Albimonas pacifica sp. nov., isolated from seawater of the Pacific, and emended description of the genus Albimonas

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A Gram-stain-negative, aerobic, catalase- and oxidase-positive, non-flagellated, rod-shaped bacterium, designated strain P-50-3T, was isolated from seawater of the Pacific. The strain grew at 10–40 °C (optimum at 30 °C) and with 0–12 % (w/v, optimum 2 %) NaCl. It reduced nitrate to nitrite but did not hydrolyse gelatin, starch or Tween 80. Analysis of 16S rRNA gene sequences showed that strain P-50-3T clustered tightly with the genus Albimonas and shared the highest 16S rRNA gene sequence similarity (94.3 %) with the type strain of Albimonas donghaensis. The major respiratory quinone was Q-10 and the major cellular fatty acids were C18:1ω7c, C18:0, 11-methyl C18:1ω7c and C16:0. Polar lipids included phosphatidylglycerol (PG), phosphatidylcholine (PC), two unidentified aminolipids and an unidentified lipid. The genomic DNA G+C content of strain P-50-3T was 69.0 mol%. On the basis of the data obtained in this polyphasic study, strain P-50-3T represents a novel species within the genus Albimonas, for which the name Albimonas pacifica sp. nov. is proposed. The type strain of Albimonas pacifica is P-50-3T (=KACC 16527T=CGMCC 1.11030T). An emended description of the genus Albimonas Lim et al. 2008 is also proposed.

The genus Albimonas within the family Rhodobacteraceae in the class Alphaproteobacteria was proposed by Lim et al., (2008) and at the time of writing contains a single species, Albimonas donghaensis (Lim et al., 2008). The type strain of A. donghaensis was isolated from seawater of the East Sea in Korea and is a non-pigmented, non-photosynthetic and slightly halophilic alphaproteobacterium whose cells are rod-shaped and devoid of flagella (Lim et al., 2008). During an investigation into the diversity of culturable bacteria in seawater samples from the Pacific, a bacterial strain, designated P-50-3T, was isolated. According to the preliminary analysis of its 16S rRNA gene sequence, the strain was most closely related to the genus Albimonas. We report here the taxonomic characterization of the novel isolate using a polyphasic approach and the proposal to classify it as the representative of a novel species in the genus Albimonas: Albimonas pacifica sp. nov.

A seawater sample was collected from a water depth of 50 m (temperature 28.6 °C; salinity 35.0 %; pH 8.3) at the CTD04 site (156.85° E, 12.85° N) of the Pacific Ocean in August 2009 during cruise DY-115-21 of R/V Da Yang Yi Hao. Strains were isolated using the dilution-plating method on TYS agar [0.5 % tryptone (Oxoid), 0.1 % yeast extract (Oxoid), 1.5 % agar and artificial seawater (Sigma sea salts, 3 %)] at 30 °C. A total of four isolates exhibiting different colony morphologies on the plates were isolated from the seawater sample and three of these strains respectively shared >99.1 % 16S rRNA gene sequence similarities with type strains of known species in the genera Marinobacter, Thalassospira and Erythrobacter. The fourth isolate, strain P-50-3T, was routinely cultivated on TYS agar or in TYS broth (0.5 % tryptone, 0.1 % yeast extract and artificial seawater) at 30 °C and stored at −80 °C in TYS broth supplemented with 20 % (v/v) glycerol.

Abbreviations: AL, unidentified aminolipid; L, unidentified lipid; ML, maximum-likelihood; MP, maximum-parsimony; NJ neighbour-joining; PC, phosphatidylcholine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Albimonas pacifica P-50-3T is JN837486.

Four supplementary figures are available with the online version of this paper.
Artificial seawater was prepared using Sigma sea salts (3%). *A. donghaensis* KCTC 12586^T^, used as a reference strain in tests for fatty acids, polar lipids and some phenotypic characteristics, was routinely cultivated in TYS broth or on TYS agar at 30°C.

For the 16S rRNA gene amplification, genomic DNA of strain P-50-3^T^ was extracted using a genomic DNA isolation kit (BioTeke). The 16S rRNA gene sequence of the strain was amplified by PCR from the genomic DNA using primers 27F (5'-AGAGTTTGATCTTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3') (Lane, 1991) and then sequenced using an Applied Biosystems model 3730 DNA sequencer at Biosune (Shanghai, China). The resultant 16S rRNA gene sequence was compared with those of type strains with validly published names in the Eztaxon-e database (http://eztaxon-e.ezbiocloud.net; Kim *et al.*, 2012) using BLASTN (Altschul *et al.*, 1997) to find phylogenetic neighbours of strain P-50-3^T^.

Pairwise sequence similarity values calculated using the global alignment algorithm were obtained through the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim *et al.*, 2012). Phylogenetic trees were reconstructed using neighbour-joining (NJ) (Saitou & Nei, 1987), maximum-parsimony (MP) (Fitch, 1971) and maximum-likelihood (ML) (Felsenstein, 1981) methods with MEGA version 5 (Tamura *et al.*, 2011). Evolutionary distances were computed according to the Kimura two-parameter model (Kimura, 1980). The topologies of the phylogenetic trees obtained were evaluated by bootstrap analyses (1000 replications).

The nearly full-length 16S rRNA gene sequence of strain P-50-3^T^ (1427 bp) was obtained, and analysis of the sequence

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain P-50-3^T^ and representatives of related genera. Bootstrap values (>50%) based on 1000 replicates are indicated at nodes. Bar, 0.01 substitutions per nucleotide position.](image-url)
revealed that strain P-50-3T belonged to the class \textit{Alphaproteobacteria}. The strain shared the highest 16S rRNA gene sequence similarity with the type strain of \textit{A. donghaensis} (94.3\%), followed by that of \textit{Oceanicella actignis} (93.5\%) and displayed <92.0\% 16S rRNA gene sequence similarities with those of other species with validly published names within the class \textit{Alphaproteobacteria}. Phylogenetic trees of 16S rRNA gene sequences built using three different treeing algorithms (NJ, MP and ML) all showed that strain P-50-3T clustered tightly with \textit{A. donghaensis}, forming a distinct phylogenetic branch with strong bootstrap support (\(\geq 93\%\)) within the family \textit{Rhodobacteraceae} (Fig. 1 and Figs. S1 and S2 available in IJSEM online).

Cellular fatty acids were analysed using GC and the Sherlock Microbial Identification Software (version 4.5) with the TSBA 40 database. For the analysis, strain P-50-3T and the reference strain \textit{A. donghaensis} KCTC 12586\(^T\) were both cultivated in TYS broth at 30\(^\circ\)C for 2 days. Polar lipids were extracted according to the method of Komagata & Suzuki (1987) and analysed using two-dimensional TLC on Merck Kieselgel 60 F\(_{254}\) aluminium-backed plates. Lipids on the plates were detected using various spraying reagents including ethanolic molybdophosphoric acid (total lipids), ninhydrin (aminolipids) and Zinnadze reagent (phospholipids) (Collins & Jones, 1980). Quinones were extracted as described by Komagata & Suzuki (1987) and analysed using reversed-phase HPLC. Genomic DNA was extracted according to the method of Marmur (1961) and the G+C content of the genomic DNA was determined by the thermal denaturation method (Marmur & Doty, 1962) using a DU800 spectrophotometer (Beckman).

The major fatty acids (\(\geq 5\%\)) detected in strain P-50-3T were \(C_{18:1}\) \(\omega7c\) (67.5\%), \(C_{18:0}\) (7.7\%), 11-methyl \(C_{18:1}\) \(\omega7c\) (6.3\%) and \(C_{16:0}\) (5.4\%) (Table 1). The fatty acid profile of strain P-50-3T was basically similar to that of \textit{A. donghaensis} KCTC 12586\(^T\), the nearest relative. Polar lipids of strain P-50-3T included major amounts of phosphatidylglycerol (PG), an unidentified aminolipid (AL1) and phosphatidylcholine (PC) and moderate amounts of an unidentified aminolipid (AL2) and an unidentified lipid (L4) (Fig. S3). PG, PC, AL1 and L4 were also detected in \textit{A. donghaensis} KCTC 12586\(^T\) (Fig. S3). The major respiratory lipoquinone of strain P-50-3T was \(Q-10\), as reported for most species of the class \textit{Alphaproteobacteria}. The genomic DNA G+C content of strain P-50-3T was 69.0 mol\%, lower than that of \textit{A. donghaensis} (72.0 mol\%).

Cell morphology was examined using a transmission electron microscope (JEM-100CX II, JEOL). Gram-staining was performed according to the method of Murray \textit{et al.} (1994). Oxidase activity was examined by using oxidase test strips (Merck) while catalase activity was determined by bubble production in 3\% (v/v) \(H_2O_2\) solution. Hydrolysis of casein, starch and Tween 80 was tested on TYS agar according to the method of Smibert & Krieg (1994). Growth at different temperatures (4, 6, 8, 10, 15, 20, 25, 30, 35, 40 and 45\(^\circ\)C) and \(pH\) (5–10.0, at 0.5 \(pH\)-unit intervals, adjusted with 1 M NaOH or HCl) was checked in TYS broth. The NaCl concentration range for growth (0\%, 0.5\% and 1–15\% with 1\% increments, w/v) was determined in a medium containing 0.5\% tryptone, 0.1\% yeast extract, 0.5\% MgCl\(_2\).6H\(_2\)O, 0.6\% MgSO\(_4\).7H\(_2\)O, 0.1\% CaCl\(_2\), 0.06\% KCl and distilled water supplemented with appropriate amounts of NaCl (Lim \textit{et al.}, 2008). Anaerobic growth was examined after incubation for 21 days at 30\(^\circ\)C in TYS broth supplemented with 0.1\% potassium nitrate, 0.05\% cysteine hydrochloride and 0.05\% sodium sulfide in Hungate tubes. Enzymic activities and additional biochemical properties were examined using API ZYM and API 20NE strips (bioMérieux) following the manufacturer’s instructions except that cells for the inoculation of the strips were suspended in artificial seawater.

Cells of strain P-50-3T were Gram-stain-negative, non-flagellated rods (Fig. S4). Growth of strain P-50-3T under anaerobic condition was not observed. Other morphological, physiological and biochemical characteristics are given in the species description. Characteristics that distinguished strain P-50-3T from \textit{A. donghaensis} are listed in Table 2. Results from phylogenetic analysis and chemotaxonomic and phenotypic characterizations indicate that strain P-50-3T represents a novel species within the genus \textit{Albimonas}, for which the name \textit{Albimonas pacifica} sp. nov. is proposed.

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Fatty acid} & \textbf{1} & \textbf{2} \\
\hline
\(C_{14:0}\) & 1.4 & 1.4 \\
iso-\(C_{15:1}\) G & 1.4 & 0.8 \\
\(C_{16:0}\) & 5.4 & 5.6 \\
\(C_{17:0}\) & 1.5 & 0.4 \\
\(C_{18:0}\) & 7.7 & 6.5 \\
\(C_{18:0}\) 3-OH & 3.1 & 4.0 \\
\(C_{18:1}\) \(\omega7c\) & 67.5 & 66.1 \\
\(C_{18:1}\) \(\omega9c\) & 0.6 & 3.0 \\
11-methyl \(C_{18:1}\) \(\omega7c\) & 6.3 & 4.4 \\
10-methyl \(C_{19:0}\) & – & 1.5 \\
\(C_{19:0}\) cyclo \(\omega8c\) & 1.0 & – \\
Summed feature 2* & 2.1 & 2.4 \\
Unknown ECL 14.502† & 1.5 & 1.3 \\
\hline
\end{tabular}
\caption{Cellular fatty acid compositions (%) of strains P-50-3T and \textit{A. donghaensis} KCTC 12586\(^T\) }
\end{table}
Table 2. Differential characteristics of strains P-50-3^T and A. donghaensis KCTC 12586^T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (width x length, μm)</td>
<td>0.8—1.2 x 2.0—4.0</td>
<td>1.0—1.4 x 1.6—2.6^*</td>
</tr>
<tr>
<td>Ranges for growth NaCl (% w/v)</td>
<td>0—12</td>
<td>0—14^*</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>10—40</td>
<td>10—38^*</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Urease</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>C_{19:0} cyclo ω8c</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>PG, PC, two ALs, L</td>
<td>PG, PC, AL, four Ls†</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69.0</td>
<td>72.0</td>
</tr>
<tr>
<td>Source</td>
<td>Seawater from the Pacific Ocean</td>
<td>Seawater from the East Sea, Korea</td>
</tr>
</tbody>
</table>

Data different from those in the original description.
†Data different from those in the original description.

Emended description of the genus Albimonas Lim et al. 2008

The description is as given by Lim et al. (2008) with the following amendments. Major polar lipids are PG, PC and an unidentified aminolipid (AL1).

Description of Albimonas pacifica sp. nov.

Albimonas pacifica (pa.ci’fi.ca. L. fem. adj. pacifica peaceful, pertaining to the Pacific Ocean, the origin of the type strain).

Cells of the type strain are Gram-stain-negative, aerobic and non-flagellated rods (2.0—4.0 μm in length and 0.8—1.2 μm in width). Colonies grown on TYS marine agar at 30°C for 3 days are round (0.8—1.2 mm in diameter), not pigmented and convex. The type strain grows at 10—40°C (optimum at 30°C), in 0—12% NaCl (w/v, optimal in 2.0%) and at pH 6.0—9.5 (optimal at pH 7.5). Does not hydrolyse casein, starch or Tween 80. In API 20NE tests, cells of the type strain are positive for nitrate reduction but negative for arginine dihydrolase, indole production, acid production from glucose, hydrolysis of aesculin and gelatin, urease, β-galactosidase and assimilation of D-glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Detected using API ZYM strips, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-Bl-phosphohydrolase are present whereas lipase (C14), trypsin, α-chymotrypsin, x-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and x-fucosidase are absent. The major respiratory quinone is Q-10. Polar lipids comprise PG, PC, two unidentified aminolipids and an unidentified lipid. The major fatty acids are C_{18:1ω7c}, C_{18:0}, 11-methyl C_{18:1ω7c} and C_{16:0}.

The type strain is P-50-3^T (=KACC 16527^T=CGMCC 1.11030^T), which was isolated from seawater of the Pacific Ocean. The genomic G+C content of the type strain is 69.0 mol%.

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References


