DNA hybridization analysis was not considered necessary to establish these strains as members of novel *Kribbella* species.

The results of the phenetic characterization of strains HMC25^T and Q41^T are presented in Table 1 and in the species descriptions. Although neither HMC25^T nor Q41^T grew anaerobically, it is worth noting that a number of *Kribbella* species could grow weakly under anaerobic conditions on ATCC medium 172 (Table 1). Interestingly,

Table 1. Physiological tests that differentiate HMC25^T and Q41^T from the type strains of *Kribbella* species

Reference strains: 1, *Kribbella antibiotica* YIM 31530^T (unless indicated, data taken from Li *et al.*, 2004); 2, *Kribbella flavida* KACC 20248^T (Park *et al.*, 1999); 3, *Kribbella jejuensis* HD9^T (Song *et al.*, 2004); 4, *Kribbella koreensis* KACC 20250^T (Lee *et al.*, 2000; Sohn *et al.*, 2003); 5, *Kribbella lupini* LU14^T (Trujillo *et al.*, 2006); 6, *Kribbella sandramycini* KACC 20249^T (Park *et al.*, 1999); 7, *Kribbella solani* DSA1^T (Song *et al.*, 2004). Results that differ from those given in these cited references are given in parentheses. Data for antibiotic susceptibility, growth in the presence of NaCl and growth on ATCC medium 172 were obtained in this study for all strains unless indicated. Symbols: ++, good growth; +, growth; +W, weak growth; –, no growth; ND, not determined; NG, no growth on this medium. All strains including the novel strains are positive for growth at 30 °C and show no growth in the presence of ampicillin, sodium salt (100 µg ml⁻¹).

Test	HMC25 ^T	Q41 ^T	1	2	3	4	5	6	7
Utilization as sole carbon source									
Fructose	+	+	+	– (+) ^{a*}	ND	+	ND	+	ND
Inulin	–	–	ND	+	+	+	ND	+	–
Lactose	+W	+	+	– (+) ^{ab}	+	+	ND	+ (–) ^{ab}	+
Mannose	+W	+	+	– ^{cd} (+) ^{ab}	–	+	+	+ ^{cd} (–) ^{ab}	+
Melibiose	+	–	ND	+	+	+	+	+	+
Raffinose	+W	+	+	+	+	+	+	+	+
Rhamnose	+W	+	+	– ^{cd} (+) ^a	+	+	+	+	+
Xylose	+W	+	+	–	+	+	ND	+	+
Nitrate reduction	+	+	–	+ ^b	–	–	–	– ^{ab}	–
Degradation of:									
Casein	+	+	+ ^d	+	– (+W) ^d	+	+	+	–
Hypoxanthine	+	+	+	–	+	+	ND	–	+
Tween 80	+	+W	ND	+	–	– ^e	ND	+	+
L-Tyrosine	+	+	ND	ND	–	+	ND	ND	–
Urea	NG	+	ND	+	+	+	–	+	–
Hydrolysis of starch	+	+	+	–	–	+ ^{df} (–) ^{ab}	–	+W (–) ^{ab}	–
Growth at:									
20 °C	+	+	ND	+	ND	+	+	+	ND
37 °C	+	+	– ^d	+ (–) ^d	+	+ (–) ^d	+	+ (–) ^d	–
45 °C	+	–	ND	–	–	–	–	–	–
pH 5	+W	+	– ^d	+ (–) ^d	–	–	–	+	–
pH 7	+	+	ND	+	+	+	+	+	+
pH 9	+	+	ND	+	–	+	+	+	–
Antibiotic resistance (µg ml ⁻¹)									
Carbenicillin, disodium salt (100)	++	++	++	+	++	+	ND	+	++
Cefotaxime (10)	–	–	–	–	+	–	ND	–	–
Cephaloridine (10)	+	–	+	++	–	+	ND	–	–
Chloramphenicol (10)	++	+	++	++	++	++	ND	++	++
Chloramphenicol (20)	+	+	+W	++	++	+W	ND	++	++
Chloramphenicol (50)	–	–	–	++	+	–	ND	+	+
Cycloheximide (10)	++	++	++	++	+	++	ND	++	++
Erythromycin (10)	+	+	–	+	+	+	ND	+	+
Gentamicin sulphate (10)	++	–	–	+W	–	–	ND	–	–
Neomycin sulphate (10)	+W	–	–	–	+W	+W	ND	+	++
Streptomycin sulphate (10)	+	+	+	++	+	++	ND	++	++
Tobramycin sulphate (10)	–	–	–	–	–	–	ND	–	+W
Vancomycin hydrochloride (10)	–	–	+	–	++	++	ND	++	++
Growth in the presence of NaCl at:									
2 %	+	++	–	++	+	++	ND	++	++
3 %	+	+W	–	– (+) ^d	–	–	+ ^d	+	–
4 %	+W	+W	–	–	–	–	+ ^d	+W	+
5 %	–	–	–	–	–	–	+ ^d	–	+
Growth on ATCC medium 172:									
Aerobic	++	++	+	++	++	++	ND	++	++
Anaerobic	–	–	–	+W	+W	+W	ND	+W	+

*Data taken from the following sources: a, Li *et al.* (2004); b, Li *et al.* (2006); c, Park *et al.* (1999); d, Trujillo *et al.* (2006); e, Song *et al.* (2004); f, Lee *et al.* (2000) and Sohn *et al.* (2003).

Fig. 1. Unrooted 16S rRNA gene sequence phylogenetic tree showing the relationships between strains HMC25^T and Q41^T and other related actinomycete taxa. Analysis was conducted over a length of 1412 bp and the tree was constructed using the neighbour-joining method, with bootstrap percentages based on 1000 resampled datasets. *Streptosporangium roseum* DSM 43021^T was set as the outgroup.

Kribbella solani DSA1^T grew moderately well under anaerobic conditions, but not as well as on the control plate incubated under aerobic conditions. None of the kribbellae grew on ISP 9 under anaerobic conditions.

Under a scanning electron microscope, the aerial mycelium of Q41^T appeared as a fine mesh of intertwined mycelia, which formed buds at the tips (see left-hand image of Supplementary Fig. S1 in IJSEM Online). The mycelia on the agar surface fragmented into rod-shaped elements (Supplementary Fig. S1; right-hand image). HMC25^T also appeared as fine aerial mycelium, but budding was not present (Supplementary Fig. S2). The aerial mycelium of both isolates fragmented into rod-shaped elements.

Neither HMC25^T nor Q41^T showed antibiotic activity against any of the test bacteria.

Description of *Kribbella swartbergensis* sp. nov.

Kribbella swartbergensis (swart'berg.en'sis. N.L. fem. adj. *swartbergensis* pertaining to the Groot Swartberg mountain range, South Africa).

Gram-positive. Aerobic. Aerial mycelium is white and the vegetative hyphae are cream-coloured on inorganic salts/starch agar (ISP 4). Hyphae fragment into rod-shaped elements in broth and on agar. No diffusible pigments are produced on glycerol-asparagine agar (ISP 5). Melanin pigment is not produced on peptone-yeast extract-iron agar (ISP 6) or tyrosine agar (ISP 7). Nitrate is reduced. Catalase-positive. Produces H₂S. Utilizes D(−)-fructose, D(+)–glucose, maltose, D(+)–melibiose, D(−)-sorbitol and sucrose as sole carbon sources, with weak growth on L(+)–arabinose, glycerol, *myo*-inositol, lactose, D-mannitol,

D(+)–mannose, raffinose, L(+)–rhamnose, sodium acetate, sodium citrate, sodium lactate, sodium succinate and D(+)–xylose after 21 days. Grows at 20–45 °C. Grows at pH 7 and 9. Aesculin, arbutin and starch are hydrolysed. Degrades adenine, casein, gelatin, hypoxanthine, Tween 80 and L-tyrosine. Grows well on ISP 2 medium in the presence of 3 % NaCl, with weak growth at 4 % NaCl. Grows in the presence of carbenicillin disodium salt (100 µg ml^{−1}), cephaloridine (10 µg ml^{−1}), chloramphenicol (20 µg ml^{−1}), cycloheximide (10 µg ml^{−1}), erythromycin (10 µg ml^{−1}), gentamicin sulphate (10 µg ml^{−1}), neomycin sulphate (10 µg ml^{−1}) and streptomycin sulphate (10 µg ml^{−1}). The cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine (chemotype I). The whole-cell sugars are ribose and mannose.

The type strain, HMC25^T (=NRRL B-24426^T=DSM 17345^T), was isolated from soil from the banks of the River Gamka, Die Hel, Groot Swartberg mountain range, Western Cape province, South Africa.

Description of *Kribbella karoensis* sp. nov.

Kribbella karoensis (ka.roo.nen'sis. N.L. fem. adj. *karoensis* pertaining to the Karoo Desert National Botanical Garden in Worcester, South Africa).

Gram-positive. Aerobic. Aerial mycelium is pale cream/white and the vegetative hyphae are cream/yellow on inorganic salts/starch agar (ISP 4). Hyphae fragment into rod-shaped elements in broth and on agar. No diffusible pigments are produced on glycerol-asparagine agar (ISP 5). Melanin pigment is not produced on peptone/yeast extract/iron agar (ISP 6) or tyrosine agar (ISP 7). Nitrate is reduced. Catalase-positive. Produces H₂S. Utilizes D(−)-fructose,

D(+) -glucose, glycerol, *myo*-inositol, lactose, D-mannitol, D(+) -mannose, raffinose, L(+) -rhamnose, sodium succinate, sucrose and D(+) -xylose as sole carbon sources, with doubtful growth on adonitol, L(+) -arabinose, maltose, sodium acetate, sodium benzoate, sodium citrate and sodium lactate. Grows at 20–37 °C. Grows at pH 5, 7 and 9. Aesculin, arbutin and starch are hydrolysed. Degrades adenine, casein, gelatin, hypoxanthine and L-tyrosine. Produces weak degradation of Tween 80 after 14 days, but the type strain grows poorly on this test medium. Grows well on ISP 2 medium in the presence of 2 % NaCl, with weak growth in the presence of 3 and 4 % NaCl. Grows in the presence of carbenicillin disodium salt (100 µg ml⁻¹), chloramphenicol (20 µg ml⁻¹), cycloheximide (10 µg ml⁻¹), erythromycin (10 µg ml⁻¹) and streptomycin sulphate (10 µg ml⁻¹). Cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine (chemotype I). The whole-cell sugars are ribose and mannose.

The type strain Q41^T (=NRRL B-24425^T=DSM 17344^T), was isolated from soil collected from the base of a giant quiver tree, *Aloe pillansii*, growing in the Karoo Desert National Botanical Garden, Worcester, Western Cape province, South Africa.

Acknowledgements

The authors wish to thank Di James for DNA sequencing, Miranda Waldron of the Electron Microscope Unit, University of Cape Town (UCT), for assistance with scanning electron microscopy, Vanessa Davids for providing the clinical bacteria for antibiotic testing and Professor Dr Hans G. Trüper for his assistance in deriving the specific names for the isolates. We thank Professor Lafras Steyn of the Institute of Infectious Diseases and Molecular Medicine at University of Cape Town for the use of the BL3 Laboratory and Associate Professors Shez Reid and Val Abratt for use of the anaerobic chamber. B. M. K. holds a local post-graduate scholarship from the Medical Research Council of South Africa (MRC). M. L. R. holds a Scarce Skills Scholarship from the National Research Foundation (NRF) and a UCT Postgraduate Research Associateship. This work was supported by research grants to P. R. M. from the MRC, the NRF (grant no. 2054264) and the University Research Committee (UCT).

References

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Cook, A. E. & Meyers, P. R. (2003). Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S rRNA gene restriction fragment patterns. *Int J Syst Evol Microbiol* **53**, 1907–1915.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Keswani, J. & Whitman, W. B. (2001). Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. *Int J Syst Evol Microbiol* **51**, 667–678.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. Arizona State University, Tempe, USA. <http://www.megasoftware.net>
- Lee, S. D., Kang, S.-O. & Hah, Y. C. (2000). *Hongia* gen. nov., a new genus of the order Actinomycetales. *Int J Syst Evol Microbiol* **50**, 191–199.
- 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.
- Matson, J. A. & Bush, J. A. (1989). Sandramycin, a novel antitumor antibiotic produced by a *Nocardioides* sp. *J Antibiot* **42**, 1763–1767.
- Nonomura, H. & Ohara, Y. (1971). Distribution of actinomycetes in soil. VIII. Green spore group of *Microtetraspora*, its preferential isolation and taxonomic characteristics. *J Ferment Technol* **49**, 1–7.
- 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.
- Sohn, K., Hong, S. G., Bae, K. S. & Chun, J. (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, B. Y., Hong, S. B., Cho, H. S., Sohn, K., Chun, J. & Suh, J. W. (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.
- 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.
- Tormo, J. R., García, J. B., DeAntonio, M., Feliz, J., Mira, A., Díez, M. T., Hernández, P. & Peláez, F. (2003). A method for the selection of production media for actinomycete strains based on their metabolite HPLC profiles. *J Ind Microbiol Biotechnol* **30**, 582–588.
- 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.
- Williams, S. T., Goodfellow, M. & Alderson, G. (1989). Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2452–2492. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.