Ideonella paludis sp. nov., isolated from a marsh

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A bacterial strain, designated KBP-31T, was isolated from a water sample taken from the Banping Lake Wetland Park in Taiwan and characterized using a polyphasic taxonomic approach. Cells of strain KBP-31T were Gram-stain-negative, strictly aerobic, motile, light-yellow rods. Growth occurred at 10–37 °C (optimum 25 °C), at pH 6–8 (optimum pH 6) and with 0–1 % NaCl (w/v, optimum 0 %). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain KBP-31T belonged to the genus Ideonella and was most closely related to Ideonella dechloratans ATCC 51718T with a sequence similarity of 98.2 %. Strain KBP-31T contained summed feature 3 (comprising C₁₆ : 1ω₇c and/or C₁₆ : 1ω₆c) and C₁₆ : 0 as the predominant fatty acids. The major hydroxyl fatty acid was C₁₀ : 0 3-OH. The polar lipid profile consisted of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an uncharacterized aminophospholipid and two uncharacterized phospholipids. The DNA G+C content of the genomic DNA was 67.9 mol%. The DNA–DNA relatedness of strain KBP-31T with respect to recognized species of the genus Ideonella was less than 70 %. On the basis of the phylogenetic inference and phenotypic data, strain KBP-31T should be classified as a novel species, for which the name Ideonella paludis sp. nov. is proposed. The type strain is KBP-31T (=BCRC 80524T=KCTC 32238T).

The genus Ideonella, first proposed by Malmqvist et al. (1994) and significantly emended by Noar & Buckley (2009), belongs to the Rubrivivax-Roseatelles-Leptothrix-Azohydromonas-Aquincola-Ideonella branch of the order Burkholderiales in the class Betaproteobacteria (Garrity et al., 2005). At the time of writing the genus Ideonella comprises only two species with validly published names: Ideonella dechloratans, isolated from activated sludge (Malmqvist et al., 1994) and Ideonella azotifigens, isolated from grass rhizosphere soil (Noar & Buckley, 2009). Cells are Gram-stain-negative, mesophilic, aerobic, chemoheterotrophic, rod-shaped, motile by means of two or more polar or subpolar flagella. Chemotaxonomically, cells possess summed feature 3 (comprising C₁₆ : 1ω₇c and/or C₁₆ : 1ω₆c) and C₁₆ : 0 as the predominant fatty acids, C₁₀ : 0 3-OH, C₁₂ : 0 2-OH and C₁₂ : 0 3-OH as the cellular hydroxy fatty acids, and DNA G+C contents of between 67.4 and 68 mol% (Malmqvist et al., 1994; Noar & Buckley, 2009). The present study was carried out to clarify the taxonomic position of a novel species of the genus Ideonella using a polyphasic taxonomic approach.

During our investigations on the biodiversity of bacteria in the water of the Banping Lake Wetland Park [GPS location: 22° 41’ 30” N 120° 18’ 32” E; 25 °C, pH 7.3, 0.5 % (w/v) NaCl] in the vicinity of Kaohsiung city, Taiwan, a novel light-yellow bacterium, designated KBP-31T, was isolated and selected for detailed taxonomic analyses. Strain KBP-31T was isolated on R2A agar (BD Difco) after incubation at 25 °C for 3 days, subcultured under the same conditions and stored at −80 °C in R2A broth (BD Difco) with 20 % (v/v) glycerol or after lyophilization.

Genomic DNA was isolated using a bacterial genomic DNA purification kit (DP02-150; GenMark Technology) and the 16S rRNA gene was sequenced and analysed, as described previously by Chen et al. (2001). The primers, FD1 (5’-AGAGTTTGATCCTGCGCTCAG-3’) and RD1 (5’-AAGAGGTTGATCCTGAGG-3’), were used for amplification of bacterial 16S rRNA genes by PCR (Weisburg et al., 1991; Anzai et al., 1997). These primers correspond to the GenBank accession numbers D24925, D24926 and 137477568 for the R2A agar (BD Difco) and the 25 °C for 3 days culture, respectively.
to nucleotide positions 8–27 and 1524–1540 of the *Escherichia coli* 16S rRNA gene, respectively, and can be used for amplifying the nearly full-length 16S rRNA gene. The PCR product was purified, and direct sequencing was performed by using sequencing primers FD1, RD1, 520F and 800R (Weisburg et al., 1991; Anzai et al., 1997) with a DNA sequencer (ABI Prism 3730; Applied Biosystems). The sequenced length of the 16S rRNA gene was 1414 bp for strain KBP-31T and this gene sequence was compared to those available from the EzTaxon-e (Kim et al., 2012), the Ribosomal Database Project (Cole et al., 2009) and the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Analysis of the sequence data was performed by using the software package BioEdit (Hall, 1999) and MEGA version 5 (Tamura et al., 2011), after multiple alignments of the data by CLUSTAL X (Thompson et al., 1997). The resulting multiple sequence alignment was corrected manually and gaps at the 5′ and 3′ ends of the alignment were omitted for further analyses. Distances (corrected according to Kimura’s two-parameter model; Kimura, 1980) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). The maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the treeing algorithms contained in the PHYLIP software package (Felsenstein, 1993). In each case bootstrap values were calculated based on 1000 replications.

Phylogenetic analyses, based on 16S rRNA gene sequences, revealed that strain KBP-31T was closely related to species of the genera *Ideonella*, *Aquincola*, *Rubrivivax*, *Leptothrix* and *Roseateales*, with 97.0–98.2 %, 96.8 %, 96.6 %, 95.3–96.7 % and 95.3–95.8 % sequence similarities, respectively. Strain KBP-31T formed a deep phyletic cluster with *I. dechloratans* ATCC 51718T and *I. azotifigens* DSM 21438T within the *Rubrivivax*–*Roseateales*-Leptothrix-Azohydromonas-Aquincola-Ideonella branch in the neighbour-joining tree (Fig. 1). The overall topologies of the maximum-likelihood and maximum-parsimony trees were similar. Sequence similarity calculations (over 1400 bp) indicated that strain KBP-31T was closely related to *I. dechloratans* ATCC 51718T (98.2 % 16S rRNA gene sequence similarity) and *I. azotifigens* DSM 21438T (97.6 % 16S rRNA gene sequence similarity). Sequence similarities <96.8 % were observed with the type strains of all other species listed in Fig. 1.

*I. dechloratans* ATCC 51718T was obtained from the American type Culture Collection (ATCC) and *I. azotifigens* DSM 21438T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Both type strains were grown under the same conditions and used as reference strains for phenotypic and genotypic tests.

Cell morphology of strain KBP-31T was observed by phase-contrast microscopy (Leica; DM 2000) and transmission electron microscopy (Hitachi; H-7500) (Fig. S1, available in the online Supplementary Material) using cells grown in R2A agar at 25 °C for lag, exponential and stationary phases. Flagellar motility was tested using the hanging drop method, and Spot Test flagella stain (BD Difco) was used for flagellum staining. The Gram Stain Set S kit (BD Difco) and the Ryu non-staining KOH method (Powers, 1995) were used to perform the Gram reaction. Poly-hydroxyalkanoate granule accumulation was examined under light microscopy after staining of the cells with Sudan black (Schlegel et al., 1970) and visualized by UV illumination after directly staining growing bacteria on plates containing Nile red (Spiekermann et al., 1999). Colony morphology was observed on R2A agar by using a stereoscopic microscope (Nikon; SMZ 800).

The physiological characteristics of strain KBP-31T and the two reference strains were examined by growing bacteria at various pH values, temperatures and NaCl concentrations. The pH range for bacterial growth was estimated by measuring the optical densities (wavelength 600 nm) of R2A broth cultures. The pH of the medium was adjusted prior to sterilization to pH 4.0–9.0 (at intervals of 0.5 pH unit) using the following biological buffers (Breznak & Costilow, 2007): citrate/Na2HPO4 (pH 4.0–5.5); phosphate (pH 6.0–7.5); and Tris (pH 8.0–9.0). Verification of the pH values after autoclaving revealed only minor changes. The temperature range for growth was determined in R2A broth at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45 and 50 °C. To investigate the tolerance to NaCl, R2A broth was prepared according to the formula of the BD Difco medium with NaCl concentration adjusted to 0, 0.5 % and 1.0–5.0 % (w/v, at intervals of 1.0 %). Growth under anaerobic conditions was determined after incubating strain KBP-31T in the Oxoid AnaeroGen system. Growth was tested in nutrient broth, full-strength trypticase soy broth, R2A broth and LB broth (all from Difco).

Strain KBP-31T was examined for activities of catalase, oxidase, DNase, urease and lipase (corn oil), and hydrolysis of starch, casein, gelatin, lecithin and Tweens 20, 40, 60 and 80 using standard approaches (Tindall et al., 2007). Hydrolysis of alginate (1 %, w/v, sodium alginate) was examined on R2A agar. Chitin hydrolysis was assessed on chitinase-detection agar (Wen et al., 2002) and visualized by the formation of clear zones around the colonies. Hydrolysis of carboxymethylcellulose (CM-cellulose) was tested as described by Bowman (2000) using R2A agar as the basal medium. Additional biochemical tests were performed using API ZYM and API 20NE kits (all from bioMérieux). All commercial phenotypic tests were performed according to the manufacturers’ recommendations.

Sensitivity of strain KBP-31T and the two reference strains to antibiotics was tested by the disc diffusion method after spreading cell suspensions (0.5 McFarland standard) on R2A agar plates. The discs (Oxoid) contained the following antibiotics: ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), novobiocin (30 μg), penicillin G (10 U), rifampicin (5 μg), streptomycin (10 μg), sulframethoxazole (23.75 μg) plus trimethoprim (1.25 μg) and tetracycline (30 μg).
The diameter of the antibiotic discs was 8 mm. The effect of antibiotics on cell growth was assessed after 3 days at 25 °C. A strain was considered susceptible when the diameter of the inhibition zone was >13 mm, intermediate at 10–12 mm and resistant at <10 mm as described by Nokhal & Schlegel (1983). Detailed results from the phenotypic and biochemical analyses of strain KBP-31T are provided in Table 1 and in the species description.

DNA–DNA hybridization experiments were carried out by the method of Ezaki et al. (1989). The level of DNA–DNA relatedness of strain KBP-31T with I. dechloratans ATCC 51718T and I. azotifigens DSM 21438T was 24.2 ± 2.6% (25.8 ± 2.9% in a reciprocal experiment) and 31.0 ± 2.8% (38.9 ± 1.9%), respectively. The DNA–DNA relatedness between strain KBP-31T and its closest phylogenetic neighbours was below the 70% cut-off point recommended for the assignment of strains to the same genomic species (Wayne et al., 1987). This warranted the status of strain KBP-31T as a separate species in the genus Ideonella.

The fatty acid profile of strain KBP-31T, I. dechloratans ATCC 51718T and I. azotifigens DSM 21438T was analysed on cells grown on R2A agar at 25 °C for 2 days. The physiological age of the different bacterial cultures at the time of harvest was standardized by choice of sector from a quadrant streak on the R2A agar plates according to the MIDI protocol. In this study, the different species of the genus Ideonella exhibited very similar growth rates on R2A agar. Fatty acid methyl esters were prepared and separated according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0), analysed by GC (Hewlett Packard 5890 Series II) and identified by using the RTSBA6.00 database of the microbial identification system (Sasser, 1990).

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of KBP-31T and closely related strains. Numbers at nodes are bootstrap percentages (>70%) based on neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. Filled circles indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. Open circles indicate that the corresponding nodes were recovered in the tree generated with the maximum-parsimony algorithm. Tepidimonas fonticaldi AT-A2T was used as an out-group. Bar, 0.01 substitutions per nucleotide position.
The predominant cellular fatty acids of strain KBP-31T were summed feature 3 (comprising C₁₆ : 1 ω7c and/or C₁₆ : 1 ω6c; 46.4 %) and C₁₆ : 0 (28.6 %). The major hydroxyl fatty acid was C₁₀ : 0 3-OH (5.2 %). The complete fatty acid composition is shown in Table 2. The fatty acid profile of strain KBP-31T differed markedly from those of the two reference strains due to higher amounts of summed feature 3, lower amounts of C₁₈ : 1 ω7c, the presence of C₁₇ : 0 and the absence of C₁₂ : 0 2-OH and C₁₂ : 0 3-OH.

The DNA G+C content of strain KBP-31T, as determined by HPLC according to Mesbah et al. (1989), was 67.9 ± 1.0 mol%, a value within the range reported for strains of species of the genus *Ideonella* (Malmqvist et al., 1994; Noar & Buckley, 2009).

The polar lipids of strain KBP-31T, *I. dechloratans ATCC 51718T* and *I. azotifigens DSM 21438T*, were extracted and analysed by two-dimensional TLC according to Embley & Wait (1994). Chromatography was carried out using chloroform/methanol/water (65 : 25 : 3.8, by vol.) in the first direction, followed by chloroform/methanol/ acetic acid/water (40 : 7.5 : 6:1.8, by vol.) in the second. Ethanolic molybdophosphoric acid (10 %, v/v) was used as a spray reagent.
Table 2. Cellular fatty acid composition of strain KBP-31T and type strains of other species of the genus Ideonella

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0</td>
<td>1.8</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>C10:0 3-OH</td>
<td>5.2</td>
<td>2.4</td>
<td>4.0</td>
</tr>
<tr>
<td>C12:0</td>
<td>4.1</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>C12:0 2-OH</td>
<td>-</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>-</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.6</td>
<td>1.5</td>
<td>1.8</td>
</tr>
<tr>
<td>C15:096c</td>
<td>1.3</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>C16:0</td>
<td>28.6</td>
<td>31.0</td>
<td>30.3</td>
</tr>
<tr>
<td>C17:0</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C18:097c</td>
<td>1.9</td>
<td>-</td>
<td>6.4</td>
</tr>
<tr>
<td>Simulated feature 3*</td>
<td>46.4</td>
<td>39.5</td>
<td>29.5</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises C16:097c and/or C16:096c.

Strains: 1, KBP-31T; 2, I. dechloratans ATCC 51718T; 3, I. azotifigens DSM 21438T. All data were obtained from this study. All strains were grown on R2A agar at 25 ℃ for 2 days. Values are percentages of the total fatty acids present; Fatty acids comprising less than 1 % of the total are not listed or are indicated by –.

KBP-31T constitutes a novel member of the genus Ideonella for which the name Ideonella paludis sp. nov. is proposed.

**Description of Ideonella paludis sp. nov.**

*Ideonella paludis* (pa.lu’dis. L. gen. n. paludis of a swamp, of a marsh, of a bog).

Cells are Gram-stain-negative, strictly aerobic, motile and rod-shaped. Negative for poly-β-hydroxybutyrate accumulation. After 48 h of incubation on R2A agar at 25 ℃, the mean cell size is 0.4–0.8 μm in diameter and 0.8–1.4 μm in length. Colonies on R2A agar are light-yellow, slightly sticky, convex and circular with regular, curpled margins. The colony size is approximately 0.8–1.2 mm in diameter after 48 h at 25 ℃. Growth occurs 10–37 ℃ (optimum, 25 ℃), at pH 6–8 (optimum, pH 6) and with 0–1 % NaCl (w/v, optimum 0 %). Positive for oxidase activity and the hydrolysis of casein, alginate and Tween 20. Negative for catalase, lipase and urease activities, and the hydrolysis of starch, DNA, chitin, CM-cellulose, lecithin, Tweens 40, 60 and 80. In API 20NE tests, positive for gelatin hydrolysis and negative for nitrate reduction, indole production, d-glucose acidification, arginine dihydrolase, urease and β-galactosidase activities, aesculin hydrolysis and the assimilation of glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, glucosone, caprate, adipate, malate, citrate and phenyl-acetate. In the API ZYM kit, alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but C14 lipase, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and n-fucosidase activities are absent. Sensitive to ampicillin, chloramphenicol, kanamycin, nalidixic acid, novobiocin, penicillin G, rifampicin, streptomycin, tetracycline, sulfamethoxazole plus trimethoprim and gentamicin. The major fatty acids (> 10 % of the total fatty acids) are summed feature 3 (comprising C16:097c and/or C16:096c) and C16:0. The major hydroxy fatty acid is C10:0 3-OH. The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an uncharacterized aminophospholipid and two uncharacterized phospholipids.

The type strain is KBP-31T (=BCRC 80524T =KCTC 32238T), isolated from the water of the Banping Lake Wetland Park in the vicinity of Kaoshiung city, Taiwan. The DNA G+C content of the type strain is 67.9 mol%.

**References**


Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of...


