Transfer of *Hongia koreensis* Lee *et al.* 2000 to the genus *Kribbella* Park *et al.* 1999 as *Kribbella koreensis* comb. nov.

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The taxonomic status of *Hongia koreensis* was investigated by molecular systematic methods. On the basis of 16S rDNA phylogeny, *H. koreensis* was closely associated with *Kribbella flavida* and *Kribbella sandramycini* and formed a monophyletic clade with these species. These three taxa shared over 98% 16S rDNA sequence similarity and many chemotaxonomic properties, which strongly indicates that they belong to the same genus. DNA–DNA pairing was employed to elucidate the genomic relatedness among these taxa. *H. koreensis* represented a distinct genomic species that can be differentiated from members of the genus *Kribbella*. Physiological characteristics and phospholipid and cellular fatty acid compositions can be also used to separate *H. koreensis* from species of the genus *Kribbella*. On the basis of data presented in this and earlier studies, it is proposed that *H. koreensis* Lee *et al.* 2000 should be transferred to the genus *Kribbella* Park *et al.* 1999 as *Kribbella koreensis* comb. nov.

The genus *Hongia* was proposed to accommodate a nocardioform actinomycete strain isolated from gold-mine soil in Korea (Lee *et al.*, 2000). Similarly, Park *et al.* (1999) independently described the genus *Kribbella* to encompass strains previously known as '*Nocardioides fulvus*' IFO 14399 (now NBRC 14399) and *Nocardioides* sp. ATCC 39419. The proposals of these two novel genera were based on unique chemotaxonomic markers, i.e. tetrahydrogenated menaquinone with nine isoprene units, L-alanine at the first position of peptide subunits, phospholipid composition and phylogenetic analysis based on 16S rDNA. Although these genera were proposed separately, we have found that they have many common morphological and chemotaxonomic properties. Therefore, we re-examined the taxonomic status of these two genera.

16S rDNA sequences of *Hongia koreensis* (the only species in this genus) and two *Kribbella* species (namely, *Kribbella flavida* and *Kribbella sandramycini*) were determined in previous studies (Lee *et al.*, 2000; Park *et al.*, 1999). Corresponding 16S rRNA gene sequences of related actinomycetes were obtained from GenBank. Alignment, pairwise comparison and phylogenetic analysis were done using the PHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit/>) as described previously (Chun *et al.*,

2000). It is clear from phylogenetic trees based on the Jukes & Cantor (1969) model, using the neighbour-joining (Saitou & Nei, 1987; Fig. 1) and Fitch & Margoliash (1967; not shown) methods, that *H. koreensis*, *K. flavida* and *K. sandramycini* formed a significant monophyletic clade. This grouping was supported by a bootstrap value of 100%, based on the neighbour-joining algorithm. The position of *H. koreensis* was encompassed by the *Kribbella* species in the neighbour-joining tree, although *H. koreensis* was the sister group to the *Kribbella* sequences in the Fitch–Margoliash tree (data not shown). Pairwise 16S rDNA sequence similarity values of *H. koreensis* to *K. flavida* and *K. sandramycini* were 98.37 and 98.57%, respectively, whereas the corresponding value between the two *Kribbella* species was 99.12%. It is evident from 16S rDNA analysis that *H. koreensis* cannot be separated from the *Kribbella* species and belongs to the same genus. As the three species showed a high level of 16S rDNA sequence similarity, we employed a DNA–DNA pairing method (Chun *et al.*, 1998) to check whether *H. koreensis* belongs to either of the described *Kribbella* species. DNA relatedness values between *H. koreensis* IMSNU 50530^T and *Kribbella* strains were respectively 20 and 0% for *K. flavida* KCTC 9580^T and *K. sandramycini* KCTC 9609^T when DNA from *H. koreensis* was used as the probe. These values support the description of a novel species to accommodate *H. koreensis* in the genus *Kribbella*.

Fig. 1. Rooted neighbour-joining tree based on nearly complete 16S rDNA sequences, showing relationships between *Hongia koreensis* and related genera. Numbers at nodes indicate levels of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets. *Rubrobacter radiotolerans* DSM 46359^T (X87134) was used as the outgroup. Bar, 0.1 nucleotide substitution per nucleotide position.

H. koreensis shares many properties with species of the genus *Kribbella*. They contain aerial and vegetative hyphae which often fragment. Shared chemical properties include MK-9(H₄), L-alanine at position 1 of the peptide subunit, LL-diaminopimelic acids and DNA G + C contents (71 and 68–70 mol% for *H. koreensis* and *Kribbella*, respectively). However, several properties can be weighted to separate *H. koreensis* from *Kribbella* species, including phospholipid composition and utilization of several compounds as sole carbon and energy sources (Table 1). We determined the cellular fatty acid profiles of the test strains using the Sherlock system (Microbial ID), according to the manufacturer's instructions, and found that they can be readily differentiated from each other (Table 2).

From the polyphasic evidence given in this study, we

Table 1. Utilization of carbon compounds as sole carbon and energy sources by *Hongia koreensis* and *Kribbella* species

H. koreensis IMSNU 50530^T was positive, and *K. flavida* KCTC 9580^T was negative, for all reactions listed. +, Positive; –, negative; w, weakly positive reaction. Data from Park *et al.* (1999) and Lee *et al.* (2000).

Compound	<i>K. sandramycini</i> KCTC 9609 ^T
L-Arabinose	w
D-Fructose	+
D-Mannose	+
D-Xylose	+
L-Rhamnose	+
Lactose	+
Sodium acetate	–
Sodium citrate	–

Table 2. Cellular fatty acid composition (%) of *Hongia koreensis* and *Kribbella* species

Taxa: 1, *H. koreensis* IMSNU 50530^T; 2, *K. flavida* KCTC 9580^T; 3, *K. sandramycini* KCTC 9609^T.

Fatty acid	1	2	3
Saturated			
C _{15:0}	1		2
C _{16:0}	1		1
C _{17:0}	1	2	2
Unsaturated			
C _{15:1}	1		2
C _{16:1} ω _{9c}	4	5	4
C _{17:1} ω _{9c}	5	7	4
C _{18:1} ω _{9c}	1	4	1
Branched			
iso-C _{13:0}			1
iso-C _{14:0}	2	3	5
iso-C _{14:0} 3-OH			1
iso-C _{15:0}	14	5	15
anteiso-C _{15:0}	29	32	38
anteiso-C _{15:0} 2-OH	2	2	1
iso-C _{16:0}	13	14	7
iso-C _{16:0} 2-OH	1		
iso-C _{16:1}	1	2	1
iso-C _{17:0}	9	4	6
anteiso-C _{17:0}	5	10	3
9-Methyl			
C _{16:0}	8	8	7
10-Methyl			
C _{17:0}	1	2	
Summed feature 5*	1		

*Summed feature 5 contained one or more of the following fatty acids: C_{17:1} anteiso B and/or C_{17:1} anteiso I.

propose that *H. koreensis* should be transferred to the genus *Kribbella* as *Kribbella koreensis* comb. nov. The description of *K. koreensis* and emended description of the genus *Kribbella* are presented below.

Emended description of *Kribbella* Park *et al.* 1999

The description is the same as that given by Park *et al.* (1999) except for polar lipid composition. In addition to phosphatidylcholine, *K. koreensis* contains diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. DNA G + C content is 68–71 mol%.

Description of *Kribbella koreensis* (Lee *et al.* 2000) comb. nov.

Kribbella koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* pertaining to Korea, the location of the soil sample from which the type strain was isolated).

The description is identical to that of Lee *et al.* (2000).

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