Kaistia terrae sp. nov., isolated from a wetland in Korea

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An ivory-coloured bacterium, designated strain 5YN7-3^T, was isolated from a wetland, Yongneup, Korea. Cells of the strain were aerobic, Gram-stain-negative, non-motile and short rods. 16S rRNA gene sequence analysis demonstrated that strain 5YN7-3^T belongs to the order *Rhizobiales* of the class *Alphaproteobacteria* and is closely related to *Kaistia soli* 5YN9-8^T (97.8 %), *Kaistia granuli* Ko04^T (97.6 %) and *Kaistia adipata* Chj404^T (97.4 %). Strain 5YN7-3^T showed DNA–DNA hybridization values of 28, 22 and 35 % with *K. granuli* Ko04^T, *K. soli* 5YN9-8^T and *K. adipata* Chj404^T, respectively. The major fatty acids were C_{18:1}ω7c (51.2 %), C_{19:0} cyclo ω8c (25.0 %), C_{18:0} (12.9 %) and C_{16:0} (10.8 %) (>10 % of total fatty acids). Ubiquinone-10 was the major isoprenoid quinone and the DNA G + C content was 66.5 mol%. The phenotypic characteristics in combination with 16S rRNA gene sequence analysis and DNA–DNA hybridization data clearly define strain 5YN7-3^T as a novel species of the genus *Kaistia*, for which the name *Kaistia terrae* sp. nov. is proposed. The type strain is 5YN7-3^T (=KACC 12910^T =DSM 21341^T).

The genus *Kaistia* was described by Im *et al.* (2004) and contains three species at the time of writing, namely *Kaistia adipata* (Im *et al.*, 2004), *Kaistia granuli* (Lee *et al.*, 2007) and *Kaistia soli* (Weon *et al.*, 2008b). Members of the genus *Kaistia* are characterized as non-motile, mesophilic, oxidase- and catalase-positive, rod- or coccus-shaped bacteria. Ubiquinone-10 (Q-10) is the major isoprenoid quinone and the major fatty acids are C_{18:1}ω7c and C_{19:0}ω8c cyclo.

Yongneup (38° 12' 53" N 128° 07' 30" E), the only high moor in Korea, is a wetland at 1200–1280 m above sea level. It is located around the top of Mount Daeam, Seohwa-myōn, Inje-gun, Kangwon-do, Korea. The peat layers are approximately 150 cm thick and were formed over 4000–5000 years. This area has a unique type of ecosystem in terms of weather, soil and vegetation. During the course of studies on the presence of cultivable bacterial communities in Yongneup wetland, a number of novel bacterial strains were isolated (Weon *et al.*, 2008a, b; Kwon *et al.*, 2008). A peat sample was serially diluted with 0.85 % (w/v) NaCl and appropriate 10-fold dilutions were plated on R2A agar (Difco) (Reasoner & Geldreich, 1985). The

plates were incubated at 30 °C for 4 days. Strain 5YN7-3^T was one of the isolates that appeared on the R2A medium and could be distinguished in terms of ivory-coloured colonies and subjected to taxonomic investigation. In this study, we have characterized strain 5YN7-3^T. Phenotypic, chemotaxonomic and phylogenetic analyses revealed that strain 5YN7-3^T was a member of the genus *Kaistia*. On the basis of the substantial evidence obtained from the polyphasic taxonomic studies, we propose 5YN7-3^T as the type strain of a novel species of the genus *Kaistia*, namely *Kaistia terrae*.

Cell morphology and motility were observed under a phase-contrast microscope (AXIO; ZEISS) and transmission electron microscope (912AB; LEO) after cultivation of strain 5YN7-3^T on R2A at 30 °C for 1 day. Gram staining, presence of catalase and oxidase activities, accumulation of poly-β-hydroxybutyrate and hydrolysis of casein, DNA and starch were determined as described by Smibert & Krieg (1994). Hydrolysis of CM-cellulose (0.1 %, w/v), chitin from crab shells (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) were also examined. Growth at different temperatures (5, 10, 15, 20, 25, 30, 35, 37, 40 and 45 °C) and various pH (4.0–10.0 at intervals of 1.0 pH units) were assessed after 7 days of incubation in R2A broth. Salt tolerance was tested in liquid R2A medium

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supplemented with 0–7 % NaCl (0, 1, 2, 3, 5 and 7 %, w/v) after 7 days of incubation at 30 °C. Carbon source utilization and some enzyme activities were tested by using the API 20NE, API ZYM and API ID 32 GN systems (bioMérieux) according to the manufacturer's instructions. Cells of strain 5YN7-3^T were aerobic, Gram-stain-negative, non-motile and short rods. Strain 5YN7-3^T was able to grow on R2A, nutrient agar (NA), tryptic soy agar (TSA) and MacConkey agar (Difco). Results of the phenotypic and physiological characterization are given in Table 1 and the species description.

Genomic DNA was isolated by the method of Ausubel *et al.* (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA gene

was amplified by using the universal primers fD1 and rP2 (Weisburg *et al.*, 1991), and sequenced as described by Kwon *et al.* (2003). Sequence similarity levels were calculated by the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Alignment of 16S rRNA gene sequences was performed with the CLUSTAL W program (Thompson *et al.*, 1994). Phylogenetic analysis was performed using MEGA version 3.1 (Kumar *et al.*, 2004). Distances using distance options according to the Kimura two-parameter model (Kimura, 1983) and clustering using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) were determined using bootstrap values based on 1000 replicates (Felsenstein, 1985). The nearly complete 16S rRNA gene sequence (1429 bp) was determined for strain 5YN7-3^T. The 16S

Table 1. Phenotypic and chemotaxonomic characteristics that distinguish strain 5YN7-3^T from other species of the genus *Kaistia*

Strains: 1, 5YN7-3^T (data from this study); 2, *Kaistia adipata* Chj404^T; 3, *K. granuli* Ko04^T; 4, *K. soli* 5YN9-8^T. Data for columns 2–4 from Weon *et al.* (2008b) except where indicated. All strains were positive for urease, aesculin hydrolysis, β -galactosidase, esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and β -glucosidase. All strains were negative for indole production, glucose fermentation, arginine dihydrolase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase and β -glucuronidase. All strains assimilated D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, L-rhamnose, D-ribose, inositol, sucrose, salicin, melibiose, L-fucose and D-sorbitol. All strains assimilated lactic acid and potassium 2-ketogluconate weakly. None of the strains assimilated potassium gluconate, capric acid, adipic acid, trisodium citrate, phenylacetic acid, itaconic acid, suberic acid, sodium malonate, 3-hydroxybenzoic acid, L-serine, propionic acid, valeric acid, L-histidine or 4-hydroxybenzoic acid. For fatty acid analysis, cells of all strains were harvested after growth on R2A at 30 °C for 2 days. +, Positive; (+) weakly positive; –, negative.

Characteristic	1	2	3	4
Nitrate reduction	–	+	+	–
Growth at 37 °C	–	+*	–*	+
Growth on MacConkey agar*	+	+	+	–
Starch hydrolysis	–	+*	+*	–
Gelatin hydrolysis	–	+	–	–
Assimilation of:				
Malic acid	–	–	–	+
Sodium acetate	–	(+)	–	–
L-Alanine	(+)	(+)	–	–
Potassium 5-ketogluconate	(+)	(+)	+	–
Glycogen	+	(+)	–	(+)
3-Hydroxybutyric acid	–	(+)	–	–
L-Proline	–	(+)	–	–
Enzymic activities				
Alkaline phosphatase	+	–	+	–
Esterase lipase (C8)	–	–	–	+
N-Acetyl- β -glucosaminidase	–	–	–	+
α -Mannosidase	–	+	–	+
α -Fucosidase	–	–	–	+
Fatty acids (%)				
11-Methyl C _{18:1} ω 7c	–	–	–	4.2
iso-C _{11:0} 3-OH	–	3.5	–	1.1
C _{16:0}	10.8	11.2	6.4	11.4
C _{18:0}	12.9	13.7	9.5	19.7
C _{18:1} ω 7c	51.2	52.7	55.7	46.8
C _{19:0} cyclo ω 8c	25.0	18.9	28.4	16.7
DNA G + C content (mol%)	66.5	67.7	67.8	67.0

*Data from this study.

rRNA gene sequence analysis revealed that strain 5YN7-3^T was most closely related to *K. soli* 5YN9-8^T (97.8%), *K. granuli* Ko04^T (97.6%) and *K. adipata* Chj404^T (97.4%). Strain 5YN7-3^T showed less than 94% 16S rRNA gene sequence similarity to the type strains of all recognized species of the order *Rhizobiales* of the class *Alphaproteobacteria*. The neighbour-joining tree (Fig. 1) showed that strain 5YN7-3^T formed a compact cluster with *K. granuli* Ko04^T, *K. soli* 5YN9-8^T and *K. adipata* Chj404^T. The tree obtained using the maximum-parsimony method also showed that strain 5YN7-3^T was a member of the genus *Kaistia* (Fig. 1).

Cellular fatty acid methyl esters were prepared for the bacteria grown in R2A medium for 2 days at 30 °C, and analysed using gas chromatography according to the instructions of the Microbial Identification System (MIDI). The predominant fatty acids of strain 5YN7-3^T were C_{18:1}ω7c (51.2%), C_{19:0}ω8c cyclo (25.0%), C_{18:0} (12.9%) and C_{16:0} (10.8%) (Table 1). Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). Strain 5YN7-3^T had ubiquinone-10 as the predominant isoprenoid quinone. DNA G+C contents (mol%) were determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), using a reverse-phased column (Supelcosil LC-18 S; Supelco). The DNA G+C content of strain 5YN7-3^T was 66.5 mol%. DNA–DNA hybridization tests were carried out using the filter hybridization method as described previously by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High prime system (Roche). Further, the hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). Strain 5YN7-3^T showed DNA–DNA hybridization values of 28, 22 and 35% with *K. granuli* Ko04^T, *K. soli* 5YN9-8^T and *K. adipata* Chj404^T, respectively. These values are below the threshold value (70%) suggested for species delineation (Wayne *et al.*, 1987), indicating that strain 5YN7-3^T represents a novel species of the genus *Kaistia*.

Strain 5YN7-3^T can be differentiated from *K. adipata* Chj404^T by the absence of nitrate reduction, growth at 37 °C, and starch and gelatin hydrolysis, the negative response for assimilation of sodium acetate, 3-hydroxybutyric acid and L-proline, and the presence of alkaline

phosphatase activity. Strain 5YN7-3^T can be differentiated from *K. granuli* Ko04^T based on the absence of nitrate reduction and starch hydrolysis, and the assimilation of L-alanine and glycogen. Strain 5YN7-3^T was also clearly differentiated from *K. soli* 5YN9-8^T based on growth in MacConkey agar, assimilation of L-alanine and potassium 5-ketogluconate, and the presence of alkaline phosphatase activity. The fatty acid composition of 5YN7-3^T was similar to that of *K. granuli* Ko04^T. However, strain 5YN7-3^T could be differentiated from *K. adipata* Chj404^T by a lack of iso-C_{11:0} 3-OH.

Based on the data from polyphasic studies including analyses of physiological traits, fatty acid composition, 16S rRNA gene sequence and DNA–DNA hybridization, strain 5YN7-3^T represents a novel species within the genus *Kaistia*, for which the name *Kaistia terrae* sp. nov. is proposed.

Description of *Kaistia terrae* sp. nov.

Kaistia terrae (ter'rae. L. gen. n. *terrae* of the soil).

Cells are Gram-stain-negative, aerobic, non-motile, short rods, 0.8–0.9 µm in width and 1.2–1.4 µm in length. Cells grow on R2A, NA, TSA and MacConkey agar. Colonies on R2A at 30 °C are ivory-coloured, convex and round with clear margins. Grows at 5–35 °C (optimum, 30 °C), but not at 37 °C. Grows at pH 5.0–8.0 (optimum, 7.0). Able to grow without NaCl added to the medium and does not tolerate salt levels above 1%. Does not grow anaerobically on R2A. Positive for oxidase and catalase tests. Xanthine is hydrolysed, but casein, chitin, CM-cellulose, DNA, hypoxanthine, pectin, starch, Tween 80 and tyrosine are not hydrolysed. Positive for urease, aesculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and gelatin hydrolysis (API 20NE test strip). Assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, L-rhamnose, D-ribose, inositol, sucrose, glycogen, salicin, melibiose, L-fucose and D-sorbitol, weakly assimilates L-alanine, lactic acid, potassium 5-ketogluconate and potassium 2-ketogluconate, and does not assimilate potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, itaconic acid, suberic acid, sodium malonate, sodium acetate,

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain 5YN7-3^T and closely related species. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bootstrap values below 50% are not indicated. Bar, 0.02 changes per nucleotide position.

3-hydroxybenzoic acid, L-serine, propionic acid, valeric acid, L-histidine, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline (API 20NE and API ID 32GN test strips). Positive for the following enzyme activities (API ZYM test strip): alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and β -glucosidase. Negative for the following enzyme activities: esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Major fatty acids are C_{18:1} ω 7c (51.2%), C_{19:0} cyclo ω 8c (25.0%), C_{18:0} (12.9%) and C_{16:0} (10.8%) (>10% of the total fatty acids). Ubiquinone-10 is the predominant quinone. The DNA G+C content of the type strain is 66.5 mol%.

The type strain, 5YN7-3^T (=KACC 12910^T =DSM 21341^T), was isolated from wetland soil of Yongneup, Republic of Korea.

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References

- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (1987). *Current Protocols in Molecular Biology*. New York: Wiley.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 57, 2259–2261.
- 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., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* 46, 234–239.
- Im, W. T., Yokota, A., Kim, M. K. & Lee, S. T. (2004). *Kaistia adipata* gen. nov., sp. nov., a novel α -proteobacterium. *J Gen Appl Microbiol* 50, 249–254.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University 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.
- Kwon, S.-W., Kim, B.-Y., Kim, W.-G., Yoo, K.-H., Yoo, S.-H., Son, J.-A. & Weon, H.-W. (2008). *Paludibacterium yongneupense* gen. nov., sp. nov., isolated from a wetland, Yongneup, in Korea. *Int J Syst Evol Microbiol* 58, 190–194.
- Lee, H.-W., Yu, H.-S., Liu, Q.-M., Jung, H.-M., An, D.-S., Im, W.-T., Jin, F.-X. & Lee, S.-T. (2007). *Kaistia granuli* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* 57, 2280–2283.
- 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.
- Reasoner, D. J. & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* 49, 1–7.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.
- 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.
- 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.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173, 697–703.
- Weon, H.-Y., Kim, B.-Y., Yoo, S.-H., Kwon, S.-W., Go, S.-J. & Stackebrandt, E. (2008a). *Uliginosibacterium gangwonense* gen. nov., sp. nov., isolated from a wetland, Yongneup, in Korea. *Int J Syst Evol Microbiol* 58, 131–135.
- Weon, H.-Y., Lee, C.-M., Hong, S.-B., Kim, B.-Y., Yoo, S.-H., Kwon, S.-W. & Go, S.-J. (2008b). *Kaistia soli* sp. nov., isolated from a wetland in Korea. *Int J Syst Evol Microbiol* 58, 1522–1524.