Arenimonas daejeonensis sp. nov., isolated from compost

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A Gram-negative, aerobic, motile and rod-shaped bacterium, designated strain T7-07^T, was isolated from compost in Daejeon, Korea. Phylogenetic analysis based on 16S rRNA gene sequencing showed that strain T7-07^T had 99.0% gene sequence similarity with *Arenimonas malthae* KACC 14618^T and 94.7–95.9% with other recognized species of the genus *Arenimonas*. Cells formed creamy white to yellowish colonies on R2A agar and contained Q-8 as the predominant ubiquinone, C_{15:0} iso, C_{16:0} iso, C_{17:1} iso ω 9c and C_{11:0} iso 3-OH as the major fatty acids, and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine and an unknown aminolipid as the major polar lipids. The DNA G + C content of strain T7-07^T was 68.3 mol%. DNA–DNA reassociation experiments between T7-07^T and *Arenimonas malthae* KACC 14618^T resulted in a mean relatedness value of 22.2%. Combined genotypic and phenotypic data supported the conclusion that the strain T7-07^T represents a novel species, for which the name *Arenimonas daejeonensis* sp. nov. is proposed. The type strain is T7-07^T (=KCTC 12667^T=DSM 18060^T).

Compost is an important source for the isolation of thermophiles and moderate thermophiles. However, to date, only moderate thermophiles have been found and most of them belong to the genera *Bacillus*, *Geobacillus* and *Clostridium* and other related genera (Lajudie, 1956; Kane & Mullins, 1973; Winker & Woese, 1991; Moriya *et al.*, 2011). The genus *Arenimonas*, which was proposed by Kwon *et al.* (2007), is a member of the family *Xanthomonadaceae* in the class *Gammaproteobacteria*, and at the time of writing, the genus *Arenimonas* comprised four recognized species isolated from different environmental sources. *Arenimonas composti* KCTC 12666^T was isolated from compost (Jin *et al.*, 2007), *A. donghaensis* KACC 11381^T from an anaerobic sludge blanket reactor (Kwon *et al.*, 2007), *A. malthae* CC-JY-1^T from a soil sample (Young *et al.*, 2007), and *A. oryzae* KACC 14607^T from a field of rice (Aslam *et al.*, 2009). The type species of this genus is *A. donghaensis*.

Strain T7-07^T was isolated during an investigation of the bacterial community present in compost from a farm in Daejeon, Korea. A 1 g compost sample was collected and serially diluted in 0.85% saline solution. Aliquots of each serial dilution were spread on R2A agar (Difco) and incubated at 30 °C for 7 days. A creamy white to yellowish colony, designated strain T7-07^T, was isolated and sub-cultivated on R2A agar at 30 °C for 48 h. Based on phylogenetic, phenotypic and chemotaxonomic analysis, it is suggested that the new strain represents a novel species of the genus *Arenimonas*. For further classification, strain T7-07^T was investigated using a polyphasic approach.

The Gram reaction was performed with 2% (w/v) crystal violet, iodine fixation and decolorization. Decolorization was accomplished using 95% ethanol followed by a distilled water wash and counterstaining with safranin (Gerhardt *et al.*, 1994). Cell morphology and motility were observed under a phase-contrast microscope (Optiphot, Nikon; 1000× magnification) with cells grown on R2A agar for 1–3 days. The presence of flagella was determined by transmission electron microscopy (JEM-10111; JEOL)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T7-07^T is AM229325.

A supplementary table and a supplementary figure are available with the online version of this paper.

after negative staining with 2 % (w/v) uranyl acetate. Oxidase activity was tested using 1 % tetramethyl-*p*-phenylenediamine (Tarrand & Gröschel, 1982) and catalase activity was tested using 3 % H₂O₂. Growth was investigated at 4, 10, 15, 20, 25, 30, 32, 37, 42 and 45 °C, in the presence of 1, 2, 3 and 5 % (w/v) NaCl and at pH 5–10 in increments of 1 pH unit. The following biological buffers were used to adjust the pH: Na₂HPO₄/NaH₂PO₄ for pH 5–7 and Na₂CO₃/NaHCO₃ for pH 8–10 (Gomori, 1955; Bates & Bower, 1956). Degradation of DNA was investigated using DNA agar (Difco) supplemented with 0.01 % toluidine blue (Merck). Degradation of casein, chitin and starch (Atlas, 1993) and cellulose and xylan (Ten *et al.*, 2004) was also examined. Antibiotic-susceptibility tests were performed in duplicate using filter-paper discs containing the following: ampicillin (10 µg), erythromycin (30 µg), kanamycin (30 µg), neomycin (30 µg), penicillin G (10 U) and streptomycin (10 µg) (Sigma). Carbon source utilization tests, acid production tests and additional physiological analyses were performed using API 20NE (bioMérieux), Biolog GN2 (Biolog), API 50CH (bioMérieux) and API

ZYM (bioMérieux) galleries according to the manufacturers' instructions.

Fatty acid methyl esters were prepared from bacteria grown in R2A medium for 2 days at 30 °C. Samples were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by GC (6890; Hewlett Packard) and were identified according to the Microbial Identification software package (Sasser, 1990). Identification and comparison were made using the Aerobe (TSBA version 3.9) database. Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1977).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA genes and sequencing of purified PCR products were carried out according to procedures described by Rainey *et al.* (1996). The 16S rRNA gene sequences were aligned with published sequences retrieved from EMBL using CLUSTAL_X (Thompson *et al.*, 1997) and

Table 1. Phenotypic and chemotaxonomic characteristics that distinguish strain T7-07^T from other species of the genus *Arenimonas*

Strains: 1, T7-07^T; 2, *A. malthae* KACC 14618^T; 3, *A. composti* KCTC 12666^T; 4, *A. donghaensis* KACC 11381^T; 5, *A. oryzae* KACC 14607^T. All data from this study unless indicated. All strains were positive for acid phosphatase, alkaline phosphatase, α -chymotrypsin, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase. All strains were negative for nitrate and nitrite reduction, indole production, glucose fermentation, aesculin hydrolysis, *N*-acetyl- β -glucosaminidase, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase and α -mannosidase. +, Positive; –, negative; AL, unknown aminolipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid; PME, phosphatidylmethylethanolamine.

Characteristic	1	2	3	4	5
Cell size (µm)	0.4–0.5 × 1.0–1.5	0.3–0.4 × 1.6–2.7*	0.4–0.5 × 1.0–1.8	0.4–0.5 × 1.0–1.8†	0.5–0.6 × 2.3–2.6‡
Colony colour	Creamy white to yellowish	Transparent to brownish	Creamy white to brownish	Yellowish white	Yellowish to creamy white
Temperature range (°C)	15–37	15–37	20–42	4–37	15–37
Growth range of NaCl (% w/v)	0–3.0	0–2.0*	0–2.0	0–3.0†	0‡
Catalase	+	+	–	+	+
Arginine dihydrolase	+	–	+	–	–
Urease	+	–	+	–	–
Gelatin hydrolysis	–	+	–	+	–
Enzyme activities:					
Lipase (C14)	–	–	–	–	+
Leucine arylamidase	+	+	+	–	+
Trypsin	+	+	–	+	+
Valine arylamidase	–	–	–	–	+
Cystine arylamidase	–	–	–	–	+
Major polar lipid	DPG, PE, PG, PME, AL	DPG, PE, PG*	PE, PG, PME	DPG, PE, PG, PL, AL†	DPG, PE, PG‡
DNA G + C content (mol%)	68.3	70.4*	70.8	65.0†	65.8‡

*Data taken from Young *et al.* (2007).

†Data taken from Kwon *et al.* (2007).

‡Data taken from Aslam *et al.* (2009).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences using the neighbour-joining method (Saitou & Nei, 1987) showing the positions of strain T7-07^T and related taxa. Numbers at branching points refer to bootstrap values (1000 resamplings, only values >50 % shown). Bar, 2 substitutions per 100 nt positions.

edited using BIOEDIT (Hall, 1999). The phylogenetic tree was constructed on the basis of the neighbour-joining method (Saitou & Nei, 1987); distances were estimated using the Kimura two-parameter model (Kimura, 1983) with MEGA version 3.1 (Kumar *et al.*, 2004). The resultant neighbour-joining tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets. DNA G+C content was determined by HPLC after hydrolysis as described by Tamaoka & Komagata (1984) and non-methylated λ DNA (Sigma) was used as a standard. DNA–DNA hybridization to determine genomic relatedness was performed fluorometrically by the method of Ezaki *et al.* (1989) using DNA probes labelled with photobiotin (A1935; Sigma) and microdilution wells (96-well microplate; Greiner Bio-one).

Strain T7-07^T formed visible colonies on R2A agar within 48 h when incubated at 30 °C. Growth occurred at temperatures ranging from 15 to 37 °C, but no growth was observed at 42 °C or at temperatures below 10 °C. Growth occurred at pH 6–9, but no growth was observed at pH 5 or 10. The colonies were creamy white to yellowish, translucent, convex and circular with entire edges. Cells were Gram-negative-staining, catalase and oxidase-positive and were motile rods with a polar flagellum. Detailed physiological and biochemical characteristics are summarized in Table 1 and in the species description.

The only respiratory quinone was ubiquinone Q-8. The predominant fatty acids were C_{15:0} iso (26.76 %), C_{16:0} iso

(24.51 %), C_{17:0} iso ω 9c (20.54 %) and C_{11:0} iso 3-OH (7.43 %). The entire fatty acid profile is available in Table S1. The polar lipids were composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine, an unknown aminolipid and two unknown phospholipids; a two-dimensional TLC of the polar lipids from strain T7-07^T is available in Fig. S1 (in IJSEM Online).

The almost-complete 16S rRNA gene sequence (approx. 1480 nt) of strain T7-07^T was determined and compared with those of representative species within the genus *Arenimonas* (Fig. 1). Strain T7-07^T showed the highest similarity to *A. malthae* CC-JY-1^T, with value of 98.6 % and low levels of sequence similarity to *A. composti* KCTC 12666^T, *A. donghaensis* KACC 11381^T and *A. oryzae* KACC 14607^T, with values of 95.9 %, 95.1 % and 94.5 %, respectively. Strain T7-07^T showed similarity values of 82.3–92.2 % with other representative species within the family *Xanthomonadaceae*. The DNA–DNA hybridization level between strains T7-07^T and *A. malthae* CC-JY-1^T was 22.2 %, which was well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987).

On the basis of 16S rRNA gene dissimilarity to related taxa and a phylogenetically distinct position, together with distinctive phenotypic characteristics and low levels of genomic relatedness, strain T7-07^T represents a novel species, for which the name *Arenimonas daejeonensis* sp. nov. is proposed.

Description of *Arenimonas daejeonensis* sp. nov.

Arenimonas daejeonensis (dae.je.on.en'sis. N.L. fem. adj. *daejeonensis* pertaining to Daejeon, a city in South Korea, from where the type strain was isolated).

Cells are Gram-negative-staining, non-spore-forming, motile rods ($0.4\text{--}0.5 \times 1.0\text{--}1.5\ \mu\text{m}$) with a polar flagellum. Good growth is observed on R2A agar and nutrient agar but not on tryptic soy agar. Growth occurs at $15\text{--}37\ ^\circ\text{C}$ (optimum $30\ ^\circ\text{C}$) and at pH 6–9 (optimum pH 7). Growth occurs in the presence of 1 and 3 % (w/v) NaCl, but not in concentrations above 4 %. Colonies are creamy white to yellowish, translucent, convex and circular with entire edges. Oxidase- and catalase-positive. Hydrolyses casein and DNA but does not hydrolyse chitin, starch, cellulose or xylan. Positive result in tests for arginine dihydrolase and urease activities, but negative results for nitrate and nitrite reduction, indole production, glucose fermentation, aesculin hydrolysis, gelatin hydrolysis and β -galactosidase (API 20NE test strip). Positive result for the following enzyme activities (API ZYM test strip): acid phosphatase, alkaline phosphatase, α -chymotrypsin, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and trypsin, but negative result for the following enzyme activities: *N*-acetyl- β -glucosaminidase, cystine arylamidase, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -mannosidase, lipase (C14) and valine arylamidase. Acid is produced from 5-ketogluconate (weakly), but not from *N*-acetylglucosamine, adonitol, aesculin, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, fructose, D-fucose, L-fucose, galactose, gentiobiose, gluconate, glucose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, lactose, D-lyxose, maltose, mannitol, mannose, melezitose, melibiose, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -D-xylose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylose or L-xylose. Assimilates L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine (weakly), L-aspartic acid (weakly), L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, β -hydroxybutyric acid, L-proline, pyruvic acid methyl ester (weakly), succinamic acid (weakly), succinic acid monomethyl ester (weakly), L-threonine (weakly), Tween 40 (weakly) and Tween 80 (weakly) and does not assimilate acetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *cis*-aconitic acid, adonitol, D-alanine, γ -aminobutyric acid, 2-aminoethanol, L-arabinose, L-arabitol, bromosuccinic acid, 2,3-butanediol, DL-carnitine, D-cellobiose, citric acid, α -cyclodextrin, dextrin, i-erythritol, formic acid, D-fructose, L-fructose, D-galactonic acid lactone, D-galactose, D-galacturonic acid, gentiobiose, D-glucuronic acid, D-glucosaminic acid, α -D-glucose, α -D-glucose 1-phosphate, D-glucose 6-phosphate, glucuronamide, D-glucuronic acid, glycerol, DL- α -glycerol phosphate, glycogen, L-histidine, hydroxy-L-proline, α -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, inosine, *myo*-inositol, itaconic acid, α -ketobutyric

acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, α -D-lactose, lactulose, L-leucine, malonic acid, maltose, D-mannitol, D-mannose, D-melibiose, methyl β -D-glucoside, L-ornithine, L-phenylalanine, phenylethylamine, propionic acid, D-psicose, putrescine, L-pyroglutamic acid, quinic acid, D-raffinose, L-rhamnose, D-saccharic acid, sebacic acid, D-serine, L-serine, D-sorbitol, succinic acid, sucrose, thymidine, D-trehalose, turanose, uridine, urocanic acid or xylitol (Biolog GN2 system). Susceptible to kanamycin and neomycin. Resistant to ampicillin, erythromycin, penicillin G and streptomycin. The major respiratory quinone is ubiquinone Q-8. The predominant fatty acids are C_{15:0} iso, C_{16:0} iso, C_{17:0} iso ω 9c and C_{11:0} iso 3-OH.

The type strain, T7-07^T (=KCTC 12667^T=DSM 18060^T), was isolated from compost. The G+C content of the genomic DNA is 68.3 mol%.

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References

- Aslam, Z., Park, J. H., Kim, S. W., Jeon, C. O. & Chung, Y. R. (2009). *Arenimonas oryziterrae* sp. nov., isolated from a field of rice (*Oryza sativa* L.) managed under a no-tillage regime, and reclassification of *Aspromonas composti* as *Arenimonas composti* comb. nov. *Int J Syst Evol Microbiol* **59**, 2967–2972.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Bates, R. G. & Bower, V. E. (1956). Alkaline solutions for pH control. *Anal Chem* **28**, 1322–1324.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- 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.
- Gomori, G. (1955). Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.
- 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.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Jin, L., Kim, K. K., Im, W. T., Yang, H. C. & Lee, S. T. (2007). *Aspromonas komposti* gen. nov., sp. nov., a novel member of the family Xanthomonadaceae. *Int J Syst Evol Microbiol* **57**, 1876–1880.
- Kane, B. E. & Mullins, J. T. (1973). Thermophilic fungi in a municipal waste compost system. *Mycologia* **65**, 1087–1100.

- 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, B. Y., Weon, H. Y., Baek, Y. K. & Go, S. J. (2007).** *Arenimonas donghaensis* gen. nov., sp. nov., isolated from seashore sand. *Int J Syst Evol Microbiol* **57**, 954–958.
- Lajudie, J. (1956).** [Study on thermophilic soil microflora. I. Technics]. *Ann Inst Pasteur (Paris)* **91**, 778–780 (in French).
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977).** Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Moriya, T., Hikota, T., Yumoto, I., Ito, T., Terui, Y., Yamagishi, A. & Oshima, T. (2011).** *Calditerricola satsumensis* gen. nov., sp. nov. and *Calditerricola yamamurae* sp. nov., extreme thermophiles isolated from a high-temperature compost. *Int J Syst Evol Microbiol* **61**, 631–636.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996).** The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- 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.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tarrand, J. J. & Gröschel, D. H. M. (1982).** Rapid, modified oxidase test for oxidase-variable bacterial isolates. *J Clin Microbiol* **16**, 772–774.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M. S. & Lee, S.-T. (2004).** Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.
- 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* **25**, 4876–4882.
- 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.
- Winker, S. & Woese, C. R. (1991).** A definition of the domains Archaea, Bacteria and Eucarya in terms of small subunit ribosomal RNA characteristics. *Syst Appl Microbiol* **14**, 305–310.
- Young, C. C., Kämpfer, P., Ho, M. J., Busse, H. J., Huber, B. E., Arun, A. B., Shen, F. T., Lai, W. A. & Rekha, P. D. (2007).** *Arenimonas malthae* sp. nov., a gammaproteobacterium isolated from an oil-contaminated site. *Int J Syst Evol Microbiol* **57**, 2790–2793.