Acidovorax soli sp. nov., isolated from landfill soil

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A Gram-negative, aerobic, rod-shaped, non-motile strain, BL21^T, was isolated from landfill soil in Pohang, Korea. Strain BL21^T grew optimally at pH 7.0, 30 °C and 0 % NaCl (w/v). Phylogenetic analysis based on 16S rRNA gene sequence indicated that strain BL21^T belonged to the class *Betaproteobacteria* and was related to the genus *Acidovorax*. The 16S rRNA gene sequence of strain BL21^T was less than 98.30 % similar to those of other species in the genus *Acidovorax*. DNA–DNA hybridization values with phylogenetically related species of the genus *Acidovorax* were only 11.7–28.4 %. The major fatty acid components included summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{16:0}$, $C_{18:1}\omega7c$ and $C_{10:0}$ 3-OH. The DNA G+C content was 60.9 mol%. For these reasons, strain BL21^T (=KCTC 22399^T =JCM 15909^T) is proposed as a novel species in the genus *Acidovorax*, with the name *Acidovorax soli* sp. nov.

The genus *Acidovorax* was proposed by reclassification of *Pseudomonas* species by Willems *et al.* (1990). At the time of writing, the genus *Acidovorax* comprises 12 recognized species (Gardan *et al.*, 2000, 2003; Heylen *et al.*, 2008; Schaad *et al.*, 2008; Schulze *et al.*, 1999; Willems *et al.*, 1990, 1992). The species of the genus *Acidovorax* can be separated into two groups by occurrence and habitat. *Acidovorax defluvii*, *A. facilis*, *A. delafieldii*, *A. temperans* and *A. caeni* are in the group of environmental species which are found mainly in soil and water habitats. *Acidovorax citrulli*, *A. cattleyae*, *A. avenae*, *A. oryzae*, *A. anthurii*, *A. valerianellae* and *A. konjaci* are the phytopathogenic species which infect corn, oats, rice and many other plants. In this paper, we describe the morphological, biochemical and phylogenetic characteristics of a novel environmental species, *Acidovorax*-like strain BL21^T, isolated from landfill soil.

Strain BL21^T was isolated from landfill soil in Pohang, Korea, by using the standard dilution plating method on Luria–Bertani (LB, BBL) agar at 30 °C for 3 days. The culture conditions of strain BL21^T were determined by growth at a variety of temperatures, pH and NaCl concentrations for up to 5 days. Growth temperatures were monitored on LB agar at 4, 10, 15, 20, 25, 30, 37, 40, 42 and 45 °C. Optimum pH was measured in LB adjusted to pH 5.0–10.0 in increments of 0.5 units. NaCl tolerance was tested in LB containing NaCl at various concentrations (0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 %, w/v). Strain BL21^T grew at temperatures in the range 10–42 °C, with 0–1 % NaCl and at pH 6.0–9.0. Optimum conditions for growth of strain BL21^T were 30 °C, 0 % NaCl and pH 7.0. For the investigation of morphological and physiological characteristics, strain BL21^T was routinely cultivated on LB agar under optimum culture conditions. Cell morphology of strain BL21^T was observed by light microscopy (ECLIPSE 80*i*, Nikon) and transmission electron microscopy. Motility was determined using semisolid agar (Motility test medium, BBL) and observed by the spreading out of cells from the line of inoculation in the tube. Gram reaction was performed using the non-staining method described by Buck (1982). Catalase and oxidase activities of the strain were determined by bubble production in 3 % (v/v) hydrogen peroxide solution and by colour development in 1 % (w/v) *p*-tetramethyl phenylenediamine (bioMérieux), respectively. Enzyme activities and utilization of carbon sources were tested with commercial API 20NE and API ZYM kits (bioMérieux) and the Biolog GN2 MicroPlate assay, according to the manufacturers' protocols. Hydrolysis of DNA and casein were tested using DNase test agar (BBL) and skimmed milk (BBL), respectively, as described by Atlas (1993). Hydrolysis of cellulose was tested using the method of Gerhardt *et al.* (1994). The morphological, physiological and biochemical traits of strain BL21^T and closely related species of the genus *Acidovorax* are listed in Table 1 and the species description (see also Supplementary Fig. S1 available in IJSEM Online). Physiological and biochemical characteristics of BL21^T and the related strains *A. delafieldii* DSM 64^T, *A. defluvii* DSM 12644^T, *A. caeni* DSM 19327^T, *A. temperans* DSM 7270^T and *A. facilis* DSM 649^T were examined under the exact same experimental conditions.

The 16S rRNA gene of strain BL21^T was amplified by the colony PCR method with two universal primers as described by Baker *et al.* (2003). PCR products were purified using a PCR purification kit (LaboPass) according

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BL21^T is FJ599672.

Transmission electron micrographs of cells of strain BL21^T are available with the online version of this paper.

Table 1. Physiological and biochemical characteristics of strain BL21^T and the type strains of phylogenetically closely related species

Strains: 1, *Acidovorax soli* sp. nov. BL21^T; 2, *A. delafieldii* DSM 64^T; 3, *A. temperans* DSM 7270^T; 4, *A. defluvii* DSM 12644^T; 5, *A. facilis* DSM 649^T; 6, *A. caeni* DSM 19327^T. All strains were positive for reduction of nitrate to nitrite and activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS BI-phosphohydrolase. All strains were negative for glucose fermentation, utilization of capric acid and phenylacetic acid, and activities of oxidase, L-arginine hydrolase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phospatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. −, Negative; +, positive; W, weakly positive. Data were taken from this study.

Characteristic	1	2	3	4	5	6
Indole production	−	−	−	−	−	+
Hydrolysis of:						
Urea	W	+	−	W	W	+
Aesculin	−	−	+	−	−	−
Gelatin	−	+	+	+	+	+
DNA	W	W	+	+	+	−
Casein	−	+	−	+	+	+
Cellulose	−	−	−	−	+	−
Utilization of:						
D-Glucose	+	+	+	−	−	−
L-Arabinose	−	+	+	+	−	−
D-Mannose	−	+	+	+	−	−
D-Mannitol	−	+	+	+	−	−
N-Acetylglucosamine	−	−	+	−	−	−
Maltose	−	−	+	−	−	−
Potassium gluconate	−	+	−	+	−	−
Adipic acid	+	−	−	−	−	−
Malic acid	−	+	+	+	−	+
Trisodium citrate	−	−	+	−	−	−
Enzyme activity						
Catalase	−	W	+	−	W	+
β-Galactosidase	−	−	+	−	−	−
Lipase (C14)	−	W	−	−	−	+
β-Glucosidase	−	−	+	−	−	−

to the instructions of the manufacturer. Sequencing and phylogenetic analysis were performed as previously described (Choi *et al.*, 2010). The 16S rRNA gene sequence of strain BL21^T was compared with known 16S rRNA sequences in the GenBank database (NCBI) using BLAST. The sequence of strain BL21^T was aligned with those of recognized species in the genus *Acidovorax* by the multiple sequence alignment program CLUSTAL X v.1.83 (Thompson *et al.*, 1997). The phylogenetic relationships between strain BL21^T and the representative species of the genus *Acidovorax* were defined by MEGA 4 (Tamura *et al.*, 2007). A phylogenetic consensus tree was reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1981) and maximum-likelihood (Kluge & Farris, 1969) algorithms. The 16S rRNA gene sequence of strain BL21^T shared less than 98.30 % similarity with those of other members of the genus *Acidovorax* and showed high similarities to those of the type strains *A. delafieldii* ATCC 17505^T (98.26 %), *A. temperans* CCUG 11779^T (98.19 %), *A. avenae* ATCC 19860^T (97.98 %), *A. citrulli* ATCC 29625^T (97.98 %), *A. facilis* CCUG 2113^T (97.98 %), *A. defluvii* BSB411^T (97.98 %), *A. oryzae* FC-143^T (97.97 %), *A. cattleyae* NCPPB 961^T (97.91 %), *A. caeni* R-24608^T (97.70 %) and *A. valerianellae* CFBP 4730^T (97.49 %). As a result of the phylogenetic analysis, strain BL21^T was classified within the genus *Acidovorax* and, in particular, as highly related to environmental species (Fig. 1). DNA–DNA relatedness studies were carried out between strain BL21^T and phylogenetically closely related species. Genomic DNA was extracted according to Sambrook *et al.* (1989). DNA–DNA hybridization was performed by using a modification of the methods of Ezaki *et al.* (1989) and Hirayama *et al.* (1996), as previously described (Choi, *et al.*, 2010). DNA–DNA hybridization values between strain BL21^T and other *Acidovorax* strains were as follows: 28.4 % with *A. delafieldii* DSM 64^T, 26.0 % with *A. defluvii* DSM 12644^T, 20.7 % with *A. caeni* DSM 19327^T, 17.7 % with *A. temperans* DSM 7270^T and 11.7 % with *A. facilis* DSM 649^T.

The DNA G+C content was determined using the fluorimetric method proposed by Gonzalez & Saiz-Jimenez (2002) using SYBR Green and a real-time PCR thermocycler. For fatty-acid analysis, cell biomass of strain BL21^T and related species was collected from LB agar plates after incubation for 2 days at 30 °C. Cellular fatty acids were extracted according to the protocol of Sasser (1990). Cellular fatty-acid compositions were determined by gas chromatography (Hewlett Packard 6890) and the Microbial Identification System. The G+C content of the genomic DNA of strain BL21^T was 60.9 mol%. The fatty acids (>1.0 %) detected in strain BL21^T were the saturated fatty acids $C_{16:0}$ (32.1 %), $C_{14:0}$ (3.7 %) and $C_{12:0}$ (3.2 %), the 3-hydroxyoctanoic fatty acid $C_{10:0}$ 3-OH (3.3 %), the unsaturated fatty acid $C_{18:1}\omega7c$ (11.7 %) and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 43.1 %). The fatty-acid profiles of strain BL21^T and the 5 reference strains were very similar. The detailed fatty-acid compositions are shown in Table 2.

On the basis of the phenotypic, genetic and phylogenetic characteristics, strain BL21^T is considered to represent a novel species of the genus *Acidovorax*, for which the name *Acidovorax soli* sp. nov. is proposed.

Description of *Acidovorax soli* sp. nov.

Acidovorax soli (so′li. L. gen. n. *soli* of soil, the isolation source of the type strain).

Cells are Gram-negative, non-motile short rods (0.5 × 1.3 μm). Colonies are circular, convex, entire, bright yellow in colour and 2.0 mm in diameter after cultivation for 2 days on LB agar at 30 °C. Grows at 10–42 °C (optimum 30 °C), 0–1 % NaCl (optimum 0 %) and pH 6.0–9.0

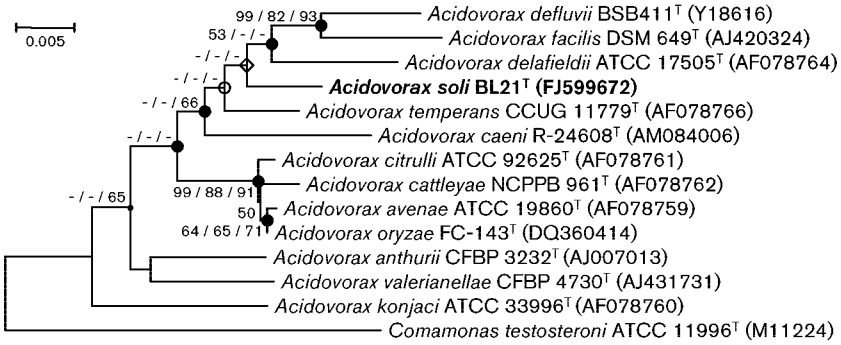

Fig. 1. Phylogenetic consensus tree based on 16S rRNA gene sequences showing the relationships between strain BL21^T and type strains of species of the genus *Acidovorax*. The tree was reconstructed based on the neighbour-joining, maximum-parsimony and maximum-likelihood methods and numbers at nodes represent bootstrap values (based on 1000, 1000 and 300 resamplings, respectively). Only bootstrap values above 50 % are shown. Filled circles indicate generic branches that were present in phylogenetic trees generated by the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms; open circles and diamonds indicate generic branches that were present in both neighbour-joining and maximum-parsimony, and both neighbour-joining and maximum-likelihood trees, respectively. The 16S rRNA gene sequence of *Comamonas testosteroni* ATCC 11996^T was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.

(optimum pH 7.0) in LB medium. Catalase- and oxidase-negative. Nitrate is reduced to nitrite. Indole is not produced. Urea and DNA are hydrolysed, but cellulose, aesculin, gelatin and casein are not. Adipic acid is utilized as carbon source. Positive for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase (API 20NE and ZYM). The following substrates can be utilized as sole carbon and energy sources: Tween 40, Tween 80, α-D-glucose, pyruvic acid methyl ester, succinic acid monomethyl ester, *cis*-aconitic acid, formic acid, *β*-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, succinamic acid, glucuronamide, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-serine, L-threonine, *γ*-aminobutyric acid, DL-α-glycerol phosphate and α-D-glucose 1-phosphate (Biolog GN2). The following compounds are not used as carbon sources: α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, *myo*-inositol, lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl *β*-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, acetic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, *γ*-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, L-alaninamide, D-alanine, L-alanyl glycine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, L-hydroxyproline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, DL-carnitine, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol and D-glucose 6-phosphate. Major cellular fatty acids are the unsaturated fatty acid $C_{18:1}\omega7c$, the saturated fatty acid $C_{16:0}$ and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$). The DNA G+C content of the type strain is 60.9 mol%.

Table 2. Fatty acid composition of strain BL21^T and the type strains of phylogenetically closely related species

Strains: 1, *Acidovorax soli* sp. nov. BL21^T; 2, *A. delafieldii* DSM 64^T; 3, *A. temperans* DSM 7270^T; 4, *A. defluvii* DSM 12644^T; 5, *A. facilis* DSM 649^T; 6, *A. caeni* DSM 19327^T. All data were obtained in the current study. Values shown are percentages of total fatty acids. ND, Not detected; tr, trace component (less than 1 %).

Fatty acid	1	2	3	4	5	6
$C_{12:0}$	3.2	4.9	6.3	3.9	7.8	3.5
$C_{14:0}$	3.7	5.3	tr	4.6	7.4	1.5
$C_{15:0}$	tr	tr	tr	tr	tr	1.2
$C_{16:0}$	32.1	23.5	25.8	25.5	27.1	30.5
$C_{17:0}$	ND	tr	tr	tr	ND	tr
$C_{17:0}$ cyclo	1.6	1.8	ND	2.9	3.2	ND
$C_{18:0}$	tr	tr	ND	tr	ND	tr
$C_{8:0}$ 3-OH	tr	1.7	1.9	1.0	2.8	1.1
$C_{10:0}$ 3-OH	3.3	4.7	4.7	3.9	8.5	3.1
$C_{18:1}\omega7c$	11.7	18.9	15.9	22.6	11.7	16.0
11-Methyl $C_{18:1}\omega7c$	ND	tr	ND	tr	ND	ND
Summed feature 3*	43.1	38.1	43.1	34.3	31.0	41.6
Summed feature 7*	ND	ND	ND	ND	ND	tr

*Summed feature 3 contained iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$. Summed feature 7 contained $C_{19:1}$ cyclo $\omega10c$ and/or $C_{19:1}\omega6c$.

The type strain, BL21^T (=KCTC 22399^T =JCM 15909^T), was isolated from a landfill site in Pohang, Republic of Korea.

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