Kribbella hippodromi sp. nov., isolated from soil from a racecourse in South Africa

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A novel actinomycete, designated strain S1.4^T, was isolated from a soil sample collected from Kenilworth Racecourse in the Western Cape, South Africa. The strain was able to grow in the presence of 5% NaCl. It contained LL-diaminopimelic acid and glycine in the cell-wall peptidoglycan with glucose present in the whole-cell sugar profile. Strain S1.4^T was shown to be a member of either the genus *Kribbella* or the genus *Nocardioides* based on a rapid molecular identification method by using single-enzyme restriction endonuclease digestion of the PCR-amplified 16S rRNA gene with *MboI*, *VspI*, *SphI*, *SnaBI*, *SalI* and *AgeI*. Analysis of the 16S rRNA gene sequence indicated that strain S1.4^T belonged to the genus *Kribbella*. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain S1.4^T was related most closely to *Kribbella solani* DSA1^T. Strain S1.4^T was phenotypically distinct from *K. solani* DSA1^T and was shown to be a separate genomic species based on DNA–DNA hybridization experiments (40.4 ± 3.8% DNA–DNA relatedness between the two). Strain S1.4^T (=DSM 19227^T =NRRL B-24553^T) is thus presented as the type strain of a novel species, for which the name *Kribbella hippodromi* sp. nov. is proposed.

The genus *Kribbella* was proposed by Park *et al.* (1999) and contains nocardioform actinomycetes with LL-diaminopimelic acid in the cell wall. With the transfer of *Hongia koreensis* to the genus (Sohn *et al.*, 2003) and more recently the descriptions of *Kribbella karoensis* and *Kribbella swartbergensis* (Kirby *et al.*, 2006) and *Kribbella aluminosa* (Carlssohn *et al.*, 2007) there are, at the time of writing, 12 recognized *Kribbella* species (Euzéby, 2007). Here we describe the characterization of a novel member of the genus, isolated from a soil sample collected from a racecourse in the Western Cape, South Africa.

Strain S1.4^T was isolated from soil collected in the fynbos-rich area surrounded by the horse racing track at Kenilworth Racecourse, Cape Town, South Africa. The soil was ground in a pestle and mortar before being heated at 60 °C for 1 h, after which 0.1 g was added to 1 ml of sterile distilled water and agitated by vortexing for 1 min. The sample was serially diluted in sterile distilled water and spread-plated on SM1 agar (Tan *et al.*, 2006) containing cycloheximide (50 µg ml⁻¹) and nalidixic acid (10 µg ml⁻¹). Strain S1.4^T was isolated after incubation at 30 °C for 21 days and was subsequently maintained on yeast extract-malt extract agar (ISP 2 medium) (Shirling & Gottlieb, 1966).

Genomic DNA was extracted as described by Wang *et al.* (1996), with modifications to the lysis buffer to increase the

lysozyme concentration to 20 mg ml⁻¹ and to include proteinase K (0.2 mg ml⁻¹); digestion was performed overnight. Treatment with RNase A was performed overnight. To allow rapid identification of the isolate to the genus level, 16S rRNA gene sequence amplification and restriction endonuclease digestion were performed as described by Cook & Meyers (2003), by using single digestions with the restriction enzymes *MboI* (*Sau3AI* isoschizomer), *VspI* (*AsnI* isoschizomer), *SphI*, *SnaBI*, *SalI* and *AgeI*. Approximately 500 ng template DNA was used in the PCR amplification. The 16S rRNA gene PCR product was purified by using a Cleanmix kit (TA050CLN; Talent) and then sequenced. Sequence analysis was performed with DNAMAN version 4.13 (Lynnon BioSoft) and phylogenetic analysis was conducted by using MEGA version 3.1 (Kumar *et al.*, 2004).

All International *Streptomyces* Project (ISP) media were prepared according to Shirling & Gottlieb (1966). Morphological properties were determined on ISP 2 and inorganic salts-starch agar (ISP 4). Physiological tests were carried out as described by Williams *et al.* (1989). All plates were incubated at 30 °C, for the recommended periods, unless otherwise stated. Carbon source utilization was tested according to the methods of Shirling & Gottlieb (1966), with all carbon sources being filter-sterilized and tested at a concentration of 1% (w/v), with the exception of sodium acetate, sodium succinate and sodium citrate, which were tested at 0.1% (w/v). Nitrogen source utilization was assessed according to Williams *et al.* (1989). All nitrogen sources were filter-sterilized and tested

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S1.4^T is EF472955.

at a concentration of 0.1% (w/v). Salt tolerance was determined on ISP 2 medium incubated for 14 days. Antibiotic resistance was tested on Bennett's medium (Atlas, 2004) containing the indicated concentration of antibiotic after incubation for 7 days. Growth at different pH values (pH 4.3, 5, 7 and 9) and at different temperatures (20, 30, 37 and 45 °C) was determined on Bennett's medium incubated for 14 days.

The ability to grow under anaerobic conditions was determined on ATCC medium 172 and ISP 9 with glucose as the sole carbon source by incubating the plates at 37 °C for 21 days in an anaerobic chamber (model 1024; Forma Scientific) containing an atmosphere of H₂/CO₂/N₂ (5:10:85). Before incubation, plates were opened inside the chamber for 5 s to remove all residual oxygen and then sealed in a plastic bag.

Chemotaxonomic analysis was conducted as described by Hasegawa *et al.* (1983) by using freeze-dried cells of a culture grown in ISP 2 medium, with moderate shaking, for 3 days at 30 °C. For analysis of whole-cell sugars, the solvent system used was ethyl acetate/pyridine/distilled water (100:35:25, v/v).

Antimicrobial testing was performed against *Mycobacterium aurum* A+ in standard agar overlays with 9-day-old stab-inoculated cultures of strain S1.4^T on ISP 2, Difco Middlebrook 7H9 agar (Becton Dickinson) containing 10 mM glucose (albumin–dextrose–catalase supplement omitted), MC agar (Nonomura & Ohara, 1971) and MC agar containing glycerol as the carbon source. Luria sloppy agar (Sambrook *et al.*, 1989) was used for the overlays.

DNA–DNA hybridization experiments were performed as a service by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) by using the spectrophotometric method of De Ley *et al.* (1970) and incorporating the modifications of Huß *et al.* (1983).

Analysis of the 16S rRNA gene restriction fragment patterns of strain S1.4^T indicated that it belonged to either the genus *Kribbella* or the genus *Nocardioides* (Cook & Meyers, 2003). A nucleotide–nucleotide BLAST search (Altschul *et al.*, 1997) based on 1389 bp of the 16S rRNA gene sequence indicated that strain S1.4^T belonged to the genus *Kribbella* and was most closely related to *Kribbella solani* DSA1^T (99.57% similar over 1389 bp, by local alignment). This association was confirmed by constructing a neighbour-joining phylogenetic tree (Saitou & Nei, 1987) in which strain S1.4^T grouped with *K. solani* DSA1^T (Fig. 1). Minimum-evolution and maximum-parsimony (Takahashi & Nei, 2000) trees were also constructed, which further supported these results (see Fig. 1).

DNA–DNA hybridization experiments between *K. solani* DSA1^T and strain S1.4^T indicated that these strains shared only 40.4 ± 3.8% DNA–DNA relatedness and thus represent two separate genomic species (based on the threshold value of 70% used to delineate bacterial species; Wayne *et al.*, 1987). Results from the phenotypic characterization of strain S1.4^T are given in Table 1 and in the species description.

Description of *Kribbella hippodromi* sp. nov.

Kribbella hippodromi (hip.po.dro'mi. Gr. masc. n. *hippodromos* horse racecourse, N.L. gen. masc. n. *hippodromi* of/ from a horse racecourse, referring to the source of isolation of the type strain, Kenilworth Racecourse, Cape Town, Western Cape, South Africa).

Cells are Gram-positive and aerobic (unable to grow on ATCC medium 172 or ISP 9 with glucose under anaerobic conditions). Colonies appear convoluted with irregular edges on most media. Vegetative mycelium appears cream in colour with highly branched hyphae, which fragment in both liquid and agar cultures. Aerial mycelium appears white on ISP 4. No diffusible pigments are produced.

Fig. 1. Unrooted phylogenetic tree showing the position of strain S1.4^T within the genus *Kribbella* and its relationship to other actinomycetes. The tree was constructed by using the neighbour-joining method based on 1369 bp of the 16S rRNA gene sequence. Values at nodes are bootstrap percentages based on 1000 replications (only values above 40% are shown), with asterisks indicating clades that were conserved in the neighbour-joining, minimum-evolution and maximum-parsimony trees. Accession numbers are indicated in parentheses. *Actinopolymorpha singaporensis* IM7744^T, *Aeromicrobium erythreum* NRRL B-3381^T, *Nocardioides albus* KCTC 9186^T and *Streptosporangium roseum* DSM 43021^T were used as outgroups. Bar, 1 nucleotide substitution per 100 nucleotides.

Table 1. Differential phenotypic characteristics between strain S1.4^T and closely related *Kribbella* type strains

Strain: 1, S1.4^T; 2, *K. solani* DSA1^T; 3, *K. swartbergensis* HMC25^T (Kirby *et al.*, 2006); 4, *K. jejuensis* HD9^T (Song *et al.*, 2004); 5, *K. aluminosa* HKI 0478^T (Carlsohn *et al.*, 2007). Conflicting data are indicated in parentheses. ++, Strongly positive; +, positive; +w, weakly positive; –, negative; ND, no data available; NG, no growth.

Test	1	2	3	4	5
Nitrate reduction	+	+	+	–	–
Degradation of:					
Adenine	+	+	+	–	+
Casein	+	+	+	–	+
Hypoxanthine	+	+	+	– (+*)	+
Tween 80	+	+	+	–	+
Tyrosine	+	+	+	–	+
Urea	–	–	NG	+	+
Xanthine	–	–	ND	–	+
Hydrolysis of starch	+w	+w	+	–	+
Growth at/with:					
37 °C	+	–	+	+	+
45 °C	–	–	+	–	–
pH 4.3	+w	–	ND	–	ND
pH 9	+	+	+	–	ND
4 % NaCl	+	+	+w	–	–
5 % NaCl	+	+w	–	–	–
6 % NaCl	+w	–	–	–	–
Utilization as sole carbon source of:					
Adonitol	+	+	ND	–	ND
myo-Inositol	++	+	+w	–	+
Inulin	++	–	–	+	ND
α-Lactose	–	++	+w	+	ND
D(–)-Mannitol	+	+	+w	–	+
D(+)-Mannose	+	+	+w	–	ND
Salicin	+	+	ND	–	ND
D(+)-Xylose	–	++	+w	+	+
Utilization as sole nitrogen source of:					
DL-α-Amino- <i>n</i> -butyric acid	+w	+	ND	+	ND
4-Hydroxy-L-proline	+w	+	ND	+	ND
L-Methionine	–	+	ND	ND	ND
L-Phenylalanine	–	+	ND	ND	ND
L-Valine	+w	++	ND	ND	ND
Growth on ATCC medium 172:					
Under aerobic conditions	+	+	++	++*	ND
Under anaerobic conditions	–	+w	–	+w*	ND

*Data taken from Kirby *et al.* (2006).

Melanin is not produced on peptone-yeast extract-iron agar (ISP 6) or on tyrosine agar (ISP 7). Nitrate is reduced to nitrite. Produces H₂S. Utilizes adonitol, L(+)-arabinose, cellobiose, D(–)-fructose, D(+)-galactose, D(+)-glucose, glycerol, myo-inositol, inulin, maltose, D(–)-mannitol, D(+)-mannose, melezitose, melibiose, raffinose, L(+)-rhamnose, salicin, sucrose and trehalose as sole carbon sources, with weak growth on sodium acetate, sodium citrate and sodium succinate and doubtful growth on α-

lactose and D(+)-xylose. Unable to utilize xylitol as a sole carbon source. Utilizes L-arginine, L-asparagine, L-histidine, potassium nitrate and L-threonine as sole nitrogen sources, with weak growth on DL-α-amino-*n*-butyric acid, L-cysteine, L-4-hydroxyproline, L-serine and L-valine and doubtful growth on L-methionine and L-phenylalanine. Grows at 20, 30 and 37 °C, but not at 45 °C. Grows at pH 5, 7 and 9 (weakly at pH 4.3). Aesculin and arbutin are hydrolysed, starch is weakly hydrolysed but pectin is not hydrolysed. Adenine, casein, gelatin, hypoxanthine, Tween 80 and L-tyrosine are degraded. Allantoin, cellulose, guanine, urea, xanthine and xylan are not degraded. Grows in the presence of 5 % NaCl, with very weak growth at 6 % NaCl and no growth at 7 % NaCl. Resistant to lincomycin hydrochloride (100 µg ml^{–1}), neomycin sulfate (50 µg ml^{–1}), oleandomycin phosphate (100 µg ml^{–1}), penicillin G (10 IU ml^{–1}), rifampicin (50 µg ml^{–1}) and streptomycin sulfate (100 µg ml^{–1}), but sensitive to cephaloridine (100 µg ml^{–1}), tobramycin sulfate (50 µg ml^{–1}) and vancomycin hydrochloride (50 µg ml^{–1}). No antibacterial activity is detected against *M. aurum* A+. The cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine (chemotype I of Lechevalier & Lechevalier, 1970). Glucose and an unidentified sugar are present in the whole-cell sugar hydrolysate.

The type strain, S1.4^T (=DSM 19227^T =NRRL B-24553^T), was isolated from soil from Kenilworth Racecourse, Cape Town, South Africa.

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