Pontibacter akesuensis sp. nov., isolated from a desert soil in China

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A Gram-negative, non-motile, rod-shaped bacterium, designated strain AKS 1^T, was isolated from a desert soil sample collected from Akesu, XinJiang Province, China. A taxonomic study, including phylogenetic analysis based on 16S rRNA gene sequences and phenotypic characteristics, was performed on the novel isolate. The predominant menaquinone of strain AKS 1^T was MK-7. The major fatty acids included i-C_{15:0}, ai-C_{17:1} B/i-C_{17:1} I and i-C_{17:0} 3-OH. The G + C content of the DNA was 51.4 mol%. Based on the results of phenotypic and genotypic characteristics, strain AKS 1^T should be assigned as representing a novel species of the genus *Pontibacter*, for which the name *Pontibacter akesuensis* is proposed. The type strain is AKS 1^T (=KCTC 12758^T = CCTCC AB 206086^T).

The genus *Pontibacter* belongs to the phylum *Bacteroidetes* and was first described by Nedashkovskaya *et al.* (2005b) based on a single isolate from an unidentified sea anemone. The main characteristics of members of the phylum *Bacteroidetes* include the ability to move by gliding (many lack motility), a rod-shaped or ring-shaped morphology, possession of Gram-negative cell walls and pigmentation. The majority of the members of the phylum *Bacteroidetes* have been isolated from seawater, sediment or algae. Some marine bacteria of the phylum *Bacteroidetes* possess menaquinone 7 (MK-7) as their main respiratory quinone (Bowman *et al.*, 2003; Brettar *et al.*, 2004a, b; Nedashkovskaya *et al.*, 2003, 2004, 2005a, b; Raj & Maloy, 1990; Van Trappen *et al.*, 2004; Yi & Chun, 2004; Yoon *et al.*, 2004, 2005a, b, c). The present investigation was designed to establish the taxonomic position of a novel isolate that formed a common clade with the genus *Pontibacter* within the phylum *Bacteroidetes*. Although the novel isolate has some properties characteristic of the phylum *Bacteroidetes*, it also has many special properties that are distinct from other

members of the phylum. Genotypic and phenotypic data show that the strain represents a novel species of the genus *Pontibacter*.

Strain AKS 1^T was isolated from a desert soil sample collected from Akesu, XinJiang Province, north-west China, by using the traditional dilution plating method. LB agar (Sambrook & Russell, 2002) was used for selective isolation and cultures were incubated at 30 °C for 1 week. The novel strain was maintained on LB agar slants at 4 °C and as glycerol suspensions (20 %, v/v) at –80 °C. Biomass for molecular systematic and chemotaxonomic studies was obtained after incubation in LB at 30 °C for 2 days in shake flasks (about 150 r.p.m.), with the pH adjusted to pH 7.8 using NaOH.

Cell morphology of the novel isolate was observed by light microscopy (BH2; Olympus) and by transmission electron microscopy (H-7650; Hitachi) after 24 h growth on LB medium. Acid production from carbohydrates was tested using the media and methods described by Gordon *et al.* (1974). Sole carbon and sole carbon/nitrogen source utilization was investigated using the Biolog GN2 Microplate system (Nedashkovskaya *et al.*, 2005b) according to the manufacturers' instructions. Growth was tested at 4,

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AKS 1^T is DQ672723.

Fig. 1. Transmission electron micrographs of cells of strain AKS 1^T grown on LB agar medium for 24 h at 30 °C. Bars, 500 nm (a, d) and 1 µm (b, c).

10, 27, 30, 36, 37, 40 and 45 °C on LB medium. Requirement for and tolerance of NaCl (1, 4, 7, and 10 %) and pH (3.0, 6.0, 7.0, 7.8, 8.0, 10.0, 11.0, 12.0) were tested using modified LB medium. Susceptibility to antibiotics was examined as described by Nedashkovskaya *et al.* (2003, 2005b) with results recorded after incubation at 30 °C for up to 3 days. Gram-staining and other phenotypic characteristics were determined according to the method described by Gerhardt *et al.* (1994).

Menaquinones were extracted from lyophilized cells and determined using the procedures reported by Akagawa-Matsushita *et al.* (1992) and Hu *et al.* (2001). Biomass for the quantitative fatty acid analysis was prepared by collecting growth from shake flasks of LB medium that had been incubated for 2 days at 30 °C. Fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system as described by Sasser (1990).

Table 1. Phenotypic characteristics that differentiate strain AKS 1^T from *Pontibacter actin-iarum* KMM 6156^T

Both strains have a similar colony colour (pink) and give a positive result in tests for β -galactosidase activity. Both strains are able to hydrolyse aesculin and trypsin, but not Tweens 40 or 80. +, Positive; –, negative. Data are from Nedashkovskaya *et al.* (2005b) and this study.

Characteristic	Strain AKS 1 ^T	<i>P. actiniarum</i> KMM 6156 ^T
Habitat	Soil	Marine
Cell size (µm)	0.7–0.75 × 1.5–1.6	0.3–0.4 × 1.2–1.9
Gliding motility	–	+
α -Galactosidase activity	+	–
Salinity range (%)	0–4	0–10
Temperature range (°C)	4–36	6–43
Hydrolysis of:		
Agar	+	–
Starch	–	+
DNA G+C content (mol%)	51.4	48.7

Table 2. Cellular fatty acid content (%) of strain AKS 1^T and its closest relative, *Pontibacter actiniarum* KMM 6156^T

Summed fatty acids are those could not be separated by the Microbial Identification System. Data are from Nedashkovskaya *et al.* (2005b) and this study. Values are percentages of total fatty acids and values of <1% are not shown.

Fatty acid	AKS 1 ^T	KMM 6156 ^T
i-C _{15:1} F	1.97	—
i-C _{15:1} I/C _{13:0} 3-OH	1.72	2.3
i-C _{15:0}	25.08	28.8
C _{15:0}	1.77	—
i-C _{16:0}	1.46	—
i-C _{15:0} 2-OH/C _{16:1} ω7 <i>c</i> /C _{16:1} ω7 <i>t</i>	1.94	14.7
C _{16:0}	1.68	0.2
i-C _{15:0} 3-OH	3.33	3.0
i-C _{17:1} ω9 <i>c</i>	7.69	—
ai-C _{17:1} B/i-C _{17:1} I	19.97	31.3
Unknown 16.582	1	—
ai-C _{18:0} /C _{18:2} ω6, 9 <i>c</i>	—	1.8
i-C _{17:0}	6.06	2.2
C _{17:1} ω6 <i>c</i>	2.87	1.4
C _{17:0} /C _{18:0}	2.41	—
i-C _{18:1} H/C _{18:1} ω9 <i>c</i> /C _{18:1} ω7 <i>c</i> /C _{18:1} ω5 <i>c</i>	2.30	—
i-C _{17:0} 3-OH	11.46	6.5

For the determination of G + C composition, genomic DNA was prepared according to the method of Marmur (1961). The DNA G + C content of strain AKS 1^T was determined using the thermal denaturation method (Mandel & Marmur, 1968).

PCR amplification of the 16S rRNA gene was performed as described by Xu *et al.* (2003). Phylogenetic analysis was performed using the PHYLIP (Felsenstein, 1993) and MEGA version 3.1 (Kumar *et al.*, 2001) software packages after

multiple alignment of data by using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model; Kimura, 1980, 1983) and clustering were based on the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1500 resamplings (Felsenstein, 1985).

Cells of strain AKS 1^T were collected for morphological observations after being cultured on LB agar for 24 h. Cells were prepared for transmission electron microscopy as described by Nedashkovskaya *et al.* (2005b). Cells of strain AKS 1^T ranged from 0.7 to 0.75 μm in width and from 1.5 to 1.6 μm in length and an unknown rosy substance was observed around the cells (Fig. 1).

Colonies of strain AKS 1^T were circular, convex, shiny, smooth and pink-coloured with diameters of 1–2 mm on LB agar for 48 h. Colonies of strain AKS 1^T were rosy and wet. Other physiological and biochemical characteristics are given in Table 1 and in the species description.

The predominant menaquinone was MK-7. The major fatty acids of strain AKS 1^T were i-C_{15:0}, ai-C_{17:1} B/i-C_{17:1} I and i-C_{17:0} 3-OH; detailed fatty acid profiles are shown in Table 2. The G + C content of the DNA was 51.4 mol%.

The almost complete 16S rRNA gene sequence (1408 bp) of strain AKS 1^T was determined and was used to construct a phylogenetic tree with reference sequences selected from the GenBank database following BLAST searches (unidentified and unpublished sequences were not considered). Phylogenetic analyses revealed that strain AKS 1^T belongs to the phylum *Bacteroidetes* and is most closely related to the type strain of the only recognized species of the genus *Pontibacter*, *Pontibacter actiniarum* KMM 6156^T (Fig. 2). The two strains formed a common group supported by a high bootstrap value (100%).

Fig. 2. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain AKS 1^T and representative members of the phylum *Bacteroidetes*. Numbers at nodes indicate bootstrap values (%). Bar, 0.02 substitutions per nucleotide position.

Although *P. actiniarum* KMM 6156^T and strain AKS 1^T shared up to 95 % 16S rRNA gene sequence similarity and had similar menaquinone fingerprints (MK-7), they could be differentiated by their habitats and physiological and biochemical characteristics (Table 1). Strain AKS 1^T was isolated from a desert soil, but *P. actiniarum* KMM 6156^T was isolated from a marine habitat. Biolog GN2 tests indicated that about 70 different compounds could be utilized as sole carbon or sole carbon/nitrogen sources by strain AKS 1^T, while *P. actiniarum* KMM 6156^T could only use 20 different compounds. Strain AKS 1^T was resistant to kanamycin and sensitive to neomycin and gentamicin, but strain KMM 6156^T was sensitive to kanamycin and resistant to neomycin and gentamicin. The features that differentiate strain AKS 1^T from its nearest phylogenetic neighbour, *P. actiniarum* KMM 6156^T, are shown in Table 1.

In conclusion, genotypic, chemotaxonomic and phenotypic data demonstrate that strain AKS 1^T represents a novel species of the genus *Pontibacter*, for which we propose the name *Pontibacter akesuensis* sp. nov.

Description of *Pontibacter akesuensis* sp. nov.

Pontibacter akesuensis (a.ke.su.en'sis. N.L. masc. adj. *akesuensis* pertaining to Akesu, a city of XinJiang Province in the north-west of China from where the type strain was isolated).

Cells range from 0.7 to 0.75 µm in width and from 1.5 to 1.6 µm in length. Colonies are circular, 1–2 mm in diameter, convex, shiny, pink and smooth on nutrient agar plates. Gram-negative. Cells are non-motile, non-spore-forming, aerobic and chemoorganotrophic. A ropy substance is observed around cells. Grows well in very dry environmental conditions, does not require Na⁺ or other special substances for growth. Growth occurs at between 4 and 36 °C, with an optimum at 28–30 °C. Growth occurs at pH 7–11, with optimal growth at pH 7.6–8.0. Growth occurs at 0–4 % NaCl. Pigment can be extracted with organic solvents. Gelatin, DNA and starch are decomposed, but casein, Tweens 20, 40 and 80, cellulose and chitin are not hydrolysed. Forms acid from aesculin, D-fructose, D-sucrose, inositol, L-raffinose, D-maltose, N-acetylglucosamine, L-fucose and D-glucose, but not from D-cellobiose, D-galactose, D-lactose, D-melibiose, L-rhamnose, L-sorbose or DL-xylose. In Biolog GN tests, the type strain utilizes α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, L-arabinose, D-arabitol, N-acetyl-D-glucosamine, D-cellobiose, D-fructose, L-fucose, L-alanine, D-galactose, gentiobiose, α-D-glucose, myo-inositol, maltose, D-mannitol, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, lactulose, xylitol, methyl pyruvate, monomethyl succinate, acetic acid, D-galactonic acid lactone, cis-aconitic acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, propionic

acid, quinic acid, D-saccharic acid, succinic acid, bromo-succinic acid, succinamic acid, L-alaninamide, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyrroglutamic acid, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, inosine, thymidine, putrescine, 2,3-butanediol and glucose 6-phosphate. However, it does not utilize Tweens 20, 40, or 80, adonitol, i-erythritol, uridine, urocanic acid, α-D-lactose, D-mannose, citric acid, formic acid, *p*-hydroxyphenylacetic acid, malonic acid, sebacic acid, glucuronamide, D-alanine, D-serine, phenyl-ethylamine, 2-aminoethanol, DL-α-glycerol phosphate or glucose 1-phosphate. Nitrate is not reduced. Indole and H₂S (triple sugar iron reaction) are negative. Susceptible to the following antibiotics: ampicillin, benzylpenicillin, chloramphenicol, neomycin, gentamicin, erythromycin, carbenicillin, lincomycin and tetracycline. Resistant to kanamycin, polymyxin B and streptomycin. The predominant menaquinone is MK-7. The fatty acid profile contains i-C_{15:0} (25.08 %), ai-C_{17:1} B/i-C_{17:1} I (19.97 %), i-C_{17:0} 3-OH (11.46 %), i-C_{17:1} ω9c (7.69 %), i-C_{17:0} (6.06 %), i-C_{15:0} 3-OH (3.33 %), C_{17:1} ω6c (2.87 %), i-C_{15:1} F (1.97 %), i-C_{15:0} 2-OH/C_{16:1} ω7c/C_{16:1} ω7t (1.94 %), C_{15:0} (1.77 %), i-C_{15:1} I/C_{13:0} 3-OH (1.72 %), C_{18:0} (1.71 %), C_{16:0} (1.68 %), and i-C_{16:0} (1.46 %). The DNA G + C content is 51.4 mol%.

The type strain, AKS 1^T (=KCTC 12758^T=CCTCC AB 206086^T), was isolated from the surface layer of a desert soil from Akesu, XinJiang Province, China.

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References

- Akagawa-Matsushita, M., Itoh, T., Katayama, Y., Kuraishi, H. & Yamasato, K. (1992). Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J Gen Microbiol* **138**, 2275–2281.
- Bowman, J. P., Nichols, C. M. & Gibson, J. A. E. (2003). *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. *Int J Syst Evol Microbiol* **53**, 1343–1355.
- Brettar, I., Christen, R. & Höfle, M. G. (2004a). *Belliella baltica* gen. nov., sp. nov., a novel marine bacterium of the *Cytophaga-Flavobacterium-Bacteroides* group isolated from surface water of the central Baltic Sea. *Int J Syst Evol Microbiol* **54**, 65–70.
- Brettar, I., Christen, R. & Höfle, M. G. (2004b). *Aquiflexum balticum* gen. nov., sp. nov., a novel marine bacterium of the *Cytophaga-Flavobacterium-Bacteroides* group isolated from surface water of the central Baltic Sea. *Int J Syst Evol Microbiol* **54**, 2335–2341.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

- Felsenstein, J. (1985). Conference limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Gordon, R. E., Barnett, D. A., Handerman, J. E. & Pang, C. H. N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- Hu, H. Y., Lim, B. R., Goto, N. & Fujie, K. (2001). Analytical precision and repeatability of respiratory quinones for quantitative study of microbial community structure in environmental samples. *J Microbiol Methods* **47**, 17–24.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. *J Mol Evol* **16**, 111–120.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003). *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga-Flavobacterium-Bacteroides*. *Int J Syst Evol Microbiol* **53**, 81–85.
- Nedashkovskaya, O. I., Vancanneyt, M., Van Trappen, S., Vandemeulebroecke, K., Lysenko, A. M., Rohde, M., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Swings, J. (2004). Description of *Algoriphagus aquimarinus* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus winogradskyi* sp. nov., from sea water and algae, transfer of *Hongiella halophila* Yi and Chun 2004 to the genus *Algoriphagus* as *Algoriphagus halophilus* comb. nov. and emended descriptions of the genera *Algoriphagus* Bowman *et al.* 2003 and *Hongiella* Yi and Chun 2004. *Int J Syst Evol Microbiol* **54**, 1757–1764.
- Nedashkovskaya, O. I., Kim, S. B., Lee, D. H., Lysenko, A. M., Shevchenko, L. S., Frolova, G. M., Mikhailov, V. V., Lee, K. H. & Bae, K. S. (2005a). *Roseivirga ehrenbergii* gen. nov., sp. nov., a novel marine bacterium of the phylum ‘*Bacteroidetes*’, isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **55**, 231–234.
- Nedashkovskaya, O. I., Kim, S. B., Suzuki, M., Shevchenko, L. S., Lee, M. S., Lee, K. H., Park, M. S., Frolova, G. M., Oh, H. W. & other authors (2005b). *Pontibacter actiniarum* gen. nov., sp. nov., a novel member of the phylum ‘*Bacteroidetes*’, and proposal of *Reichenbachella* gen. nov. as a replacement for the illegitimate prokaryotic generic name *Reichenbachia* Nedashkovskaya *et al.* (2003). *Int J Syst Evol Microbiol* **55**, 2583–2588.
- Raj, H. D. & Maloy, S. R. (1990). Proposal of *Cyclobacterium marinus* gen. nov., comb. nov. for a marine bacterium previously assigned to the genus *Flectobacillus*. *Int J Syst Bacteriol* **40**, 337–347.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sambrook, J. & Russell, D. W. (2002). *Molecular Cloning: a Laboratory Manual*, pp. 1595–1596, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 1–6.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**, 4876–4882.
- Van Trappen, S., Vandecandelaere, I., Mergaert, J. & Swings, J. (2004). *Algoriphagus antarcticus* sp. nov., a novel psychrophile from microbial mats in Antarctic lakes. *Int J Syst Evol Microbiol* **54**, 1969–1973.
- Xu, P., Li, W. J., Xu, L. H. & Jiang, C. L. (2003). A microwave-based method for genomic DNA extraction from *Actinomycetes*. *Microbiology (Beijing)* **30**, 82–84 (in Chinese).
- Yi, H. & Chun, J. (2004). *Hongiella manitolivorans* gen. nov., sp. nov., *Hongiella halophila* sp. nov. and *Hongiella ornithinivorans* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **54**, 157–162.
- Yoon, J.-H., Yeo, S.-H. & Oh, T.-K. (2004). *Hongiella marincola* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1845–1848.
- Yoon, J.-H., Kang, S.-J., Lee, C.-H. & Oh, T.-K. (2005a). *Marinicola seohaensis* gen. nov., sp. nov., isolated from sea water of the Yellow Sea, Korea. *Int J Syst Evol Microbiol* **55**, 859–863.
- Yoon, J.-H., Kang, S.-J., Jung, S.-Y., Lee, C.-H. & Oh, T.-K. (2005b). *Algoriphagus yeomjeoni* sp. nov., isolated from a marine solar saltern in the Yellow Sea, Korea. *Int J Syst Evol Microbiol* **55**, 865–870.
- Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2005c). *Algoriphagus locisalis* sp. nov., isolated from a marine solar saltern. *Int J Syst Evol Microbiol* **55**, 1635–1639.