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}:1ω7c), C_{16}:0, C_{18}:1ω7c 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 defluii, A. facilis, A. delafeldii, 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 80i, 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. delafeldii DSM 64^{T}, A. defluii 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 to the protocol of the manufacturer. The PCR products were purified, then amplified with two universal primers and sequencing primers. The 16S rRNA gene sequence of strain BL21^{T} was aligned with other strains in the genus Acidovorax. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BL21^{T} is FJS99672.
Table 1. Physiological and biochemical characteristics of strain BL21T and the type strains of phylogenetically closely related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole production</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA</td>
<td>w</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cellulose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

| 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 BL21T was compared with known 16S rRNA sequences in the GenBank database (NCBI) using BLAST. The sequence of strain BL21T 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 BL21T 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 BL21T 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. delafeldii ATCC 17505T (98.26%), A. temperans CCUG 11779T (98.19%), A. avenae ATCC 19860T (97.98%), A. citrulli ATCC 29625T (97.98%), A. facilis CCUG 2113T (97.98%), A. defluvii B8411T (97.98%), A. oryzae FC-143T (97.97%) and A. cattleyae NCPPB 961T (97.91%), A. caeni R-24608T (97.70%) and A. valerianellae CFBP 4730T (97.49%). As a result of the phylogenetic analysis, strain BL21T 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 BL21T 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 BL21T and other Acidovorax strains were as follows: 28.4% with A. delafeldii DSM 64T, 26.0% with A. defluvii DSM 12644T, 20.7% with A. caeni DSM 19327T, 17.7% with A. temperans DSM 7270T and 11.7% with A. facilis DSM 649T.

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 BL21T 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 BL21T was 60.9 mol%. The fatty acids (>1.0%) detected in strain BL21T were the saturated fatty acids C16:0 (32.1%), C14:0 (3.7%) and C12:0 (3.2%), the 3-hydroxyoctanoic fatty acid C10:0 3-OH (3.3%), the unsaturated fatty acid C18:1ω7c (11.7%) and summed feature 3 (iso-C15:0 2-OH and/or C16:1ω7c 43.1%). The fatty-acid profiles of strain BL21T 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 BL21T 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 x 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
Acidovorax soli sp. nov.

(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-Bl-phosphohydrolase (API 20NE and ZYM). The following substrates can be utilized as sole carbon and energy sources: Tween 40, Tween 80, \( \alpha-D \)-glucose, pyruvic acid methyl ester, succinic acid mono-methyl ester, cis-aconitic acid, \( \beta \)-hydroxybutyric acid, \( \alpha \)-ketoglutaric acid, DL-lactic acid, succinamic acid, glucuronamide, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-serine, L-threonine, \( \gamma \)-aminobutyric acid, DL-\( \alpha \)-glycerol phosphate and \( \alpha \)-D-glucose 1-phosphate (Biolog GN2). The following compounds are not used as carbon sources: \( \alpha \)-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-ara-binose, D-arabitol, cellobiose, \( \alpha \)-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, myo-inositol, lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl \( \beta \)-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xyitol, acetic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-glucuronic acid, D-glucosaminic acid, D-glucuronic acid, \( \alpha \)-hydroxybutyric acid, \( \gamma \)-hydroxybutyric acid, \( p \)-hydroxyphenylacetic acid, itaconic acid, \( \alpha \)-ketobutyric acid, \( \alpha \)-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, L-alaninamide, L-alanine, L-alanyl glycine, glycyll L-aspartic acid, glycyll L-glutamic acid, L-histidine, L-hydroxyproline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-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 C18:1 \( v \) 7c, the saturated fatty acid C16:0 and summed feature 3 (iso-C 15:0 2-OH and/or C16:1 \( v \) 7c). 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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>3.2</td>
<td>4.9</td>
<td>6.3</td>
<td>3.9</td>
<td>7.8</td>
<td>3.5</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.7</td>
<td>5.3</td>
<td>tr</td>
<td>4.6</td>
<td>7.4</td>
<td>1.5</td>
</tr>
<tr>
<td>C15:0</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>1.2</td>
</tr>
<tr>
<td>C16:0</td>
<td>32.1</td>
<td>23.5</td>
<td>25.8</td>
<td>25.5</td>
<td>27.1</td>
<td>30.5</td>
</tr>
<tr>
<td>C17:0</td>
<td>ND</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>ND</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>1.6</td>
<td>1.8</td>
<td>ND</td>
<td>2.9</td>
<td>3.2</td>
<td>ND</td>
</tr>
<tr>
<td>C18:0</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>ND</td>
<td>tr</td>
<td>ND</td>
</tr>
<tr>
<td>C20:0 cyclo</td>
<td>1.7</td>
<td>1.9</td>
<td>1.8</td>
<td>1.9</td>
<td>1.0</td>
<td>tr</td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>3.3</td>
<td>4.7</td>
<td>3.9</td>
<td>8.5</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>C18:1(\omega7c)</td>
<td>11.7</td>
<td>18.9</td>
<td>15.9</td>
<td>22.6</td>
<td>11.7</td>
<td>16.0</td>
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<tr>
<td>11-Methyl C18:1(\omega7c)</td>
<td>ND</td>
<td>tr</td>
<td>ND</td>
<td>tr</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>43.1</td>
<td>38.1</td>
<td>43.1</td>
<td>34.3</td>
<td>31.0</td>
<td>41.6</td>
</tr>
<tr>
<td>Summed feature 7*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>tr</td>
<td></td>
</tr>
</tbody>
</table>

*Summed feature 3 contained iso-C15:0 2-OH and/or C16:1\(\omega7c\). Summed feature 7 contained C19:1 cyclo \(\omega10c\) and/or C19:1\(\omega6c\).

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.

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

<table>
<thead>
<tr>
<th>Strains:</th>
<th>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%).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid</td>
<td>1</td>
</tr>
<tr>
<td>C12:0</td>
<td>3.2</td>
</tr>
<tr>
<td>C14:0</td>
<td>3.7</td>
</tr>
<tr>
<td>C15:0</td>
<td>tr</td>
</tr>
<tr>
<td>C16:0</td>
<td>32.1</td>
</tr>
<tr>
<td>C17:0</td>
<td>ND</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>1.6</td>
</tr>
<tr>
<td>C18:0</td>
<td>tr</td>
</tr>
<tr>
<td>C20:0 cyclo</td>
<td>1.7</td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>3.3</td>
</tr>
<tr>
<td>C18:1(\omega7c)</td>
<td>11.7</td>
</tr>
<tr>
<td>11-Methyl C18:1(\omega7c)</td>
<td>ND</td>
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<tr>
<td>Summed feature 3*</td>
<td>43.1</td>
</tr>
<tr>
<td>Summed feature 7*</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Summed feature 3 contained iso-C15:0 2-OH and/or C16:1\(\omega7c\). Summed feature 7 contained C19:1 cyclo \(\omega10c\) and/or C19:1\(\omega6c\).

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The type strain, BL21T (=KCTC 22399T =JCM 15909T), was isolated from a landfill site in Pohang, Republic of Korea.

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References


