Knoellia aerolata sp. nov., isolated from an air sample in Korea

Hang-Yeon Weon,¹ Byung-Yong Kim,² Peter Schumann,³
Reiner M. Kroppenstedt,³ Hyung-Jun Noh,¹ Chan-Won Park⁴
and Soon-Wo Kwon²

Correspondence

Soon-Wo Kwon
swkwon@rda.go.kr

¹Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Republic of Korea

²KACC – Korean Agricultural Culture Collection, Microbial Genetics Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-707, Republic of Korea

³DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstrasse 7b, D-38124 Braunschweig, Germany

⁴Soil Management Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Republic of Korea

An aerobic, Gram-positive, non-motile, non-spore-forming, rod-coccus-shaped bacterium, strain 5317S-21^T, was isolated from an air sample from Suwon city, Republic of Korea. The isolate was able to grow within a pH range of 5.0–9.0 and a temperature range of 5–35 °C and it tolerated up to 2% (w/v) NaCl. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid as diagnostic diamino acid. The predominant isoprenoid quinone was MK-8(H₄). The major polar lipids were phosphatidylinositol, phosphatidylethanolamine and diphosphatidylglycerol; phosphatidylglycerol and several unknown phospholipids were also detected. Mycolic acids were absent. The only whole-cell sugar was glucose. The major cellular fatty acids were iso-C_{16:0}, C_{17:1}ω8c and iso-C_{15:0}. 16S rRNA gene sequence analysis indicated that strain 5317S-21^T was related phylogenetically to members of the genus *Knoellia*, with 97.4% sequence similarity to the type strains of *Knoellia sinensis* and *Knoellia subterranea*. The G + C content of the genomic DNA of strain 5317S-21^T was 73 mol%. Levels of DNA–DNA relatedness between strain 5317S-21^T and the type strains of *Knoellia sinensis* and *Knoellia subterranea* were 37 and 41%, respectively. It was concluded that strain 5317S-21^T represents a novel species of the genus *Knoellia*, for which the name *Knoellia aerolata* sp. nov. is proposed. The type strain is 5317S-21^T (=KACC 20583^T =DSM 18566^T).

The genus *Knoellia* is a member of the family *Intrasporangiaceae* within the class *Actinobacteria* and contains organisms that are Gram-positive, non-motile, irregular rods or cocci. The peptidoglycan is of type A1γ, the predominant menaquinone is MK-8(H₄) and the major fatty acids are iso-C_{15:0}, iso-C_{17:0}, iso-C_{16:0} and anteiso-C_{17:0}. The major polar lipids are phosphatidylethanolamine (PE), phosphatidylinositol (PI) and diphosphatidylglycerol

(DPG). Currently, the genus is represented by two recognized species, *Knoellia sinensis* and *Knoellia subterranea* (Groth *et al.*, 2002).

An air sample was collected in Suwon city, Republic of Korea, using an MAS-100 air sampler (single-stage multiple-hole impactor; Merck), which contained Petri dishes with R2A agar (BBL) supplemented with 200 µg cycloheximide ml⁻¹ (Sigma). Strain 5317S-21^T was isolated after incubation for 5 days at 30 °C. The isolate was subcultured on R2A medium at 30 °C and maintained on the same medium.

Genomic DNA was extracted using a DNeasy Tissue kit (Qiagen) following the manufacturer's instructions. Sequencing of the 16S rRNA gene was performed as described by Kwon *et al.* (2003). Multiple alignments were performed by using the program CLUSTAL W (Thompson

Abbreviations: A₂pm, diaminopimelic acid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5317S-21^T is EF553529.

Polar lipid profiles and fatty acid compositions of strain 5317S-21^T and members of the genus *Knoellia* are available as supplementary material with the online version of this paper.

et al., 1994). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed with neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Fitch, 1971) using the program MEGA3 (Kumar *et al.*, 2004), with bootstrap values based on 1000 replications (Felsenstein, 1985). The 16S rRNA gene sequence of strain 5317S-21^T was based on a continuous stretch of 1401 bp. Comparison of 16S rRNA gene sequences revealed that strain 5317S-21^T shared 97.4 % similarity with both *K. sinensis* DSM 12331^T and *K. subterranea* DSM 12332^T, 96.8 % similarity with *Janibacter limosus* DSM 11140^T, 96.7 % similarity with *Tetrasphaera elongata* DSM 14184^T, 96.6 % similarity with both *Janibacter melonis* DSM 16063^T and *Janibacter terrae* DSM 13876^T and less than 96.5 % similarity with all the other type strains included in Fig. 1. According to the neighbour-joining tree (Fig. 1), strain 5317S-21^T clustered with the two recognized species of the genus *Knoellia* with a 68 % bootstrap value. This grouping was also supported by maximum-parsimony (Fig. 1).

DNA–DNA hybridization was carried out according to the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted using the non-radioactive DIG-High prime system (Roche) and hybridized DNA was visualized with the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). The DNA–DNA hybridization values of 5317S-21^T with *K. sinensis* DSM 12331^T and *K. subterranea* DSM 12332^T were 37 and 41 %, respectively.

After incubation for 3 days on R2A agar, cell morphology was examined by phase-contrast microscopy (AXIO; Zeiss), as well as with an electron microscope (model 912AB; LEO) after negative staining with uranyl acetate. Methods described by Smibert & Krieg (1994) were used for Gram staining and to detect catalase, oxidase and hydrolysis of casein, DNA, hypoxanthine, starch, Tween 80 and xanthine. Hydrolysis of CM-cellulose (0.1 %; Sigma), chitin from crab shells (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was checked after 14 days incubation. Growth was assessed at 5, 10, 20, 25, 30, 35, 40 and 45 °C,

at pH 4, 5, 6, 7, 8, 9 and 10 and at 0, 1, 3, 5, 7 and 10 % NaCl after 14 days incubation on R2A medium. Anaerobic growth was checked using BBL anaerobic jars (Becton Dickinson). Further physiological properties and enzyme activities were tested using the API 20NE, API ID 32GN and API ZYM systems (bioMérieux). The results of API 20NE and API ID 32GN were recorded after 5 days incubation. Differential phenotypic characteristics of strain 5317S-21^T and the type strains of *K. sinensis* and *K. subterranea* on the basis of API 20NE and API ID 32GN test strips are shown in Table 1.

The peptidoglycan was analysed as described by Schleifer & Kandler (1972); phospholipids and menaquinones were analysed by the method of Minnikin *et al.* (1984). Sugar analysis of whole cells and mycolic acid determinations were carried out as described by Stanek & Roberts (1974) and Minnikin *et al.* (1975), respectively. Analyses of the whole-cell fatty acid pattern followed the described methods for the MIDI system (Microbial ID) (Kroppenstedt, 1985; Sasser, 1990). The G+C content of the DNA was determined by the method of Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18 S; Supelco).

Whole-cell hydrolysates contained *meso*-diaminopimelic acid (*meso*-A₂pm) as the diagnostic dibasic amino acid in the peptidoglycan. Polar lipids included PI, PE, DPG, phosphatidylglycerol (PG) and several unknown phospholipids (PLs) (see Supplementary Fig. S1 available in IJSEM Online). Cells contained MK-8(H₄) as the predominant menaquinone and glucose as the only whole-cell sugar; mycolic acids were not detected. Cellular fatty acid analysis revealed predominant amounts of iso-C_{16:0} (20.7 %), C_{17:1}ω8c (18.5 %) and iso-C_{15:0} (15.5 %), and smaller amounts of C_{17:0} (9.5 %), anteiso-C_{17:0} (6.7 %) and C_{18:1}ω9c (5.2 %). The complete fatty acid profile is given in Supplementary Table S1. The DNA G+C content of strain 5317S-21^T was 73 mol%.

Strain 5317S-21^T and the type strains of the two species of *Knoellia* gave the same enzymic reactions with the API ZYM method and the same reactions for several tests such as nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and hydrolysis of aesculin,

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain 5317S-21^T within the radiation encompassing related genera of the family *Intrasporangiaceae*. Asterisks indicate branches replicated with maximum-parsimony tree-making algorithms. Numbers at nodes indicate bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 1 substitution per 100 nt.

Table 1. Phenotypic characteristics of members of the genus *Knoellia*

According to the results from API 20NE and API ZYM test strips, all strains were positive for nitrate reduction, aesculin hydrolysis, gelatin hydrolysis and β -galactosidase and all strains were negative for indole production, glucose fermentation, arginine dihydrolase and urease. According to the results from API 20NE and API ID 32GN test strips, all strains assimilated D-glucose, maltose, sucrose, sodium acetate, glycogen, salicin, valeric acid, 3-hydroxybutyric acid and L-proline. None of the strains assimilated L-arabinose, capric acid, trisodium citrate, phenylacetic acid, L-rhamnose, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, D-melibiose, L-fucose, potassium 2-ketogluconate or 4-hydroxybenzoic acid.

Assimilation of:	Strain 5317S-21 ^T	<i>K. sinensis</i> DSM 12331 ^T	<i>K. subterranea</i> DSM 12332 ^T
D-Mannose	+	–	+
D-Mannitol	+	–	+
N-Acetylglucosamine	+	–	+
Potassium gluconate	+	–	–
Adipic acid	–	+	–
Malic acid	–	–	+
D-Ribose	–	–	+
Inositol	+	–	–
Lactic acid	–	+	+
L-Alanine	+	–	+
L-Serine	–	+	+
D-Sorbitol	+	–	+
Propionic acid	+	+	+
L-Histidine	–	+	+

gelatin and urea in the API 20NE test strip. Furthermore, the peptidoglycan type (*meso*-A₂pm), major menaquinone [MK-8(H₄)], polar lipid pattern (PE, PI, DPG, PG and PLs) and whole-cell sugar pattern (only glucose) of strain 5317S-21^T were all consistent with its being a member of the genus *Knoellia*. However, strain 5317S-21^T could be differentiated from the two related *Knoellia* species on the basis of assimilation patterns in API 20NE and API ID 32GN, fatty acid profiles, phylogenetic analysis and low levels of DNA–DNA relatedness (below 41 %) (Table 1, Fig. 1 and Supplementary Table S1).

In conclusion, phenotypic features and phylogenetic analyses suggest that strain 5317S-21^T represents a novel species of the genus *Knoellia*, for which the name *Knoellia aerolata* sp. nov. is proposed.

Description of *Knoellia aerolata* sp. nov.

Knoellia aerolata (ae.ro.la'ta. Gr. n. *aer* air; L. part. adj. *latus* carried; N.L. fem. part. adj. *aerolata* airborne).

Cells are aerobic, Gram-positive, non-motile and non-spore-forming, growing as irregular rods (1.0 × 1.5–2.0 µm) and cocci (1.0–1.5 µm diameter). Grows on

R2A, tryptic soy agar (Difco) and nutrient agar (Difco), but does not grow on MacConkey agar. Growth occurs at pH 5.0–9.0 and in 0–2 % NaCl, with optimal growth at pH 6.0–7.0 and 0–1 % NaCl. Grows at 5–35 °C, with optimum growth at 30 °C. Starch and Tween 80 are hydrolysed, but casein, chitin, CM-cellulose, DNA, hypoxanthine, pectin, tyrosine and xanthine are not. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, β -glucuronidase, α -glucosidase and β -glucosidase activities are detected with the API ZYM system, but not lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase. Cell-wall peptidoglycan contains *meso*-A₂pm as diagnostic diamino acid. The predominant isoprenoid quinone is MK-8(H₄). The major polar lipids are PI, PE and DPG. Mycolic acids are absent. The only whole-cell sugar is glucose. The major cellular fatty acids are iso-C_{16:0}, C_{17:1}ω8c and iso-C_{15:0}.

The type strain is 5317S-21^T (=KACC 20583^T =DSM 18566^T), isolated from an air sample from Suwon city, Korea. The DNA G+C content of the type strain is 73 mol%.

Acknowledgements

This study was supported by a programme of international collaborative research (NIAB grant no. 06-4-11-19-3) between the Rural Development Administration (Suwon, Republic of Korea) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany).

References

- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Groth, I., Schumann, P., Schütze, B., Augsten, K. & Stackebrandt, E. (2002). *Knoellia sinensis* gen. nov., sp. nov. and *Knoellia subterranea* sp. nov., two novel actinobacteria isolated from a cave. *Int J Syst Evol Microbiol* **52**, 77–84.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (SAB Technical Series no. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kwon, S. W., Kim, J. S., Park, I. C., Yoon, S. H., Park, D. H., Lim, C. K. & Go, S. J. (2003). *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. *Int J Syst Evol Microbiol* **53**, 21–27.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanoly-sates. *J Gen Microbiol* **88**, 200–204.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Seldin, L. & Dubnau, D. (1985). Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int J Syst Bacteriol* **35**, 151–154.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.