Kribbella solani sp. nov. and *Kribbella jejuensis* sp. nov., isolated from potato tuber and soil in Jeju, Korea

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Two actinomycete strains, DSA1^T and HD9^T, were isolated from a potato tuber and soil from a potato-cultivating field in Jeju, Korea, respectively. A comprehensive 16S rDNA analysis revealed that the isolates belong to the genus *Kribbella* and share 97.7–98.6% sequence similarity to *Kribbella* species. The strains also contained typical chemotaxonomic markers of the genus *Kribbella*: LL-diaminopimelic acid, alanine, glycine and glutamic acid in the cell wall peptidoglycan; mannose, glucose, galactose and ribose as whole cell sugars; diphosphatidylglycerol, phosphatidylcholine and phosphatidylinositol as characteristic phospholipids; and the major menaquinone MK-9 (H₄). DNA–DNA hybridization experiments showed that the isolates represented two distinct genomic species. A number of phenotypic properties can be used to differentiate the two isolates from *Kribbella* species. On the basis of polyphasic evidence, two novel species are proposed: *Kribbella solani* sp. nov. for strain DSA1^T (=KACC 20196^T=JCM 12205^T) and *Kribbella jejuensis* sp. nov. for strain HD9^T (=KACC 20266^T=JCM 12204^T).

The genus *Kribbella* established by Park *et al.* (1999) contains LL-diaminopimelic acid (LL-DAP) in its cell wall peptidoglycan and lies within the family *Nocardioideae* (Nesterenko *et al.*, 1985, 1990). Two species, *Kribbella flava* and *Kribbella sandramycini*, included in this genus were originally assigned to the genus *Nocardioideae* (*Nocardioideae fulvus* IFO 14399 and *Nocardioideae* sp. ATCC 39419, respectively) based on 16S rDNA sequence analysis. Recently, *Hongia koreensis* (Lee *et al.*, 2000) was transferred to this genus as *Kribbella koreensis* on the basis of its molecular systematics (Sohn *et al.*, 2003). Here, we present polyphasic characterization of two novel *Kribbella* strains isolated during a taxonomic survey of the potato scab caused by *Streptomyces* spp. from Jeju, Korea (Song *et al.*, 2004).

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Abbreviation: LL-DAP, LL-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains KACC 20196^T, KACC 20248^T, KACC 20249^T, KACC 20250^T and KACC 20266^T are AY253862–AY253866, respectively.

An extended neighbour-joining tree and a maximum-parsimony tree for the isolates and related actinomycete taxa are available as supplementary data in IJSEM Online.

Strain DSA1^T was isolated from a potato tuber with scab lesions. For isolation, the tuber was washed twice with distilled water and scraped lesions were suspended in distilled water using a vortex mixer. The suspension was plated on GYM agar (DSMZ medium no. 65; <http://www.dsmz.de/media/media.htm>) using the dilution method and cultured at 30 °C for 5 days. Strain HD9^T was isolated from soil of a potato-cultivating field using the same growth conditions and the dilution plating method.

Growth and morphological properties of the isolates were recorded from GYM agar, ISP media (Shirling & Gottlieb, 1966) and trypticase soy agar (TSA; BBL 11768). Melanin formation was examined on ISP media 6 and 7. For scanning electron microscopy, cells grown at 30 °C for 5 days on GYM agar were prepared by cutting agar blocks fixed with 1% osmium tetroxide and observed under a scanning electron microscope (Hitachi S-2460N). Both strains grew on GYM agar, TSA, nutrient agar and all ISP media at 28 °C. The two strains did not produce melanin pigment. Their colonies were sand-pasty and had lichenous shapes with irregular edges on GYM agar. Aerial mycelium of the isolates was white and consisted of hyphae that fragmented into rod-shaped elements.

Physiological characteristics were examined using standard

procedures (Lee *et al.*, 2000; Williams *et al.*, 1983) at 30 °C for up to 5 days. In addition, catalase activity, nitrate reduction, urease activity, hydrogen sulfide production and hydrolysis of arbutin, casein, aesculin, gelatin and starch were tested as described by MacFaddin (2000). Antimicrobial activity was examined against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Streptomyces scabiei*, *Candida albicans*, *Aspergillus niger*, *Trichoderma harzianum*, *Fusarium acuminatum* and *Colletotrichum gloeosporioides*. Physiological characteristics are given in the species descriptions and in Table 1.

Genomic DNA extraction, PCR amplification of 16S rDNA and cloning were done as described previously (Song *et al.*, 2004). 16S rDNA sequences were analysed using an Applied Biosystems DNA sequencer (ABI3100) and aligned using CLUSTAL W software (Thompson *et al.*, 1994). Nucleotide similarity values were calculated from the alignment. An evolutionary distance matrix was constructed using the algorithm of Jukes & Cantor (1969) and phylogenetic trees for the datasets were inferred from the neighbour-joining method of Saitou & Nei (1987) using MEGA version 2.1 (Kumar *et al.*, 2001). The stability of relationships was evaluated by

Table 1. Differential physiological characteristics of isolates DSA1^T and HD9^T and type strains of *Kribbella* species

Strains: 1, DSA1^T; 2, HD9^T; 3, *K. flavida* KACC 20248^T (data from Park *et al.*, 1999 unless indicated); 4, *K. sandramycini* KACC 20249^T (Park *et al.*, 1999); 5, *K. koreensis* KACC 20250^T (Lee *et al.*, 2000). +, Positive; –, negative; w, weakly positive; ND, not done.

Characteristic	1	2	3	4	5
Use of sole carbon sources:					
D-Xylose	+	+	–	+	+
D-Arabinose	+	+	–	w	+
D-Mannose	+	–	–	+	+
L-Rhamnose	+	+	–	+	+
α-Lactose	+	+	–	+	+
Galactose	–	+	–	+	+
Adonitol	–	–	+	+	+
Xylitol	–	–	–	+	–
myo-Inositol	–	–	+	+	ND
Mannitol	–	–	+	+	+
Salicin	+	–	ND	ND	+
Inulin	–	+	+	+	+
Hydrolysis of:					
Tween 80*	+	–	+	+	–
Elastin	–	–	+	+	+
Urea	–	+	+	+	+
Starch	–	–	–	w	+
Casein	–	w	+	+	+
Growth at:					
15 °C*	–	–	–	–	+
37 °C	–	+	+	+	+

*Data determined in this study.

performing bootstrap analysis of the neighbour-joining data based on 1000 resamplings.

Phylogenetic analysis based on 16S rDNA sequences indicated that our isolates belong to the genus *Kribbella* with 100 % bootstrap support (Fig. 1). Strain DSA1^T showed the highest similarity to *K. sandramycini* KACC 20249^T (98·6 %), followed by *K. flavida* KACC 20248^T (98·5 %), strain HD9^T (98·2 %) and *K. koreensis* KACC 20250^T (98·1 %). Strain HD9^T showed the closest similarity to strain DSA1^T (98·2 %), followed by *K. sandramycini* KACC 20249^T (98 %), *K. flavida* KACC 20248^T (97·9 %) and *K. koreensis* KACC 20250^T (97·7 %). An extended neighbour-joining tree and a maximum-parsimony tree using PAUP version 4.0b10 (Swofford, 2000) are available as supplementary data in IJSEM Online.

DNA–DNA relatedness between the isolates and type strains of the *Kribbella* species were determined using the slot-blot hybridization method of Chun *et al.* (1998). Our isolates showed relatively low DNA–DNA relatedness of 42·5 %. Isolate DSA1^T showed a low level of DNA–DNA relatedness with *K. sandramycini* (0 %), *K. koreensis* (16·4 %) and *K. flavida* (50·4 %). Similarly, isolate HD9^T showed a low level of DNA–DNA relatedness with *K. sandramycini* (0 %), *K. koreensis* (20·9 %) and *K. flavida* (47·8 %).

Biomass for most of the chemotaxonomic studies was prepared following growth of the isolates and standard strains in shake flasks of GYM for 5 days at 30 °C; after a check for purity, biomass was harvested by centrifugation, washed twice in distilled water and freeze-dried. Integrated lipid and wall analysis were carried out by previously described methods: wall amino acids (O'Donnell *et al.*, 1985), diaminopimelic acids (Staneck & Roberts, 1974), sugars (Saddler *et al.*, 1991), menaquinone (O'Donnell *et al.*, 1985) and polar lipids (Minnikin *et al.*, 1984). Whole-cell fatty acid profile was determined on TSA (BBL 11768) using the MIDI (Microbial Identification) system. Both strains had LL-DAP, alanine, glycine and glutamic acid in the cell wall peptidoglycan, contained mannose, glucose, galactose and ribose as whole cell sugars and MK-9 (H₄) as the major menaquinone, as for other *Kribbella* species. Polar lipid profiles of

Fig. 1. Phylogenetic tree showing the relationship of isolates DSA1^T and HD9^T and related actinomycete taxa. Numbers at branching points indicate percentages of occurrence based on neighbour-joining analyses of 1000 resampled dataset bootstrapped trees. Bar, one nucleotide substitution per 100 nucleotides.

the two strains revealed diphosphatidylglycerol, phosphatidylcholine and phosphatidylinositol. Whole-cell fatty acid profiles of our isolates and other *Kribbella* species are given in Table 2. The G + C content of the DNA was determined using the thermal denaturation method of Marmur & Doty (1962): values were 69.2 and 68 ± 1 mol%, for strains DSA1^T and HD9^T, respectively.

From genotypic and phenotypic characterization, it is evident that our isolates not only belong to the genus *Kribbella* but also represent novel species. Along with molecular data, a number of phenotypic characters can be used to differentiate the isolates from recognized *Kribbella* species and

from each other (Tables 1 and 2). We therefore propose two novel species, *Kribbella solani* sp. nov. for strain DSA1^T and *Kribbella jejuensis* sp. nov. for strain HD9^T.

Description of *Kribbella solani* sp. nov.

Kribbella solani (so.la'ni. N.L. gen. n. *solani* of *Solanum*, the genus of the potato, *Solanum tuberosum*, from which the type strain was isolated).

Aerial mycelium is white and consists of hyphae that fragment into rod-shaped elements. Vegetative mycelium is creamy and shows hyphae that are broadly branched. Colonies are sand-pasty and have lichenous shapes with irregular edges. No diffusible pigment is formed. Non-motile. Nitrate is not reduced to nitrite. Catalase-positive. Urease-negative. Utilizes glucose, sucrose, D-xylose, D-arabinose, D-melezitose, D-mannose, L-rhamnose, α -lactose, trehalose, melibiose, cellobiose, raffinose and salicin as carbon sources. Utilizes L-histidine, DL-arginine, DL- α -amino-*n*-butyric acid, *trans*-4-hydroxy-L-proline and potassium nitrate as nitrogen sources. Grows at 28 – 30 °C but not at 15 or 37 °C. Grows at pH 7 but not at pH 4 or 10. DNA, Tween 80, aesculin, arbutin and tributyrin are hydrolysed. Xanthine, hypoxanthine, xylan, elastin, adenine, guanine, tyrosine, allantoin, casein, chitin and starch are not hydrolysed. Tolerant to 0.01 % lysozyme, 2 % NaCl, 0.1 % phenyl ethanol and 0.001 % potassium tellurite but not to 0.01 % sodium azide, 0.01 % thallos acetate and 0.1 % phenol. Resistant to 4 μ g tetracycline, 10 μ g gentamicin, 80 μ g neomycin, 4 μ g rifamycin, 20 μ g streptomycin, 4 μ g vancomycin and 4 μ g tobramycin ml⁻¹, but susceptible to 10 μ g treoleandomycin ml⁻¹. Weakly resistant to 10 U penicillin G ml⁻¹. Antimicrobial activity is not observed. Cell wall peptidoglycan contains LL-DAP, alanine, glycine and glutamic acid. Mannose, glucose, galactose and ribose are present as whole cell sugars. The predominant fatty acids are 12-methyltridecanoic acid, 12-methyltetradecanoic acid and 14-methylpentadecanoic acid. The genomic DNA G + C content is about 69 mol%.

The type strain, DSA1^T (=KACC 20196^T=JCM 12205^T), was isolated from a potato tuber with scab lesions from Jeju, Korea. The species description is based on the type strain only.

Description of *Kribbella jejuensis* sp. nov.

Kribbella jejuensis (je.ju.en'sis. N.L. fem. adj. *jejuensis* referring to Jeju, Korea).

Aerial mycelium is white and consists of hyphae that fragment into rod-shaped elements. Vegetative mycelium is creamy and shows hyphae that are broadly branched. Colonies are sand-pasty and have lichenous shapes with irregular edges. No diffusible pigment is formed. Non-motile. Nitrate is not reduced to nitrite. Catalase- and urease-positive. Utilizes glucose, sucrose, D-xylose, D-arabinose, D-melezitose, galactose, L-rhamnose, α -lactose, trehalose, melibiose, cellobiose, raffinose and inulin as carbon

Table 2. Cellular fatty acid profiles of isolates DSA1^T and HD9^T and type strains of *Kribbella* species on TSA medium

Strains: 1, DSA1^T; 2, HD9^T; 3, *K. flavida* KACC 20248^T; 4, *K. san-dramycini* KACC 20249^T; 5, *K. koreensis* KACC 20250^T. Values of less than 1 % are not shown. TBSA, Tuberculostearic acid.

Fatty acid	1	2	3	4	5
Saturated fatty acids:					
C _{15:0}				1.4	
C _{16:0}				4.23	
C _{17:0}			1.61	1.13	
Unsaturated fatty acids:					
C _{15:1} B*	3.98			2.31	
C _{16:1} ω 7c			2.21	2.83	2.87
C _{17:1} ω 8c	3.98		10.57	3.85	2.98
C _{18:1} ω 9c				4.99	
Branched fatty acids:					
C _{14:0} iso	12.58	7.14	5.72	6.59	2.87
C _{15:0} iso	5.81	10.84	5.06	12.5	20.09
C _{15:0} anteiso	21.59	31.22	28.7	32.23	36.4
C _{15:1} anteiso A*	1.94				
C _{16:0} iso	19.47	16.17	21.27	5.63	8.44
C _{16:0} 9-methyl	3.27	9.3	5.71	6.97	12.65
C _{17:0} iso		3.31	1.75	1.55	4.53
C _{17:0} anteiso		1.81	6.43	1.07	3.07
C _{17:0} 10-methyl			2.94		
C _{18:0} 10-methyl (TBSA)				4	
Hydroxy fatty acids:					
C _{16:0} 2-OH				2.74	
Mixed functional groups:					
C _{16:1} iso H*	6.2	2.68	5.33	2.39	2.13
C _{15:0} anteiso 2-OH	2.93	4.53	2.69		2.44
C _{16:0} iso 2-OH	4.2	6.53			
C _{16:1} 2-OH		1.25			
C _{17:0} anteiso 2-OH		1.03			
Summed feature 4†		4.22			

*The double bond position indicated by a capital letter is unknown.

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained one or more of C_{15:0} iso 2-OH/C_{16:1} ω 9t.

sources. Utilizes L-histidine, DL-arginine, DL-homoserine, DL- α -amino-*n*-butyric acid, *trans*-4-hydroxy-L-proline and potassium nitrate as nitrogen sources. Grows at 28–37 °C but not at 15 or 45 °C. Grows at pH 7 but not at pH 4 or 10. Gelatin, aesculin, arbutin and tributyrin are hydrolysed. Tween 80, xanthine, hypoxanthine, xylan, elastin, adenine, guanine, tyrosine, allantoin, casein, chitin and starch are not hydrolysed. Tolerant to 0.01 % lysozyme, 1 % NaCl, 0.1 % phenyl ethanol and 0.001 % potassium tellurite but not to 0.01 % sodium azide, 0.01 % thallos acetate or 0.1 % phenol. Resistant to 1 µg tetracycline, 0.4 µg gentamicin, 4 µg rifamycin, 1 µg streptomycin, 4 µg vancomycin and 1 µg tobramycin ml⁻¹, but susceptible to 4 µg neomycin, 20 µg streptomycin and 10 µg treoleandomycin ml⁻¹. Weakly resistant to 4 µg tobramycin, 4 µg streptomycin and 10 U penicillin G ml⁻¹. Weak antimicrobial activity is observed towards *Streptomyces scabiei*. Cell wall peptidoglycan contains LL-DAP, alanine, glycine and glutamic acid. Mannose, glucose, galactose and ribose are present as cell wall sugars. The predominant fatty acids are 13-methyltetradecanoic acid, 12-methyltetradecanoic acid and 14-methylpentadecanoic acid. The genomic DNA G+C content is about 68 mol%.

The type strain, HD9^T (=KACC 20266^T=JCM 12204^T), was isolated from soil from Jeju, Korea. The species description is based on the type strain only.

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