Arenimonas daejeonensis sp. nov., isolated from compost

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A Gram-negative, aerobic, motile and rod-shaped bacterium, designated strain T7-07T, was isolated from compost in Daejeon, Korea. Phylogenetic analysis based on 16S rRNA gene sequencing showed that strain T7-07T had 99.0 % gene sequence similarity with Arenimonas malthae KACC 14618T 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, C16 : 0 iso, C16 : 0 iso, C17 : 1 iso 9c and C11 : 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 T7-07T was 68.3 mol%. DNA–DNA reassociation experiments between T7-07T and Arenimonas malthae KACC 14618T resulted in a mean relatedness value of 22.2 %. Combined genotypic and phenotypic data supported the conclusion that the strain T7-07T represents a novel species, for which the name Arenimonas daejeonensis sp. nov. is proposed. The type strain is T7-07T (=KCTC 12667T=DSM 18060T).

Strain T7-07T 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 T7-07T, was isolated and subcultivated 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 T7-07T 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-1011; JEOL).
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 (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 T7-07T from other species of the genus Arenimonas

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

*Data taken from Young et al. (2007).
†Data taken from Kwon et al. (2007).
‡Data taken from Aslam et al. (2009).
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 bootstrapped 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 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 T7-07T 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 C15:0 iso (26.76 %), C16:0 iso (24.51 %), C17:0 iso ω9c (20.54 %) and C11:0 iso 3-OH (7.43 %). The entire fatty acid profile is available in Table S1. The polar lipids comprised of diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylethanolamine, an unknown aminolipid and two unknown phospholipids; a two-dimensional TLC of the polar lipids from strain T7-07T is available in Fig. S1 (in IJSEM Online).

The almost-complete 16S rRNA gene sequence (approx. 1480 nt) of strain T7-07T was determined and compared with those of representative species within the genus Arenimonas (Fig. 1). Strain T7-07T showed the highest similarity to A. malthae CC-JY-1T, with value of 98.6 % and low levels of sequence similarity to A. composti KCTC 12666T, A. donghaensis KACC 11381T and A. oryziterrae KACC 14607T, with values of 95.9 %, 95.1 % and 94.5 %, respectively. Strain T7-07T showed similarity values of 82.3–92.2 % with other representative species within the family Xanthomonadaceae. The DNA–DNA hybridization level between strains T7-07T and A. malthae CC-JY-1T 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 T7-07T 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 (0.4–0.5 × 1.0–1.5 μm) with a polar flagellum. Good growth is observed on R2A agar and nutrient agar but not on tryptic soy agar. Growth occurs at 15–37 °C (optimum 30 °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 nitrates 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-glucuronidase, β-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, arbutin, cellobiose, dulcitol, erythritol, fructose, D-fucose, L-fucose, galactose, gentiobiose, gluconate, gluco-β-arabitol, 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-glucuronidase, β-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, arbutin, cellobiose, dulcitol, erythritol, fructose, D-fucose, L-fucose, galactose, gentiobiose, gluconate, glucose, glycerol, glycerin, inositol, inulin, 2-ketogluconate, lactose, D-lyxose, maltose, mannitol, mannose, melezitose, rhamnose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylene or L-xylene. Assimilates L-alanine, L-alanine, D-alanine glycine, L-asparagine (weakly), L-aspartic acid (weakly), L-glutamic acid, glycol L-aspartic acid, glycol L-glutamic acid, β-hydroxybutyric acid, L-proline, pyruvic acid methyl ester (weakly), succinic 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-aminoimidobutyric acid, 2-aminoethanol, L-arabinose, L-arabitol, bromosuccinic acid, 2,3-butanediol, DL-carnitine, D-cellobiose, citric acid, α-cyclodextrin, dextrin, L-erythritol, formic acid, DL-fructose, L-fructose, D-galactonic acid lactone, D-galactose, D-galacturonic acid, gentiobiose, D-glucuronic acid, D-glucosaminic acid, α-D-glucoside, α-D-glucose 1-phosphate, D-glucose 6-phosphate, glucuronamide, D-glucuronic acid, glycerol, DL-α-glycerol phosphate, glycoll, L-histidine, hydroyl-L-proline, α-hydroxybutyric acid, α-hydroxybutyric acid, p-hydroxyphenylacetic acid, inosine, myo-inositol, itaconic acid, α-keto- butyric 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-rafinsose, L-rhamnose, D-saccharic acid, sebacic acid, D-serine, L-serine, D-sorbitol, sucrin acid, sucrose, thymidine, D-trehalose, turanose, uridine, urocanic acid or xyitol (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 C15:0 iso, C16:0 iso, C17:0 iso 3-OH and C11:0 iso 3-OH.

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

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References


