

## ***Knoellia sinensis* gen. nov., sp. nov. and *Knoellia subterranea* sp. nov., two novel actinobacteria isolated from a cave**

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**Two novel strains of the class *Actinobacteria* were isolated from a cave in China. Cells of both strains were Gram-positive, non-motile, non-spore-forming and not acid-fast and exhibited a rod/coccus growth cycle. Both isolates grew well on complex organic media under aerobic conditions. Their cell wall peptidoglycan contained meso-diaminopimelic acid as diagnostic diamino acid. The acyl type of the glycan chain of peptidoglycan was acetyl. The major respiratory quinone was MK-8(H<sub>4</sub>). The cellular fatty acid profile was characterized by the predominance of 13-methyltetradecanoic (i-C<sub>15:0</sub>), 15-methylhexadecanoic (i-C<sub>17:0</sub>), 14-methylpentadecanoic (i-C<sub>16:0</sub>) and 14-methylhexadecanoic (ai-C<sub>17:0</sub>) acids. The major polar lipids were phosphatidylethanolamine, phosphatidylinositol and diphosphatidylglycerol. Mycolic acids were absent. The DNA G+C composition was 68–69 mol%. 16S rDNA-based phylogenetic analysis revealed an intermediate phylogenetic position of the cave isolates between the genera *Janibacter* and *Tetrasphaera*, which did not permit their unambiguous affiliation to either genus. Differences in morphological, physiological and chemotaxonomic properties between the two isolates and their closest phylogenetic neighbours support the proposal of a new genus and two novel species, *Knoellia sinensis* gen. nov., sp. nov. and *Knoellia subterranea* sp. nov. The type and only strains of the species are respectively HKI 0119<sup>T</sup> (= DSM 12331<sup>T</sup> = CIP 106775<sup>T</sup>) and HKI 0120<sup>T</sup> (= DSM 12332<sup>T</sup> = CIP 106776<sup>T</sup>).**

**Keywords:** *Knoellia sinensis* gen. nov., sp. nov., *Knoellia subterranea* sp. nov., meso-diaminopimelic acid-containing actinomycetes

### **INTRODUCTION**

Mycolate-less members of the class *Actinobacteria* containing both meso-diaminopimelic acid (meso-A<sub>2</sub>pm) in the peptidoglycan and the respiratory quinone MK-8(H<sub>4</sub>) as the major menaquinone fall into various phylogenetic lineages within the order *Actinomycetales*. They comprise the genera *Dermatophilus*, family *Dermatophilaceae* (Stackebrandt *et al.*, 1983), *Pseudonocardia*, family *Pseudonocardiaceae* (Warwick *et al.*, 1994), *Microsphaera*, family *Microsphaeraceae* (Stackebrandt *et al.*, 1997), *Janibacter* (Martin *et al.*,

1997) and the recently described genus *Tetrasphaera* (Maszenan *et al.*, 2000), the latter two still without a family affiliation. Members of these genera are characterized morphologically either by the formation of a branched mycelium with sporulating hyphae (*Dermatophilus* and *Pseudonocardia*) or by coccoid and rod-shaped cells (*Janibacter*, *Microsphaera*, *Tetrasphaera*). All strains of the latter three genera were isolated from deposited or activated sludge and polluted environments.

In this paper, we describe two strains (HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup>) that originate from soil samples collected in a cave (Reed Flute Cave, Guilin, China), a poorly studied hypogean habitat. These isolates were found to be closely related to the genera *Janibacter* and *Tetrasphaera*. However, it has become evident from our

**Abbreviation:** A<sub>2</sub>pm, diaminopimelic acid.

The EMBL accession numbers for the 16S rDNA sequences of DSM 12331<sup>T</sup> (= HKI 0119<sup>T</sup>) and DSM 12332<sup>T</sup> (= HKI 0120<sup>T</sup>) are AJ294412 and AJ294413.

studies that their intermediate phylogenetic position does not justify a taxonomic affiliation to either genus, due to the differences observed in the 16S rDNA sequence data supported by morphological, physiological and chemotaxonomic properties. Furthermore, the low level of DNA–DNA relatedness and phenotypic differences between the two strains indicate two separate species. Therefore, it is proposed the cave isolates be classified in a new genus and two novel species, *Knoellia sinensis* gen. nov., sp. nov. and *Knoellia subterranea* sp. nov.

## METHODS

**Bacterial strains and cultural conditions.** Strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> were isolated from two soil samples collected at different sites in the Reed Flute Cave near Guilin, Guangxi (China), on casein mineral medium (Altenburger *et al.*, 1996) and, in the case of strain HKI 0120<sup>T</sup>, on peptone/yeast extract/brain–heart infusion medium (Yokota *et al.*, 1993), by using the standard dilution agar plating method. Routine cultivation was performed on rich (R) agar (Yamada & Komagata, 1972) at 28 °C. Biomass for biochemical analyses was obtained by growing the strains in liquid R medium or Bacto tryptic soy broth (Difco) and harvesting the cells by centrifugation.

**Morphological and physiological characteristics.** Morphological features were examined by light and electron microscopy as described by Groth *et al.* (1997, 1999). Characterization of physiological parameters was done as described previously (Groth *et al.*, 1997). The pH range for growth was determined in nutrient agar (Difco) adjusted to pH values between 5 and 9 with HCl or NaOH and incubation at 28 °C. In addition to the classical physiological tests, the ability of the strains to utilize a broad range of carbon sources was determined by using the panel of 95 carbon-containing compounds provided in the BIOLOG-SFP microplates (BIOLOG). The inocula for the microplates were grown on the recommended BIOLOG universal growth medium (BUGM) and on tryptic soy agar (TSA) (Difco) according to the instructions of the manufacturer. Susceptibility to antibiotics was tested by placement of antibiotic discs (Oxoid) on R agar plates seeded with a suspension of strain HKI 0119<sup>T</sup> or HKI 0120<sup>T</sup> (Groth *et al.*, 1997).

**Analysis of chemotaxonomic characteristics.** Cell wall amino acid, sugar and lipid analyses were carried out by using the methods of Schleifer & Kandler (1972), Saddler *et al.* (1991), MacKenzie (1987), Uchida & Aida (1984), Stead *et al.* (1992), Collins *et al.* (1977), Minnikin *et al.* (1975, 1979) and Collins & Jones (1980), as described previously (Groth *et al.*, 1996).

**DNA base composition.** DNA was isolated and its G+C content determined by HPLC of deoxyribonucleosides as described by Groth *et al.* (1996).

**DNA–DNA hybridization.** DNA–DNA hybridization studies were carried out by the spectrophotometric method as described previously (Maszenan *et al.*, 2000).

**16S rDNA sequence determination and phylogenetic analysis.** Preparation of genomic DNA from strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> was done as described previously (Rainey *et al.*, 1996). Sequences were aligned manually to the DSMZ sequence database, consisting of several thousand 16S rDNA sequence entries, including those from the Ribosomal Database Project (Maidak *et al.*, 1997) and EMBL. Similarity values were transformed into phylogenetic distance

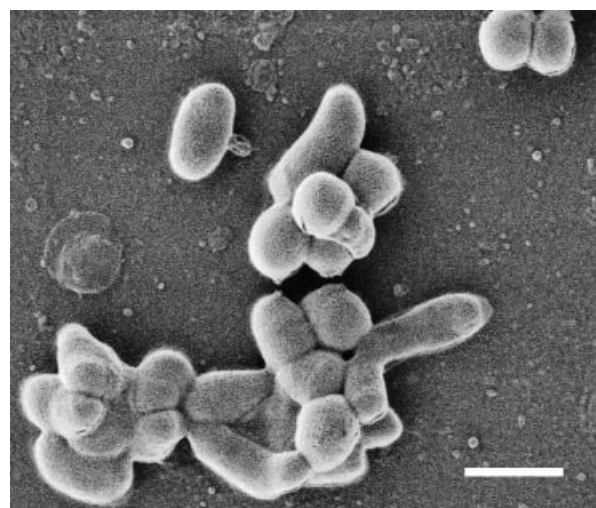
values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). Two different distance matrix methods [De Soete, 1983; and neighbour-joining (Felsenstein, 1993)], as well as the maximum-likelihood method (Felsenstein, 1993), were used in the construction of phylogenetic dendrograms. Bootstrap values were determined according to Felsenstein (1993). All analyses were done on a SUN SparcII workstation.

## RESULTS AND DISCUSSION

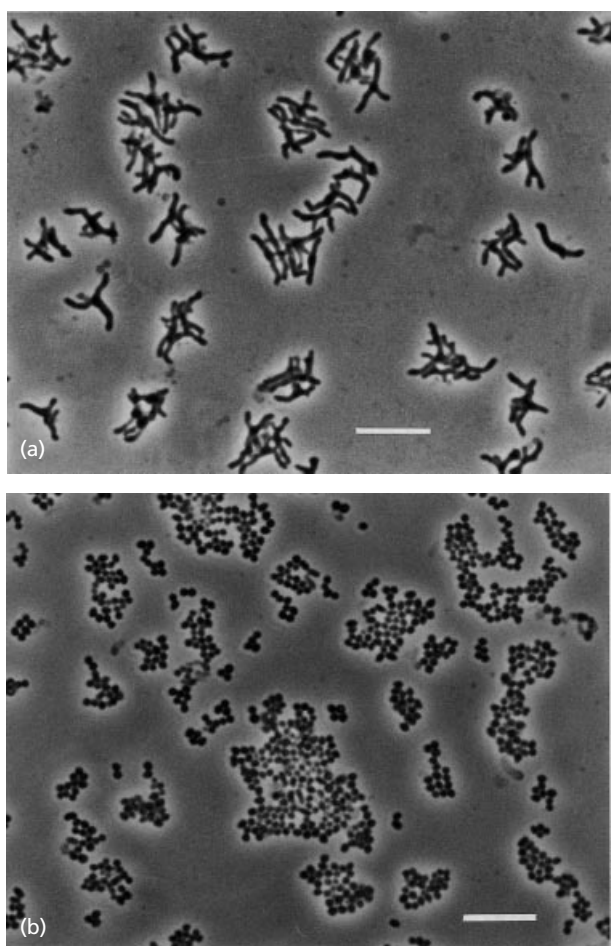
### Morphological characteristics

Isolates HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> formed similar smooth, circular, convex, opaque, white to cream-coloured colonies with entire margins that varied in size from 0.4 to 1.7 mm. For strain HKI 0119<sup>T</sup>, two colony types that differed in transparency and size were observed on R medium. In contrast, strain HKI 0120<sup>T</sup> exhibited mainly uniform, opaque colonies. Cells of both strains were Gram-positive, not acid-fast, non-motile, non-sporulating, irregular rods and cocci (Fig. 1). The dimensions of the rods were 1.7–4.5 by 0.4–0.9 µm for strain HKI 0119<sup>T</sup> and 1.9–6.0 by 0.5–1.2 µm for strain HKI 0120<sup>T</sup>. Cocci varied in diameter from 0.6 to 0.9 µm (HKI 0119<sup>T</sup>) and 0.8 to 1.4 µm (HKI 0120<sup>T</sup>). In complex media, a pronounced rod/coccus growth cycle was observed in both strains. Cells of the stationary growth phase were coccoid, occurring singly or arranged in pairs, short chains and small, irregular clusters (Fig. 2b). After transfer to fresh agar medium (slides coated with an agar film), the cocci grew out with several germ tubes (up to six, Fig. 2a) to give rise to irregular rods. As the outgrowing cells remained in their positions within their chains or clusters and the developed germ tubes sometimes became rather long, the impression of a rudimentary mycelial development was given.

Cell morphology clearly differentiates the cave isolates HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> from the phylogenetically



**Fig. 1.** Scanning electron micrograph of cells from a 3-d-old culture of strain HKI 0119<sup>T</sup> grown on R agar at 28 °C. Bar, 1 µm.



**Fig. 2.** Micrographs of strain HKI 0119<sup>T</sup> grown at 28 °C on R medium for 24 h (a) and in liquid R medium for 72 h (b). Bars, 10 µm.

neighbouring genera *Janibacter* and *Tetrasphaera*. Cells of all described *Janibacter* species, *Janibacter limosus*, *Janibacter terrae* and *Janibacter brevis*, are irregular short rods and/or cocci. The lengths of the rods do not exceed 1.8 µm and a similar kind of branching in outgrowing cells (Fig. 2a) has never been reported from these species (Martin *et al.*, 1997; Yoon *et al.*, 2000; Imamura *et al.*, 2000). Strains of the genus *Tetrasphaera* grow exclusively as irregular cocci in pairs, tetrads or clusters of tetrads. Arrangement of the cocci in chains and multiplication by the development of germ tubes have not been observed (Maszenan *et al.*, 2000).

### Physiological properties

Strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> grew well on complex organic media at 28 °C within 24–48 h under aerobic conditions. Differences in growth between the two strains were observed when the supply of oxygen was restricted and at the incubation temperature of 37 °C. While strain HKI 0119<sup>T</sup> did not grow at 37 °C, growth of isolate HKI 0120<sup>T</sup> was not restricted and

**Table 1.** Physiological properties that differentiate strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup>

–, Negative; +, positive; w, weakly positive.

Property	HKI 0119 <sup>T</sup>	HKI 0120 <sup>T</sup>
Decomposition of:		
Casein	–	+
Gelatin	–	+
Urea	–	w
Acid produced from:		
Dextrin	–	+
Sucrose	+	–
Potato starch	–	+
Trehalose	–	w
Utilization of:		
Formate	–	w
Malate	–	+
Growth at 37 °C	–	+
Susceptibility to ampicillin (10 µg)	+	–
BIOLOG test results:		
D-Mannitol	–	+
Xylitol	–	+
γ-Hydroxybutyric acid	+	–
p-Hydroxyphenyl acetic acid	+	–
D-Lactic acid methyl ester	+	–
Succinamic acid	–	+
D-Alanine	–	+
L-Alanyl glycine	–	+
Adenosine	–	+
Glucose 1-phosphate	+	–
Glucose 6-phosphate	+	–

appeared, in contrast, to be slightly enhanced. A similar effect occurred when the strains were cultivated in an aerobic atmosphere with reduced oxygen; growth of strain HKI 0119<sup>T</sup> was restricted while strain HKI 0120<sup>T</sup> grew equally well. At an incubation temperature of 42 °C under anaerobic conditions and in the presence of 6% (w/v) NaCl in the culture medium, growth of both strains was inhibited completely.

Further physiological properties of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> are indicated in the species descriptions. Differentiating characteristics between the two strains are given in Table 1. Concerning the results of the BIOLOG test, it should be mentioned that only those data are shown that were obtained with inocula from both media, TSA and BUGM. In a few cases (data not shown), the utilization of selected carbon sources was dependent on the medium used in pre-cultivation. This fact indicates variability in the utilization of certain compounds.

The influence of increased concentrations of NaCl in the culture medium and acid production from several sugars distinguished the cave isolates from *Janibacter* strains (*Tetrasphaera* strains were not examined). While growth of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> was inhibited completely by a concentration of 6%

**Table 2.** Cellular fatty acid composition of HKI 0119<sup>T</sup>, HKI 0120<sup>T</sup>, *Janibacter limosus*, *Tetrasphaera japonica* and *Tetrasphaera australiensis*

Values are percentages; values less than 1% are not shown. Abbreviations for fatty acids are as follows: C<sub>16:0</sub>, hexadecanoic acid; C<sub>18:1</sub>, octadecenoic acid; i-C<sub>15:0</sub>, 13-methyl tetradecanoic acid; ai-C<sub>15:0</sub>, 12-methyl tetradecanoic acid; TSBA, tuberculostearic acid; i, iso; ai, anteiso. Data not derived in this study were obtained from Martin *et al.* (1997) (*J. limosus*) and Maszenan *et al.* (2000) (*T. japonica* and *T. australiensis*).

Fatty acid	HKI 0119 <sup>T</sup>	HKI 0120 <sup>T</sup>	<i>J. limosus</i> HKI 0083 <sup>T</sup>	<i>T. japonica</i> ACM 5116 <sup>T</sup>	<i>T. australiensis</i> ACM 5118 <sup>T</sup>
i-C <sub>13:0</sub>	1.1	1.2	–	–	–
i-C <sub>14:0</sub>	2.4	2.0	–	–	2.6
i-C <sub>15:0</sub>	18.5	25.9	1.7	6.4	12.1
ai-C <sub>15:0</sub>	1.9	8.4	–	3.0	7.6
C <sub>15:0</sub>	–	1.2	3.8	1.1	4.0
i-C <sub>16:0</sub>	18.3	11.1	17.5	25.8	27.1
C <sub>16:0</sub>	2.4	4.3	3.9	–	5.9
i-2OH-C <sub>16:0</sub>	–	–	–	6.1	–
i-C <sub>16:1</sub>	–	–	–	3.2	9.7
C <sub>16:1</sub>	2.9	1.4	2.4	1.2/4.4*	8.4
i-C <sub>17:0</sub>	18.2	19.6	1.3	–	–
ai-C <sub>17:0</sub>	10.6	15.5	–	20.5	14.1
i-C <sub>17:1</sub>	19.6	6.2	–	2.3	–
ai-C <sub>17:1</sub>	–	–	–	6.9	4.7
C <sub>17:0</sub>	–	1.8	20.5	1.3	–
2OH-C <sub>17:0</sub>	–	–	–	5.3	–
C <sub>17:1</sub>	–	–	29.7	–	–
i-C <sub>18:0</sub>	–	–	1.4	5.3	–
i-C <sub>18:1</sub>	4.1	–	–	1.3	–
C <sub>18:0</sub>	–	–	2.4	–	–
C <sub>18:1</sub>	–	–	8.1	2.2	3.7
TSBA	–	–	–	1.5	–

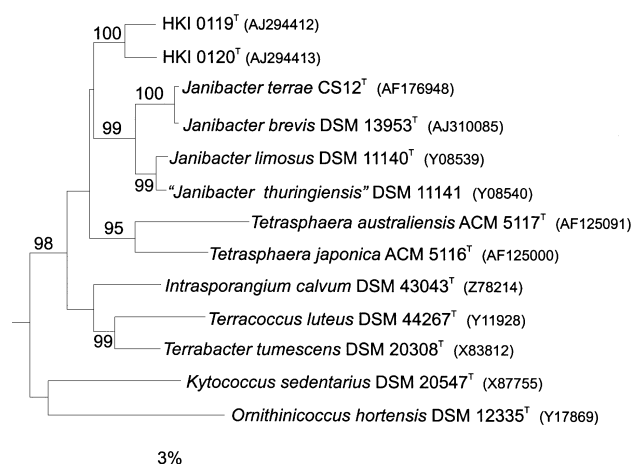
\* Two isomers of C<sub>16:1</sub>.

(w/v) NaCl on R agar, *Janibacter* strains grew equally well at 10% (w/v) NaCl (Martin *et al.*, 1997). Strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> produced acid from D-cellobiose, D-galactose, D-glucose, maltose, D-mannose and D-ribose; *Janibacter* strains do not produce acid from these sugars (Martin *et al.*, 1997; Imamura *et al.*, 2000; Yoon *et al.*, 2000). In contrast to members of the genus *Tetrasphaera*, the two cave isolates grew within 48 h; *Tetrasphaera* strains grew slowly, needing 3–4 weeks for the appearance of visible colonies or any turbidity in submerged cultures. Furthermore, *Tetrasphaera* strains grew neither at pH 5.5 and below nor at pH 8.5 and above (Maszenan *et al.*, 2000), whereas the growth of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> was reduced only slightly at pH 5 and pH 9. Generally, the cave isolates are metabolically more active than members of *Tetrasphaera*, a fact that is supported convincingly by negative results for *Tetrasphaera* strains in the reduction of nitrate, production of H<sub>2</sub>S and the BIOLOG test (Maszenan *et al.*, 2000).

Strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> were maintained as liquid cultures at a temperature of –80 °C by adding glycerol and in the vapour phase of liquid nitrogen by adding 5% (w/v) DMSO to the culture broth. The latter is recommended for long-term storage.

### Chemotaxonomic characteristics

Whole-cell hydrolysates contained *meso*-A<sub>2</sub>pm as the diagnostic constituent of the peptidoglycan. The acyl type was acetyl. The major menaquinone was MK-8(H<sub>4</sub>). MK-7(H<sub>4</sub>) and MK-6(H<sub>4</sub>) were present only in trace amounts. The polar lipids were composed of phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol, a small amount of phosphatidylglycerol and an unknown phospholipid. The presence of phosphatidylethanolamine differentiates strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> from the type strains of the species of the genera *Janibacter* and *Tetrasphaera*. The cellular fatty acid profiles of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> were similar and characterized by the predominance of 13-methyltetradecanoic (i-C<sub>15:0</sub>), 15-methylhexadecanoic (i-C<sub>17:0</sub>), 14-methylpentadecanoic (i-C<sub>16:0</sub>) and 14-methylhexadecanoic (ai-C<sub>17:0</sub>) acids. Both strains lack heptadecenoic acid, which is the main component of the fatty acid profile of *J. limosus*, while 14-methylhexadecanoic acid, present in large amounts in the cave isolates, is absent from *J. limosus*. Iso- and anteiso-branched-chain fatty acids dominate the fatty acid patterns of *Tetrasphaera* strains and of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup>. However, the patterns of



**Fig. 3.** 16S rDNA sequence-based phylogenetic dendrogram constructed from evolutionary distances (De Soete, 1983) showing the phylogenetic position of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> next to members of the family *Intrasporangiaceae*. Sequences of members of the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997) were used as references. Accession numbers are given in parentheses. Numbers at branching points refer to bootstrap values (500 trees resampled); only values above 70% are indicated. Bar, 3 inferred nucleotide substitutions per 100 nucleotides.

strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> differ from those of *Tetrasphaera* strains in the occurrence of large amounts of 15-methylhexadecanoic acid and the absence of 14-methylpentadecenoic, 14-methylhexadecenoic and octadecenoic acids (Table 2). Mycolic acids were absent. No diagnostic sugars were found. The DNA G+C contents of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> were respectively 68 and 69 mol%.

### Phylogenetic analysis

Totals of 1465 nucleotides of the 16S rDNA sequences of strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> were analysed. The degree of similarity was found to be 98.6%. The sequences were included in detailed phylogenetic analyses with actinomycete reference sequences of members of the suborder *Micrococcineae* and the genus *Tetrasphaera*. The highest similarity values were found to strains of *Janibacter*, i.e. *J. limosus* DSM 11140<sup>T</sup> and '*Janibacter thuringiensis*' DSM 11141 and *J. terrae* CS 12<sup>T</sup> (96.2–96.7%); similarities to sequences of species of *Tetrasphaera* (94.9–95.3%) and of *Intrasporangium calvum* (95.0%) were slightly lower. These two strains possess a deviation from the 16S rDNA signature of members of the suborder *Micrococcineae* that is shared with members of the genus *Tetrasphaera* (nt 952–1229, T–A base pair instead of C–G; Maszenan *et al.*, 2000).

In the 16S rDNA dendrograms generated by the distance matrix algorithms of De Soete (1983) and Saitou & Nei (1987), which are identical concerning the branching point of members of *Intrasporangiaceae*, *Tetrasphaera* and *Janibacter*, strains HKI 0119<sup>T</sup> and HKI 0120<sup>T</sup> branched intermediate to the genera

*Janibacter* and *Tetrasphaera* (Fig. 3). This branching is not supported by a high bootstrap value. In these dendrograms, the latter organisms form a phylogenetically coherent cluster, showing high (98%) statistical significance. This is in contrast to the pattern obtained from maximum-likelihood analysis (Felsenstein, 1993). Here, the genera *Intrasporangium*, *Terrabacter* and *Terracoccus* are disconnected from *Janibacter* and *Tetrasphaera*, which cluster separately (not shown). The branching point of the novel isolates is moved slightly below the bifurcation point of *Janibacter* and *Tetrasphaera* species. As shown above, the distinct phylogenetic position, combined with the presence of different chemotaxonomic and physiological diagnostic properties, allows us to propose a new genus for these two strains.

### Taxonomic conclusions

In all of the phylogenetic dendrograms, the two cave isolates are unrelated to species of their phylogenetic neighbours *Janibacter* and *Tetrasphaera*. The intermediate phylogenetic position does not permit an unambiguous taxonomic affiliation of the isolates to either genus. The diagnostic properties that distinguish this taxon from other taxa containing *meso*-A<sub>2</sub>pm and menaquinone MK-8(H<sub>4</sub>) are shown in Table 3. This separateness, supported by the above-mentioned physiological and chemotaxonomic properties, suggest the accommodation of the two strains in a novel genus, *Knoellia* gen. nov. The low level of DNA–DNA relatedness (29.2%) between the two isolates, together with differentiating phenotypic characteristics (Table 1), justify a further taxonomic discrimination into two separate species, *Knoellia sinensis* sp. nov. and *Knoellia subterranea* sp. nov. As the proposed novel species are represented in each case by single strains, the phylogenetic depth and the phenotypic variability of an entire taxon cannot be reflected by the descriptions given.

### Description of *Knoellia* gen. nov.

*Knoellia* [Knoell'i.a. N.L. fem. n. *Knoellia* after Hans Knöll (1913–1978), a German pioneer in antibiotic research].

Cells are irregular rods and cocci, occurring singly, in pairs, short chains or clusters. Rods vary in their dimensions from 1.7 to 6.0 by 0.4 to 1.2 μm and cocci in diameter from 0.6 to 1.4 μm. Gram-positive, not acid-fast, non-motile, non-sporulating and exhibit a rod/coccus growth cycle. Aerobic to microaerophilic. Catalase-positive, oxidase-negative. The peptidoglycan is of the A-1γ type (based on *meso*-A<sub>2</sub>pm, direct cross-linkage). The acyl type is acetyl. Mycolic acids are absent. The major menaquinone is MK-8(H<sub>4</sub>). MK-7(H<sub>4</sub>) and MK-6(H<sub>4</sub>) may occur in trace amounts. The cellular fatty acid profile is characterized by the predominance of 13-methyltetradecanoic (i-C<sub>15:0</sub>), 15-methylhexadecanoic (i-C<sub>17:0</sub>), 14-methylpentadecanoic (i-C<sub>16:0</sub>) and 14-methylhexadecanoic (ai-

**Table 3.** Differential characteristics of strain HKI 0119<sup>T</sup> and taxa with murein type A-1<sub>γ</sub> and major menaquinone MK-8(H<sub>4</sub>)

Data not obtained in this study were taken from Martin *et al.* (1997) (*Janibacter*), Maszenan *et al.* (2000) (*Tetrasphaera*), Yoshimi *et al.* (1996) (*Microsphaera*), Stackebrandt *et al.* (1983) (*Dermatophilus*) and Warwick *et al.* (1994) (*Pseudonocardia*). ND, Not determined.

Characteristic	HKI 0119 <sup>T</sup>	<i>Janibacter</i>	<i>Tetrasphaera</i>	<i>Microsphaera</i>	<i>Dermatophilus</i>	<i>Pseudonocardia</i>
Cell morphology	Cocci to rod-shaped, non-motile, non-sporulating	Cocci to rod-shaped, non-motile, non-sporulating	Cocci, non-motile, non-sporulating	Cocci, non-motile, non-sporulating	Substrate and aerial mycelium, sporulating, motile spores	Substrate and aerial mycelium, sporulating, non-motile spores
Habitat	Soil of a cave	Sludge, sewage waste	Sludge, sewage waste	Sludge	Skin of mammals	Soil, rotting plants
G + C content (mol%)	68–69	70	68–71	67.5	57–59	68–79
Fatty acid types*	S, I, A	S, I, U	S, I, A, U	S, A, I, U	S, U	A, I
Polar lipids†	PE, PI, DPG, PG, PL	DPG, PG, PI	DPG, PG, PI, PL	ND	DPG, PG, PI	DPG, PG, PC, PE, PI, GL

\* A, Anteiso-methyl-branched; I, iso-methyl-branched; S, straight-chain saturated; U, monounsaturated.

† DPG, Diphosphatidylglycerol; GL, unknown glycolipid(s); PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s).

C<sub>17:0</sub>) acids. The major polar lipids are phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol. The DNA G + C content is 68–69 mol%. Phylogenetically, this genus is a member of the suborder *Micrococcineae*, order *Actinomycetales*. The type species is *Knoellia sinensis*.

#### Description of *Knoellia sinensis* sp. nov.

*Knoellia sinensis* (si.nen'sis. N.L. adj. *sinensis* of China, the country of origin of the type strain).

Cells are irregular rods and cocci, occurring singly, in pairs, short chains or clusters. Rods vary in their dimensions from 1.7 to 4.5 by 0.4 to 0.9 µm and cocci in diameter from 0.6 to 0.9 µm. A rod/coccus growth cycle is exhibited on complex media. Gram-positive, not acid-fast, non-motile, non-sporulating. Colonies are smooth, circular, convex and opaque with entire margins and are white to cream-coloured. Aerobic to microaerophilic. Good growth occurs between pH 5 and 9 and at 28 °C; no growth at 37 °C or under anaerobic conditions. In R medium, NaCl is tolerated up to 4% (w/v) but not at 6% (w/v). Catalase-positive, oxidase-negative. Nitrate is reduced to nitrite. H<sub>2</sub>S is produced; indole is not produced. Acids are produced from D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, D-ribose and sucrose. No acid production from L-arabinose, dextrin, D-glucitol, glycerol, inulin, lactose, D-mannitol, D-raffinose, L-rhamnose, potato starch, salicin, trehalose or D-xylose. Methyl red and Voges-Proskauer reactions are negative. Aesculin, hippurate (weak), potato starch, Tween 80 and tyrosine are decomposed, while adenine, casein, gelatin, hypoxanthine, urea and xanthine are not. Acetate and succinate are utilized as carbon sources, while aconitate, benzoate, citrate, formate, malate and DL-tartrate are not. Susceptible to ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (2 µg), neo-

mycin (30 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg), penicillin G (2 IU), polymyxin B (300 IU), rifampicin (2 µg), streptomycin (10 µg) and sulfonamide (300 µg). Not susceptible to oxacillin (5 µg). Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and α-glucosidase are detected by the APIZYM enzyme assay; trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase are negative. Weak reaction for lipase (C14), naphthol-AS-BI-phosphohydrolase, β-glucosidase and acid phosphatase. In addition to the chemotaxonomic properties described for the genus, a small amount of phosphatidylglycerol and an unknown phospholipid are present. No diagnostic sugars. The DNA G + C content is 68 mol%. Habitat: soil from a hypogean environment, Reed Flute Cave, Guilin, China. The type strain is HKI 0119<sup>T</sup> (= DSM 12331<sup>T</sup> = CIP 106775<sup>T</sup>).

#### Description of *Knoellia subterranea* sp. nov.

*Knoellia subterranea* (sub.ter.ra'ne.a. L. pref. *sub* below, underneath; L. fem. n. *terra* soil; L. adj. *subterranea* under the earth, referring to the place of isolation).

Cells are irregular rods and cocci, occurring singly, in pairs, short chains or clusters. Rods vary in their dimensions from 1.9 to 6.0 by 0.5 to 1.2 µm and cocci in diameter from 0.8 to 1.4 µm. A rod/coccus growth cycle is exhibited on complex media. Gram-positive, not acid-fast, non-motile, non-sporulating. Colonies are smooth, circular, convex and opaque with entire margins and are white to cream-coloured. Aerobic to microaerophilic. Good growth occurs between pH 5 and 9 and at 28–37 °C; no growth at 42 °C or under anaerobic conditions. In R medium, NaCl is tolerated up to 4% (w/v) but not at 6% (w/v). Catalase-positive, oxidase-negative. Nitrate is reduced to nitrite.

H<sub>2</sub>S is produced; indole is not produced. Acids are produced from D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, maltose, D-mannose, potato starch, D-ribose and trehalose (weak). No acid production from L-arabinose, D-glucitol, glycerol, inulin, lactose, D-mannitol, D-raffinose, L-rhamnose, salicin, sucrose or D-xylose. Methyl red and Voges–Proskauer reactions are negative. Casein, aesculin, gelatin, hippurate (weak), potato starch, Tween 80, tyrosine and urea (weak) are decomposed, while adenine, hypoxanthine and xanthine are not. Acetate, formate (weak), malate and succinate are utilized as carbon sources, while aconitate, benzoate, citrate and DL-tartrate are not. Susceptible to chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (2 µg), neomycin (30 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg), penicillin G (2 IU), polymyxin B (300 IU), rifampicin (2 µg), streptomycin (10 µg) and sulfonamide (300 µg). Not susceptible to ampicillin (10 µg) or oxacillin (5 µg). Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase are detected by the APIZYM enzyme assay but trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. Weak reaction for lipase (C14) and acid phosphatase. In addition to the chemotaxonomic properties described for the genus, a small amount of phosphatidylglycerol and an unknown phospholipid are present. No diagnostic sugars. The DNA G + C content is 69 mol%. Habitat: soil from a hypogean environment, Reed Flute Cave, Guilin, China. The type strain is HKI 0120<sup>T</sup> (= DSM 12332<sup>T</sup> = CIP 106776<sup>T</sup>).

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