Paludibacterium purpuratum sp. nov., isolated from wetland soil

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A novel bacterium, designated KJ031T, was isolated from a wetland soil sample taken from Jeju island, Republic of Korea. Cells were Gram-stain-negative, curved rod shaped, oxidase- and catalase-positive, motile, and facultatively anaerobic. Growth was observed at pH 6.0–8.0 and at 20–37°C on R2A agar. Comparative analysis of 16S rRNA gene sequences revealed that strain KJ031T is a member of the genus Paludibacterium, sharing highest sequence similarities with Paludibacterium paludis KBP-21T (96.2%) and Paludibacterium yongneupense 5YN8-15T (96.0%). The major fatty acids were summed feature 3 (C16:1ω7c and/or C16:1ω6c), C16:0 and summed feature 8 (C18:1ω7c and/or C18:1ω6c). The predominant respiratory quinone was Q-8. The major polar lipids of strain KJ031T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminophospholipid, two unidentified phospholipids and one unidentified polar lipid. The DNA G+C content was 59.2 mol%. On the basis of the evidence presented in this study, strain KJ031T represents a novel species of the genus Paludibacterium, for which the name Paludibacterium purpuratum sp. nov. is proposed. The type strain is KJ031T (=KCTC 42852T =CECT 8976T).

The genus Paludibacterium was classified into the family Neisseriaceae of the class Betaproteobacteria. The genus Paludibacterium was established by Kwon et al. (2008) with a single species, Paludibacterium yongneupense, isolated from wetland peat. Subsequently, a second species, Paludibacterium paludis (Sheu et al., 2014), was isolated from wetland water. The genus Paludibacterium was Gram-negative, facultatively anaerobic, non-spore forming, curved-rod shaped with a single polar flagellum, motile, and oxidase- and catalase-positive. The major fatty acids are summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), C16:0 and C18:1ω7c. The predominant isoprenoid quinone is Q-8 (Kwon et al., 2008). In the present study, a novel strain, KJ031T, was isolated from wetland soil and its taxonomic position has been determined using a polyphasic approach, which included phenotypic, phylogenetic and chemotaxonomic features.

The species was found during the course of a study on the microbial diversity of a soil sample collected from wetlands located in Jeju island, Republic of Korea (33°21′25″ N, 126°27′48″ E). The mountainous wetland (altitude above sea level, 1100 m), consisting of a number of unique freshwater marshes and pools, is located on Halla mountain and is a designated Ramsar site. The sample was taken from the upper (0–10 cm) layer of soil. A purple colony, designated KJ031, was isolated using the standard dilution plating technique on R2A agar at 30°C. For storage, strain KJ031T was maintained at −80°C in distilled water supplemented with 20% (v/v) glycerol and lyophilized in 20% (w/v) skimmed milk.

The nearly complete sequence (1473 bp) of the 16S rRNA gene of strain KJ031T was obtained. PCR amplification was conducted with the universal primers 27F and 1492R. Amplified products were sequenced using primers 27F, 518R, 785F and 1492R (Lane, 1991). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were performed using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). The SILVA Incremental Aligner (SINA; v1.2.11) (Pruesse et al., 2012) was used to align the sequence data.
Trees were reconstructed in MEGA version 6 (Tamura et al., 2013) using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony algorithms (Fitch, 1971) all with 1000 bootstrap iterations. A neighbour-joining tree was reconstructed with the Jukes-Cantor model. A maximum-likelihood tree was reconstructed with the Tamura-Nei (TN93) model. The neighbor-joining tree indicated that strain KJ031 formed a clade with \( P. \) paludis and \( P. \) yongneupense (Fig. 1). Strain KJ031 also clustered with species of the genus \( P. \) paludis and other closely related members of the family \( Neisseriaceae \). Numbers at nodes are bootstrap values (% of 1000 replicates); only values >70% are shown. This phylogenetic inference, together with the level of 16S rRNA gene sequence similarity (<97%) (Wayne et al., 1987; Stackebrandt & Goebel, 1994) between strain KJ031 and the type strains of \( P. \) paludis and \( P. \) yongneupense, suggested that it represents a novel species of the genus \( Paludibacterium \). So, \( P. \) paludis KCTC 32182\(^\text{T} \) and \( P. \) yongneupense KACC 11601\(^\text{T} \) were obtained from the Korean Collection for Type Cultures (KCTC) and the Korean Agricultural Culture Collection (KACC), respectively, and used as reference strains.

Colony morphology was examined following growth of the strain on R2A agar at 30\(^\circ\)C for 3 days. Cell morphology and size were investigated by transmission electron microscopy (LIBRA120; Carl Zeiss) after cells had been negatively stained with 1% (w/v) phosphotungstic acid. The hanging-drop technique was used to assess gliding motility (Brown, 1921). Oxidase activity was evaluated via the oxidation of 1% \( N,N,N',N' \)-tetramethyl-p-dihydrochloride (Sigma). Catalase activity was determined by bubble production in 3% (v/v) \( H_2O_2 \) solution. The temperature range for growth was determined on R2A agar at 4\(^\circ\)C and 10–30\(^\circ\)C (at intervals of 5\(^\circ\)C), 37\(^\circ\)C and 42\(^\circ\)C. The pH range for growth was determined on R2A agar at 4\(^\circ\)C and 10–30\(^\circ\)C (at intervals of 5\(^\circ\)C), 37\(^\circ\)C and 42\(^\circ\)C.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of strain KJ031 and other closely related members of the family \( Neisseriaceae \). Numbers at nodes are bootstrap values (% of 1000 replicates); only values >70% are shown. \( Noviherbaspirillum malthae \) CC-AFH3\(^\text{T} \) was used as an out-group. Bar, 0.01 substitutions per nucleotide position.
range for growth was determined by adding 0.1 M sodium acetate buffer (pH 4.0–6.0), 0.1 M phosphate buffer (pH 7.0 and 8.0) and 0.1 M sodium carbonate buffer (pH 9.0 and 10.0) to R2A broth. Tolerance to NaCl was examined in R2A broth with 0.5 % and 1.0–5.0 % (in increments of 1.0 %) NaCl (w/v). Anaerobic growth was tested on R2A agar at 30 °C for 5 weeks using the GasPak EZ Anaerobic Container System (BD). The ability of strain KJ031\(^1\) to utilize terminal acceptors was assessed using basal freshwater medium and the procedure described by Lovley (2006). The electron donor used to evaluate potential electron acceptors was glucose (5 mM). The following potential electron acceptors were tested: 

\[ \text{Table 1} \]. Differential characteristics of strain KJ031\(^1\) and the type strains of two species of the genus *Paludibacterium*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Purple</td>
<td>Yellow</td>
<td>Cream</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>20–37</td>
<td>15–42</td>
<td>15–37</td>
</tr>
<tr>
<td>NaCl concn. range for growth (% w/v)</td>
<td>0–1.5</td>
<td>0–2</td>
<td>0–0.5</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6-8</td>
<td>6-8</td>
<td>7-8</td>
</tr>
<tr>
<td>Growth on MacConkey agar</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNase</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>API 20NE results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activities (API ZYM)</td>
<td></td>
<td></td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase, (\alpha)-glucosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>(\beta)-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-(\beta)-glucosaminidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbon Source oxidation (BIOLOG GN2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 40, Tween 80, N-acetyl-D-glucosamine, D-fructose, L-alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, D-aspartic acid, (\alpha)-glutamic acid, L-proline, D-proline, D-(\alpha)-glucose-1-phosphate, D-glucose-6-phosphate, D-Trehalose, bromosuccinimic acid, glycol-L-aspartic acid, Dextrin, glycogen, acetic acid, (\alpha)-hydroxybutyric acid, (\beta)-hydroxybutyric acid, propionic acid, L-alanine, L-serine, L-(\gamma)-amino butyric acid, thymidine</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>(\alpha)-Keto butyric acid</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Malate, succrose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Kanamycin, streptomycin</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>59.2</td>
<td>61.1</td>
<td>63.6</td>
</tr>
</tbody>
</table>

*(Data from Sheu et al., 2014; †Data from Kwon et al., 2008.)*
acceptors were tested: ferric citrate (50 mM), potassium nitrate (5 mM), sodium nitrite (5 mM), sodium thiosulfate (5 mM), sodium sulfate (5 mM) and sodium sulfate (5 mM). The growth of strain KJ031T with potential electron acceptors was monitored by measuring OD600. Hydrolysis of casein [3% skimmed milk, w/v (Difco)], carboxymethylcellulose [1% CM-cellulose, w/v (Sigma)], cellulose [1% filter paper, w/v (Whatman # 1)], dextrin [1% dextrin, w/v (Sigma)], and starch [1% soluble starch, w/v (Sigma)] was tested using 1/10 strength R2A agar as the basal medium. DNase activity was assessed on DNase test agar (Difco). Other biochemical tests were carried out using API 20NE and API ZYM kits (bioMérieux) and the oxidation of carbon compounds was evaluated with GN2 MicroPlates (Biolog) according to the manufacturer’s instructions. Growth was also evaluated on nutrient (NA; Difco), marine 2216 (MA; Difco), tryptic soy (TSA; Difco), blood (Difco) and MacConkey (Difco) agars at 30°C. Susceptibility to antibiotics was investigated on R2A agar plates using antibiotic discs (Liofilchem) containing ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamycin (10 µg), kanamycin (30 µg), penicillin G (10 IU), rifampicin (30 µg), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg). Genomic DNA was extracted using a Bacterial Genomic DNA prep kit (Solgent) (30 µg) and vancomycin (30 µg). Genomic DNA was extracted according to the standard protocols of MIDI (Sherlock Microbial Identification System, version 6.1). Fatty acids were analyzed by GC (Hewlett Packard 7890) and identified using the RTSBA6 database of the Microbial Identification System (Sasser, 1990). The cellular biomass of strain KJ031T for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown on R2A agar at 30°C for 3 days. Isoprenoid quinones and polar lipids of strain KJ031T were extracted and analyzed from freeze-dried cells as described by Minnikin et al. (1984). The respiratory quinone was analyzed by HPLC according to Collins (1985). Lipids were separated using two-dimensional TLC (plates coated with silica gel, 10×10 cm; Merck), using chloroform/methanol/water (65:25:4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, by vol.) in the second dimension. The plates were sprayed with 10% (w/v) ethanolic molybdophosphoric acid (Sigma) for detection of total polar lipids, ninhydrin (Sigma) for aminolipids, α-naphthol for glycolipids, molybdenum blue (Sigma) for phospholipids, and periodic acid-Schiff (Sigma) for α-glycols.

The fatty acid compositions of strain KJ031T and the two reference strains are compared in Table 2. The major fatty acids of strain KJ031T were summed feature 3 (C16:1ω7c and/or C16:1ω6c), C10:0 and summed feature 8 (C18:1ω7c and/or C18:1ω6c). The fatty acid composition of strain KJ031T was

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>C10:0 3-OH</td>
<td>3.5</td>
<td>3.6</td>
<td>2.5</td>
</tr>
<tr>
<td>C12:0</td>
<td>7.3</td>
<td>6.8</td>
<td>9.5</td>
</tr>
<tr>
<td>C12:0 2-OH</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>3.4</td>
<td>2.4</td>
<td>4.2</td>
</tr>
<tr>
<td>C14:1ω5c</td>
<td>TR</td>
<td>TR</td>
<td>1.1</td>
</tr>
<tr>
<td>C14:0</td>
<td>4.8</td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>C16:0</td>
<td>27.8</td>
<td>23.9</td>
<td>35.9</td>
</tr>
<tr>
<td>C17:1ω6c</td>
<td>1.5</td>
<td>1.4</td>
<td>ND</td>
</tr>
<tr>
<td>C17:0</td>
<td>1.3</td>
<td>1.5</td>
<td>ND</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>ND</td>
<td>19.8</td>
<td>ND</td>
</tr>
<tr>
<td>C19:0ω9c cyclo</td>
<td>ND</td>
<td>ND</td>
<td>1.8</td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>28.0</td>
<td>29.9</td>
<td>10.7</td>
</tr>
<tr>
<td>Summed feature 8</td>
<td>18.4</td>
<td>21.7</td>
<td>6.2</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately. Summed feature 3 was listed as C16:1ω7c and/or C16:1ω6c. Summed feature 8 was listed as C18:1ω7c and/or C18:1ω6c.

Table 2. Cellular fatty acid composition (as percentages of the total) of strain KJ031T and the type strains of the two species of the genus Paludibacterium

Strains: 1, KJ031T; 2, P. paludis KCTC 32182T; 3, P. yongneupense KACC 11601T. All data are from the present study. Only fatty acids amounting to at least 1.0% of the total cellular fatty acids of at least one of the strains are shown. TR, Trace (<1.0%); ND, not detected.

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similar to those of the two reference strains, with only minor quantitative differences. Strain KJ031$^T$ and P. paludis KCTC 32182$^T$ mainly differ from P. yongneupense KACC 11601$^T$ by the absence of C$_{17:0}$ cyclo. The predominant quinone of strain KJ031$^T$ was determined to be ubiquinone 8, which was in accordance with all species of the genus Paludibacterium with validly published names. The polar lipid profile of strain KJ031$^T$ consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids, one unidentified aminophospholipid and one unidentified polar lipid (Fig. S2), similar to the profiles of close relatives in the genus Paludibacterium. Strain KJ031$^T$ differed from other members of the genus Paludibacterium by the presence of an unidentified lipid. (Sheu et al., 2014).

On the basis of these data and the observations described above, strain KJ031$^T$ should be assigned to the genus Paludibacterium as the type strain of a novel species, for which the name Paludibacterium purpuratum sp. nov. is proposed.

Description of Paludibacterium purpuratum sp. nov.

Paludibacterium purpuratum (pur-pu.ra’tum. L. neut. adj. purpuratum clad in purple-violet, referring to the colony colour).

Cells are Gram-stain-negative, facultatively anaerobic, curved rods, and approximately 1.8–2.5 μm in length and 0.5–0.7 μm in diameter. Colonies on R2A medium are brown to purple, circular, convex and with entire margins. Growth occurs at 20–37°C (optimum, 30°C), at pH 6–8 (optimum, pH 7.0) and in R2A broth supplemented with 0–1.5 % NaCl (w/v, optimum, 0 % NaCl). Sulfate can serve as an electron acceptor under anaerobic conditions. Good growth occurs on R2A, NA and TSA, but not on MA, blood, or MacConkey agars. Casein (skimmed milk) is hydrolyzed. DNA, starch, dextrin, CM-cellulose and cellulose (filter paper) are not hydrolyzed. Susceptible to chloramphenicol, kanamycin, rifampicin, streptomycin and tetracycline, but resistant to ampicillin, erythromycin, gentamicin, penicillin G and vancomycin. In API 20NE strips, positive for glucose fermentation, esculin hydrolysis, arginine dihydrolase and gelatinase activity, and for the assimilation of D-glucose, L-arabinose, N-acetyl-glucosamine, potassium gluconate and malic acid, but negative for nitrate reduction, indole production, urease and β-galactosidase activity (PNPG test), and assimilation of D-mannose, D-mannitol, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. In API ZYM strips, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present; lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. The following compounds are utilized as sole carbon sources in GN2 MicroPlate tests: Tween 40, Tween 80, N-acetyl-D-glucosamine, D-fructose, α-D-glucose, D-trehalose, pyruvic acid methyl ester, D,l-lactic acid, bromosuccinic acid, L-alanine, L-tyrosine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyrl-L-aspartic acid, glycyrl-L-glutamic acid, L-proline, urocanic acid, α-D-glucose-1-phosphate and D-glucose-6-phosphate. Other substrates are not utilized. The major cellular fatty acids (>10 %) are summed feature 3 (C$_{16:1}$ ω7c and/or C$_{16:1}$ ω6c), C$_{16:0}$ and summed feature 8 (C$_{18:1}$ ω7c and/or C$_{18:1}$ ω6c). UQ-8 is the only respiratory quinone. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, one unidentified aminophospholipid, two unidentified phospholipids and one unidentified polar lipid.

The type strain is KJ031$^T$ (=KCTC 42852$^T$ =CECT 8976$^T$), isolated from a wetland in Jeju island, Republic of Korea. The genomic DNA G+C content is 59.2 mol%.

Acknowledgements

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References


