Aquabacterium olei sp. nov., an oil-degrading bacterium isolated from oil-contaminated soil

Van Hong Thi Pham, Seung-Woo Jeong and Jaisoo Kim

Strain NHI-1T is a Gram-negative, motile, non-spore-forming bacterium isolated from oil-contaminated soil in South Korea. The strain was able to grow by using gasoline, diesel and kerosene as energy and carbon sources. After incubation for 14 days, cells (1 g l⁻¹) degraded approximately 58% of oil present at concentration of 1500 p.p.m. at pH 8 and 28°C. Strain NHI-1T grew well under aerobic conditions, with optimal growth at pH 7–9 and 28°C–37°C but grew poorly in the presence of ≥0.5% NaCl. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the closest relatives of strain NHI-1T were Aquabacterium fontophilum CS-6T (97.96% sequence similarity), Aquabacterium parvum B6T (96.39%), Aquabacterium commune B8T (95.76%), Aquabacterium limnoticum ABP-4T (95.72%) and Aquabacterium citrithophilum B4T (95.25%). DNA–DNA relatedness was 41–53% between strain NHI-1T and its closest type strains. The major fatty acids present in strain NHI-1T were summed feature 3 (C₁₆:1ω7c/C₁₆:1ω6c, 44.45%), summed feature 8 (C₁₈:1ω7c/C₁₈:1ω6c, 21.5%) and C₁₆:0 (16.2%), and the predominant polar lipids were phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, diphosphatidylglycerol and uncharacterized aminophospholipids. Strain NHI-1T was distinguishable from other members of genus Aquabacterium based on phenotypic, chemotaxonomic and genotypic characteristics. Therefore, strain NHI-1T represents a novel species of the genus Aquabacterium for which the name Aquabacterium olei sp. nov. is proposed. The type strain is NHI-1T (=KEMB 9005-082T= KACC 18244T=NBRC 110486T).

The genus Aquabacterium belongs to the family Comamonadaceae, order Burkholderiales, in the class Betaproteobacteria (Kalmbach et al., 1999). Five species of the genus Aquabacterium have been identified to date. Aquabacterium citrithophilum, A. commune and A. parvum were isolated from biofilm from the Berlin drinking water system (Kalmbach et al., 1999), while A. fontophilum and A. limnoticum were found in freshwater springs in Taiwan (Lin et al., 2009; Chen et al., 2012). Here, we describe the isolation and analysis of a sixth member of the genus Aquabacterium. Unlike other members of the genus, strain NHI-1T was isolated from oil-contaminated soil in South Korea, and since it was capable of degrading oil components, it may be useful for the bioremediation of oil-contaminated soils. The oil-degrading ability of strain NHI-1T was evaluated in mineral salt medium under different culture conditions, including a range of pH values and incubation temperatures, and various concentrations of oil as a carbon source.

Soil samples were collected from areas around US Army bases in South Korea. Soil debris was removed by filtration through a 2 mm mesh sieve. A soil suspension was generated by mixing 1 g soil sample in 10 ml distilled water; 100 μl of this mixture was added as a inoculum to the Transwell insert membrane along with 3 ml Reasoner’s (R)2A broth on 3 g soil at the bottom of the plate, which was incubated at 28°C with shaking for 2 weeks (Pham & Kim, 2014). R2A (0.5 g each of protease peptone, yeast extract, acid digest of casein, glucose, and soluble starch; 0.3 g dipotassium phosphate; 0.024 g magnesium sulfate; and 0.3 g sodium pyruvate) containing 1500 p.p.m. oil (500 p.p.m. each of gasoline, diesel and kerosene) was used as the culture medium to isolate strain NHI-1T. Cultures from each well were serially diluted 10-fold from 10⁻¹ to 10⁻⁶, and 100 μl each dilution was spread on R2A agar plates and incubated at 28°C for 1 week (Pham et al., 2014).

Mineral salt medium containing yeast extract and peptone as nutrients was used as growth medium, and had the...
following composition per litre: 2 g each of K$_2$HPO$_4$ and (NH$_4$)$_2$SO$_4$; 1 g each of KNO$_3$, yeast extract, and peptone; 0.5 g MgSO$_4$ . 7H$_2$O; and 0.4 g NaCl. The 10 ml culture volumes in 50 ml capped bottles contained approximately 500 p.p.m. of gasoline, kerosene and diesel for a total oil concentration of 1500 p.p.m. To determine the optimal pH and incubation temperature for bacterial growth, cultures with pH values ranging from 4–10 (in 1 pH unit increments) were inoculated and incubated at 10, 20, 28 or 37 °C with shaking at 120 r.p.m. for 2 weeks. After identifying the optimal pH and incubation temperature, the optimal cell density for the highest oil degradation rate was determined. Oil remaining in the samples was extracted by incubating with dichloromethane at a 1 : 1 ratio for 24 h in a shaker, and sample compositions were determined using a HP 5890 Series II gas chromatograph (Agilent Technologies) equipped with a flame-ionization detector and an Ultra 2 capillary column (cross-linked, 5 % phenylmethyl silicone; length, 25 m; internal diameter, 0.32 mm; and film thickness, 0.17 μm) (Vermeulen, 2007).

The morphology of cells grown for 3 days at 28 °C on R2A agar (pH 7.2) was visualized by phase-contrast microscopy (BX50 microscope; Olympus) at ×1000 magnification. Gram staining was carried out as previously described (Doetsch, 1981). Growth was assessed after 5 days at 4, 10, 15, 20, 25, 30, 37, 42, 50 or 55 °C in various media, including nutrient agar, R2A broth and R2A agar. Spore formation was determined using Schaeffer’s medium composed of 0.1 % KCl, 0.01 % MgCl$_2$, 1.0 mM Ca(NO$_3$)$_2$, 0.01 mM MnCl$_2$, 0.001 mM FeSO$_4$, and 8 g nutrient broth (1.0 g Lab-Lemco’ powder, 2.0 g yeast extract, 5.0 g peptone and 5.0 g sodium chloride) per litre distilled water (Kempf et al., 2005). The motility test was performed in R2A medium containing 0.4 % agar. The growth characteristics of strain NHI-1$^T$ as compared to those of type strains of other species of the genus Aquabacterium [A. fontiphilum CS-6$^T$ (=KCTC 22896$^T$), A. limnooticum ABP-4$^T$ (=KCTC 23306$^T$), A. citratophilum B4$^T$ (=KCTC 22897$^T$), A. commune B8$^T$ (=KCTC 22900$^T$) and A. parvum B6$^T$ (=KCTC 22991$^T$)] were determined by culturing cells at pH 4.0–12.0 (at intervals of 0.5 pH units) using the following buffers (Breznak & Costilow, 1994): citrate/Na$_2$HPO$_4$ (for pH 4.0–5.0), phosphate (pH 6.0–7.0), Tris (pH 8.0–9.0), and no buffer (pH 10.0–12.0), as well as NaCl concentrations ranging from 0–10 % (w/v) in R2A broth. These experiments were carried out at 28 °C with an incubation time of 5 days. The growth of each strain under anaerobic conditions was assessed by incubating inoculated R2A plates in a GasPak anaerobic system (BBL) at 28 °C for 7 days.

Catalase and oxidase activities of each strain were evaluated by measuring bubble production in 3 % (v/v) H$_2$O$_2$ and using 1 % (v/v) tetramethyl-$p$-phenylenediamine, respectively. To evaluate caseinase and amylase production, 1 % (w/v) skimmed milk and 1 % (w/v) starch were used, respectively (Smibert & Krieg, 1994). The API ZYM kit (bioMérieux) was used to measure constitutive enzymic activity, and carbohydrate utilization profiles were determined using the API 20NE kit (bioMérieux). Other biochemical tests were performed using the Biolog GN2 microtest system. To assess gelatin and urea hydrolysis, modified liquid R2A medium supplemented with 4 % gelatin and the same medium containing 2 % (w/v) urea and 0.001 % (w/v) Phenol Red, respectively, were inoculated with the bacterial strains and incubated at 28 °C in a shaking incubator for 7 days (Smibert & Krieg, 1994).

The antibiotic sensitivity of strain NHI-1$^T$ was examined by plating cells on R2A agar (0.5 McFarland standard) and applying 6 mm discs containing the following antibiotics to the bacterial lawn: 30 μg chloramphenicol, kanamycin, nalidixic acid, novobiocin or tetracycline; 23.75 μg sulfamethoxazole; 10 μg ampicillin, gentamicin, penicillin or streptomycin; 5 μg rifampicin; or 1.25 μg trimethoprim (Lin et al., 2009). The zone of growth inhibition surrounding each disc was measured after incubation for 3 days and the strain was judged susceptible, intermediate or resistant if the diameter of the inhibition zone was >11 mm, 8–10 mm, and <8 mm, respectively (Nokhal & Schlegel, 1983).

Cells cultured on R2A for 5 days at 28 °C were used for polar lipids and fatty acid analyses. The cells were in exponential phase growth, which was found in the third quadrant of the agar plate. Polar lipids were extracted and separated by two-dimensional thin-layer chromatography. Lipid spots were detected by spraying with the appropriate reagent for identification (Minnikin et al., 1984): ethanolic molybdophosphoric acid (phosphomolybdic acid reagent, 20 % solution in ethanol; Sigma-Aldrich) for total lipids; ninhydrin reagent for free amino group-containing lipids; and Zinzadze reagent (molybdenum blue spray reagent, 1.3 %; Sigma-Aldrich) for phosphorus-containing lipids. Fatty acids were extracted and analysed using the Sherlock Microbial Identification System (TSBA6 version 6.0; MIDI) (Sasser, 1990).

The DNA G + C content of strain NHI-1$^T$ was determined by reverse-phase high-performance liquid chromatography (Mesbah et al., 1989). Photobiotin acetate was used to label the DNA, which was then used as a probe; DNA–DNA hybridization was then carried out according to previously published protocols (Wayne et al., 1987; Mehlen et al., 2004). The DNA–DNA relatedness of strain NHI-1$^T$ to the five reference strains was measured fluorometrically, with DNA from strain NHI-1$^T$ and salmon sperm used as positive and negative controls, respectively. Additionally, each reference strain was reservedly hybridized as a probe to strain NHI-1$^T$. Protein profiles of strain NHI-1$^T$ and the reference strains were obtained after separation by SDS-PAGE and staining with Coomassie brilliant blue (Sambrook et al., 1989).

DNA was sequenced to determine the primary structure of the 16S rRNA gene. Genomic DNA was extracted using an InstaGene Matrix kit (Bio-Rad), and the 16S rRNA gene was PCR amplified using the universal bacterial primers.
27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) (Pham & Kim, 2014). The PCR product was purified with a multiscreen filter plate (Millipore) and sequenced using the primers 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′) in addition to 27F and 1492R in the PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). The reaction mixture was incubated at 95°C for 5 min, cooled on ice for 5 min, and then analysed with an ABI Prism 3730XL DNA Analyser (Applied Biosystems). SeqMan software (DNASTAR) was used to assemble the near-complete full-length sequence of the 16S rRNA gene, which was identified using the EzTaxon server (Kim et al., 2012). Related sequences were obtained from the GenBank database and edited using BioEdit software (Hall, 1999). Multiple alignments were generated using the CLUSTAL X program (Thompson et al., 1997). The neighbour-joining, maximum-likelihood and maximum-parsimony methods were used to reconstruct the phylogenetic trees with the MEGA6 software package (Tamura et al., 2013) using a bootstrap value of 1500 replicate (Felsenstein, 1985).

Strain NHI-1T formed larger colonies (ranging in size between 0.5–2.0 mm after incubation for 2 days at 28°C on R2A medium) than the reference strains that showed 0.7–1.5 mm colonies. Indeed, colonies of strain NHI-1T appeared after only 1 day of incubation, indicating more rapid growth than other members of the genus Aquabacterium. Phenotypic analysis of strain NHI-1T revealed many features and values that were found in the five reference strains in this study; however, several differences were also noted (Table 1). The unique features of strain NHI-1T were the hydrolysis of starch and the assimilation of D-glucose, potassium gluconate, adipic acid and trisodium citrate. This study found some different physiological characteristics

### Table 1. Physiological characteristics of species of the genus Aquabacterium

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Salt tolerance (%)</td>
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<td>2.5</td>
<td>3.0</td>
<td>2.0</td>
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<td>7.0–8.0</td>
<td>7.0–9.0</td>
<td>7.0–8.5</td>
<td>6.5–9.5</td>
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<td>Growth temperature (°C)</td>
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<td>15–42</td>
<td>20–40</td>
<td>10–36</td>
<td>6–34</td>
<td>14–34</td>
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<tr>
<td>Hydrolysis of:</td>
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<tr>
<td>Casein</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>w</td>
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<td>–</td>
<td>–</td>
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<td>w</td>
<td>–</td>
<td>+</td>
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<td>+</td>
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<td>Gelatin</td>
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<td>–</td>
<td>+</td>
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<td>Enzymic activity</td>
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<td>Alkaline phosphatase</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>Esterase lipase (C8)</td>
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<td>+</td>
<td>+</td>
<td>w</td>
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<td>Lipase (C14)</td>
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<td>Leucine arylamidase</td>
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<td>+</td>
<td>+</td>
<td>w</td>
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<td>w</td>
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<td>–</td>
<td>w</td>
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<td>Acid phosphatase</td>
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<td>+</td>
<td>w</td>
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<td>Naphthol-AS-BI-phosphohydrolase</td>
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<td>–</td>
<td>w</td>
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<td>+</td>
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<tr>
<td>α-Glucosidase</td>
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<td>–</td>
<td>w</td>
<td>–</td>
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<tr>
<td>β-Glucosidase</td>
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<tr>
<td>α-Mannosidase</td>
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<td>API 20NE tests</td>
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<td>NO₃ (reduction)</td>
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<td>+</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>4-nitrophenyl-β-D-galactopyranoside</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>D-glucose (fermentation)</td>
<td>–</td>
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<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
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<tr>
<td>D-glucose (assimilation)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>D-Maltose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
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<tr>
<td>Potassium gluconate</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Adipic acid</td>
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<td>Trisodium citrate</td>
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<td>–</td>
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<td>DNA G+C content (mol%)</td>
<td>68.0</td>
<td>63.4</td>
<td>68.6</td>
<td>66.0</td>
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from the previous study; weak or negative result for urea hydrolysis, and valine arylamidase, lipase (C14), alkaline phosphatase, cysteine arylamidase and naphthol-AS-BI-phosphohydrolase activities of A. fontiphilum CS-6<sup>T</sup>, which were positive in the previous study (Lin et al., 2009). In addition, some features were not matched with the previous study of A. limnoticum ABP-4<sup>T</sup> such as esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and α-glucosidase activities and adipic acid assimilation (Chen et al., 2012). All other results obtained in this study were the same as the previous studies.

The phylogenetic trees generated from 16S rRNA gene sequences depict the evolutionary relationship between strain NHI-1<sup>T</sup>, other species of the genus Aquabacterium and neighbouring taxa (Fig. 1 and Fig. S1, available in the online Supplementary Material). In the genus Aquabacterium, strain NHI-1<sup>T</sup> exhibited the highest 16S rRNA gene sequence similarity to A. fontiphilum CS-6<sup>T</sup> (97.9 %), followed by A. parvum B6<sup>T</sup> (96.39 %), A. commune B8<sup>T</sup> (95.76 %), A. limnoticum ABP-4<sup>T</sup> (95.61 %), and A. citratiphilum B4<sup>T</sup> (95.25 %). Strain NHI-1<sup>T</sup> is closely related to members of other genera that are located in separate branches of the phylogenetic tree but monophyletically with the type species of Aquabacterium (Figs. 1 and S1). DNA–DNA hybridization analyses showed that the genome of strain NHI-1<sup>T</sup> was different at the species level to those of A. limnoticum ABP-4<sup>T</sup> (57 ± 3 %, n = 3), A. commune B8<sup>T</sup> (53 ± 2 %, n = 3), A. citratiphilum B4<sup>T</sup> (52 ± 4 %, n = 3), A. fontiphilum CS-6<sup>T</sup> (48 ± 2 %, n = 3), and A. parvum B6<sup>T</sup> (41 ± 3 %, n = 3) (Table S1). These results indicate that strain NHI-1<sup>T</sup> does not belong to any known species of the genus Aquabacterium, since the recommended DNA–DNA relatedness values are between 70 % and 25 % for the definition of a species (Wayne et al., 1987).

The fatty acid profile of strain NHI-1<sup>T</sup> was similar to those of the five reference strains. The major components identified were summed feature 3 (C<sub>16 : 1</sub><sub>v7c</sub>/C<sub>16 : 1</sub><sub>v6c</sub>), Roseateles depolymerans DSM 11814<sup>T</sup> (AB003626)
Roseateles aquatilis CCUG 48205<sup>T</sup> (AM501446)
Pelomonas saccharophila DSM 654<sup>T</sup> (AB021407)
Pelomonas puraquae CCUG 52769<sup>T</sup> (AM501439)
Inhella inkyongensis IMCC1713<sup>T</sup> (DQ664238)
Inhella fonicola TNR-25<sup>T</sup> (HM013811)
Rubrivivax gelatinosus ATCC 17011<sup>T</sup> (D16213)
Rubrivivax benzoatylicus JA2<sup>T</sup> (AJ888903)
Ideonella dechloratans CCG 30898<sup>T</sup> (X72724)
Leptothrix discophora SS-1<sup>T</sup> (L33975)
Leptothrix choldnii CCM 1827 (X97070)
Leptothrix mobilis Feox-1<sup>T</sup> (X97071)
Piscinibacter aquaticus IMCC1728<sup>T</sup> (DQ664244)
Rivibacter subsaxonicus BF49<sup>T</sup> (AM774413)
'Caldimonas meghalayensis' AK31 (HF562216)
'Zhizhongheella caldifontis' YIM 78140<sup>T</sup> (KF771277)
Polyangium brachysporum' K481-B101 (AM410613)
'Polyangium brachysporum' K481-B101 (AM410613)
Aquabacterium olei NHI-1<sup>T</sup> (KC424519)
Aquabacterium fontiphilum CS-6<sup>T</sup> (EF626687)
Aquabacterium parvum B6<sup>T</sup> (AF035052)
Aquabacterium commune B8<sup>T</sup> (AF035054)
Aquabacterium citratiphilum B4<sup>T</sup> (AF035050)

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences depicting the relationship between strain NHI-1<sup>T</sup> and related taxa. The tree was reconstructed using the neighbour-joining method with Hasegawa-Kishino-Yano as a best-fit model. Bar, 0.01 substitutions per nucleotide position.
44.5%), summed feature 8 (C₁₈ : 1 ν C₁₉ : 1, 21.5%),
C₁₆ : 0 (16.2%), C₁₂ : 0 (5.9%), and the hydroxyl fatty acid
C₁₀ : 0 3-OH (4.9%). Notably, a novel component referred
to as summed feature 7 (un 18.846/C₁₉ : 1, 1.2%) was
unique to strain NHI-1ᵀ, while C₁₇ : 0 iso was detected only
in strain NHI-1ᵀ (1.3%) and A. commune B₈ᵀ (1.5%).
The prevalence of other components are listed in
Table S2. The polar lipid profile of strain NHI-1ᵀ included
phosphatidylethanolamine, phosphatidylycerine, phosphati-
dyglycerol, diphosphatidylglycerol and six uncharacterized
aminophospholipids (Fig. 2).

While strain NHI-1ᵀ and the reference strains had highly
similar protein patterns, particularly in the molecular
mass range of 35–75 kDa, a 160 kDa band was detected
only in extracts of A. parvum B₆ᵀ, A. citratiphilum B₄ᵀ,
and A. commune B₈ᵀ (Fig. S2). Also, an approximately
110 kDa band was exhibited considerably in A. limnicum
ABP-4ᵀ and A. parvum B₆ᵀ, but was faint in the other
strains (Fig. S2) (Kalmbach et al., 1999).

Strain NHI-1ᵀ was resistant to penicillin, gentamicin and
sulfamethoxazole plus trimethoprim, but sensitive to rifampi-
cin, nalidixic acid, novobiocin, ampicillin, chloramphenicol,
tetracycline, kanamycin, and streptomycin. Detailed anti-
biotic resistance profiles of strain NHI-1ᵀ and the reference
strains are provided in Table S3.

The highest rate of oil degradation was at pH 8 and 28 °C for a
cell concentration of 1 g L⁻¹. Under these conditions, strain
NHI-1ᵀ reduced the initial oil concentration (1500 p.p.m.)
by approximately 58% after 14 days (Figs S3–S5).

Based on the combined phylogenetic, phenotypic and che-
matoxicographic data, strain NHI-1ᵀ represents a novel
species of the genus Aquabacterium, for which the name
Aquabacterium olei sp. nov. is proposed.

**Description of Aquabacterium olei sp. nov.**

Aquabacterium olei (o’le.i. L. gen. neut. n. olei of/from oil,
as the oil-contaminated soil from which the type strain was
isolated).

Cells are Gram-stain-negative, short rod-shaped (0.6–
0.9 × 1.0–2 μm, width × length), motile and non-spore-
forming. After incubation for 2 days at 28 °C, colonies are
approximately 0.5–2 mm, transparent, viscous, convex, with
an entire margin. Optimal growth is observed at 28 °C
(range 10 to 37 °C), between pH 7.0–9.5, and at 0 % saline
(range 0–1.0 % NaCl) in R2A. Growth does not occur
under anaerobic conditions after incubation for 5 days.
Cells are positive for oxidase and catalase activities as well as
hydrolysis of urea, gelatin, starch and casein, but are negative
for Tween 80 hydrolysis, DNase activity, and aesculin. Exhi-
bits alkaline phosphatase, esterase lipase (C8), leucine aryla-
midase, naphthol-AS-Bl-phosphohydrolase and weak acid
phosphatase activities, but not lipase (C14), α-glucosidase,
β-glucosidase or α-mannosidase activities. Positive result in
tests for NO₃ reduction, gelatin hydrolysis, D-glucose (assim-
ilation), adipic acid, malic acid, trisodium citrate, and weakly
positive result for potassium gluconate, but negative result for
4-nitrophenyl-β-D-galactopyranoside, aesculin ferric citrate,
D-glucose (fermentation) and D-maltose. The following com-
pounds are oxidized: L-arabinose, D-arabitol, i-erythritol,
α-D-glucose, methyl pyruvate, monomethyl succinate, cis-
aconitic acid, γ-hydroxybutyric acid, succinic acid, L-aspara-
gine, L-glutamic acid, hydroxy-L-proline and inosine. Weak
oxidation of D-trehalose, turanose, xyitol, acetate acid, citric
acid, formic acid, D-galactonic acid lactone, D-galacturonic
acid, D-glucosaminic acid, D-glucuronic acid, D-hydroxybutyric acid and β-hydroxybutyric acid is also
observed. Resistant to penicillin, gentamicin and sulfa-
methoxazole plus trimethoprim, but sensitive to rifampicin,
nalidixic acid, novobiocin, ampicillin, chloramphenicol,
tetracycline, kanamycin and streptomycin. The major polar
lipids are phosphatidylglycerol, phosphatidylethanolamine,
phosphatidylycerine, diphosphatidylglycerol and uncharacte-
rized aminophospholipids. The predominant fatty acids are
summed feature 3 (C₁₆ : 1 ν C₁₇ : 1, 19.6%), summed feature 8
(C₁₈ : 1 ν C₁₉ : 1, 10.6%), and C₁₆ : 0, and notably, summed
feature 7 (un 18.846/C₁₉ : 1, 10.6%), which is not present in
type strains of other species of the genus Aquabacterium.

The type strain NHI-1ᵀ (=KEMB 9005-082ᵀ =KACC 18244ᵀ
=NBRC 110486ᵀ) was isolated from oil-contaminated soil in
South Korea. The DNA G+C content of the type strain is
68 mol%.

**Fig. 2.** Chromatogram of distinct species of polar lipids detected in
strain NHI-1ᵀ by two-dimensional thin-layer chromatography. DPG,
diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidyl-
ethanolamine; PS, phosphatidylycerine; PL₁–PL₆, uncharacterized
aminophospholipids.

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