**Pseudomaricurvus alcaniphilus** sp. nov., a marine bacterium isolated from tidal flat sediment and emended descriptions of the genus *Pseudomaricurvus*, *Pseudomaricurvus alkylphenolicus* Iwaki et al. 2014 and *Maricurvus nonylphenolicus* Iwaki et al. 2012

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A novel Gram-reaction-negative, rod-shaped, aerobic and motile strain, designated MEBiC06469T, was isolated from tidal flat sediment of the Taean province, South Korea. Strain MEBiC06469T produced ivory-coloured colonies on marine agar 2216 medium and could degrade carboxymethyl-cellulose. On the basis of 16S rRNA gene sequence similarity, the closest relative was *Pseudomaricurvus alkylphenolicus* KU41GT with 96.5 % similarity. The isolate was catalase-positive but oxidase-negative. Growth was observed at 16–38 °C (optimum, 32 °C), at pH 6.0–9.0 (optimum, pH 7.5) and in the presence of 0.0–8.0 % (w/v) NaCl (optimum, 1.5 %). The only isoprenoid quinone was Q-8. The dominant fatty acids were summed feature 3 (comprised of C15 : 02-OH and/or C16 : 1ω7c; 20.4 %) and C17 : 1ω8c (30.9 %), summed feature 8 (comprised of C18 : 1ω7c and/or C18 : 1ω6c; 9.5 %), C16 : 0 (9.0 %), C15 : 1ω8c (5.3 %), and C11 : 03-OH (5.2 %). Based on these phenotypic properties and phylogenetic data, strain MEBiC06469T should be classified as a novel species within the genus *Pseudomaricurvus* for which the name *Pseudomaricurvus alcaniphilus* sp. nov. is proposed. The type strain is MEBiC06469T (=KCCM 42976T=JCM 18313T). Emended descriptions of the genus *Pseudomaricurvus*, *Pseudomaricurvus alkylphenolicus* Iwaki et al. 2014, and *Maricurvus nonylphenolicus* Iwaki et al. 2012 are also provided.

The genus *Pseudomaricurvus* Iwaki et al. 2014 is a member of distinctly branched marine bacteria in the order *Alteromonadales*. Members of this group can degrade high-molecular-mass polymers such as cellulose (Distel et al., 2002; Ekborg et al., 2005), agar (Du et al., 2008; Shieh et al., 2008), starch (Chen et al., 2011), and a variety of hydrocarbons (Gutierrez et al., 2012; Iwaki et al., 2012, 2014), etc. Clones could be affiliated with the genus *Pseudoteredinibacter* reported from ascidian (Dishaw et al., 2014), coral (Meron et al., 2011), seawater (Na et al., 2011; Zhang et al., 2007), etc. However, the majority of clones have been reported from oil-contaminated environments (Acosta-González et al., 2013; Chan et al., 2014; Gittel et al., 2012; Maeda et al., 2005; Redmond & Valentine, 2012). These reports suggested that members of the genus *Pseudomaricurvus* could be concerned in the degradation of contaminated hydrocarbons in the environment. A marine bacterium that could utilize hexadecane and degrade carboxymethyl-cellulose (CM-cellulose) was isolated from tidal flat sediments that had experienced an oil-spill accident, and a taxonomic study using a polyphasic approach revealed that this strain is a novel member of the genus *Pseudomaricurvus*.

Strain MEBiC06469T was isolated from tidal flat sediment at Taean province, Korea (36° 46' N 126° 08' E).

Abbreviations: AL, aminolipid; APL, aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain MEBiC06469T is JQ672628.

Two supplementary figures and two supplementary tables are available with the online Supplementary Material.
Approximately 1 g sample was homogenized, diluted with sterile seawater and spread onto marine agar 2216 medium (MA; BD). Inoculated plates were incubated at 25 °C for 3 days and individual colonies were isolated from MA on the basis of morphological differences. After primary isolation and purification, strain MEBiC06469\textsuperscript{T} was cultivated at 25 °C on the same medium for biochemical and physiological characterization and stored at −80 °C in marine broth 2216 (MB; BD) supplemented with 20 % (v/v) glycerol. For phenotypic comparisons, *Maricurus nonylphenolicus* KU41ET (Iwaki et al., 2012) provided from Professor Hiroaki Iwaki and *Pseudomaricurvus alkylphenolicus* KCTC 32386\textsuperscript{T} (=KU41G\textsuperscript{T}; Iwaki et al., 2014) supplied from the Korean Collection for Type Cultures (KCTC) were cultivated on MA at 25 °C.

Extraction of genomic DNA was conducted by using a DNA extraction kit (Gene All). The 16S rRNA gene sequence was amplified from extracted DNA by using Premix Taq Polymerase PCR solution (T&I) with bacterial primer set 27F and 1518R; the detailed procedure and conditions were described in Lee et al. (2013). The amplified 16S rRNA gene was sequenced using a model 3730 automatic DNA sequencer (ABI) according to the manufacturer's instructions. Obtained sequences were assembled by using Vector NTI version 9.1 (Life Technologies) and checked by BLAST pairwise alignment with the EzTaxon-e database (Kim et al., 2012). Strain MEBiC06469\textsuperscript{T} showed 96.5 % 16S rRNA gene sequence similarity with the type strain of *Pseudomaricurvus alkylphenolicus*, followed by *Maricurus nonylphenolicus* KU41E\textsuperscript{T} (94.2 %), *Teredinibacter turnerae* T7902\textsuperscript{T} (93.8 %), *Pseudoteredinibacter isoporae* sw-11\textsuperscript{T} (93.5 %); all other strains showed lower than 93 % 16S rRNA gene sequence similarity. Therefore, the nearly complete 16S rRNA gene sequence (1348 bp) of strain MEBiC06469\textsuperscript{T} was compared with that of the top 20 closely related strains. Recently, *Aestuariicella hydrocarbonica* SM-6\textsuperscript{T} was described and shows 95.1 % 16S rRNA gene sequence similarity (Lo et al., 2015), but was not included in this study. A phylogenetic analysis using the 16S rRNA gene sequence was conducted using MEGA software version 5.2 (Tamura et al., 2011). The neighbour-joining (Saitou & Nei, 1987) tree (Fig. 1) revealed that the isolated strain formed a coherent clade with *Pseudomaricurvus alkylphenolicus* KU41G\textsuperscript{T}, and this topology was recovered in maximum-likelihood (Felsenstein, 1981) but not in maximum-parsimony (Fitch, 1971) trees. Unless otherwise stated, physiological and morphological characterization was conducted according to the methods described in Kwon et al. (2005) and Yang et al. (2006). Transmission electron micrographs were taken using a LIBRA120 (Carl-Zeiss) electron microscope after negative-staining of fixed cells using 2 % phosphotungstic acid.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree.png}
\caption{Phylogenetic tree based on nearly complete 16S rRNA gene sequences (1348 bp) showing the relationship between strains MEBiC06469\textsuperscript{T} and closely related members of the class *Gammaproteobacteria*. The tree is based on the Juke & Cantor distances model and the neighbour-joining algorithm. Bootstrap values (>50 %) from neighbour-joining, maximum-likelihood and maximum-parsimony methods are placed on the left of the node. Bar, 0.01 nt substitutions per nucleotide position. Filled circles represent nodes recovered in 3 different treeing methods and hollow circles represent nodes recovered in 2 methods.}
\end{figure}


reagent (at pH 7.0), and a short-rod-shaped cell without a polar flagellum was observed (Fig. S1, available in the online Supplementary Material). The growth temperature was tested in MB in a temperature-gradient incubator (TWS126MA; Advantec) for up to 3 days at 12 different temperatures (5, 12, 16, 20, 23, 26, 29, 32, 38, 41, 45 and 50 °C). The tolerance range of NaCl was tested in the broth with ingredients of ZoBell 2216 (ZoBell, 1941) prepared with distilled water and supplemented with varying concentrations of NaCl (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12 and 20 %, w/v; Sigma). The tolerance range of pH was determined (pH 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9 and 10) in MB with the pH adjusted using biological buffers MES (for pH 4–6), HEPES (pH 6–8) or AMPSO (pH 8–10), each at 10 mM. The bacterial suspension used to inoculate API 20E, API 20NE and API ZYM test strips (bioMérieux) and the GN2 MicroPlate system (Biolog) was prepared in a 2 % sea salt (Sigma) solution. There were no responses by the type strains of M. nonylphenolicus and Pseudomaricurvus alkylphenolicus, therefore Daigo’s IMK-SP (Iwaki et al., 2012) solution was applied for biochemical characterization of these strains. The tolerance range of NaCl and pH, and biochemical characterization were conducted at 25 °C for 2 days. Detailed results of the biochemical, morphological and physiological tests are given in the species description and Table 1. The biochemical characteristics of strain MEBiC06469T were not markedly influenced by a small difference in cultivation temperature (25 °C and 32 °C, Table S1).

Polar lipids were extracted using a chloroform/methanol system, separated by two-dimensional TLC using silica gel 60 F254 aluminium-backed thin-layer plates (Minnikin et al., 1984) and detected according to the procedure and reagents described in Yang et al. (2013). The predominant polar lipids of strain MEBiC06469T were determined to be phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), one unidentified lipid, one unidentified aminophospholipid (APL) and one unidentified aminolipid (AL) (Fig. S2). The major polar lipids of M. nonylphenolicus KU41ET were determined to be PE, PG, DPG, one unidentified AL, two unidentified APL, and one unidentified phospholipid (PL), while those of Pseudomaricurvus alkylphenolicus KU41GT were determined to be PE, DPG, three unidentified lipids, two unidentified APL, two unidentified PL and one unidentified AL (Fig. S2).

Respiratory quinones were determined by HPLC analysis according to the method described by Collins (1985); the only respiratory quinone of strain MEBiC06469T was Q-8. The DNA G+C content of strain MEBiC06469T was 57.2 mol%, as determined by HPLC using a symmetry reversed-phase C18 column (Stackebrandt & Liesack, 1993).

Table 1. Differential characteristics of strain MEBiC06469T and closely related strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth range (optimum) for:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>16.4–38.7 (32.0)</td>
<td>15–30 (25–30)*</td>
<td>20–35 (25–30)*</td>
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<tr>
<td>pH</td>
<td>6.0–9.0 (7.5)</td>
<td>7.0–10.0 (8.0)*</td>
<td>7.0–8.0*</td>
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<tr>
<td>NaCl (% w/v)</td>
<td>0.0–8.0 (1.5)</td>
<td>2.0–3.0*</td>
<td>1.0–4.0 (2.0–3.0)*</td>
</tr>
<tr>
<td>Reduction of nitrate to nitrite</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Oxidase activity</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetoin production</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
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<tr>
<td>Aesculin</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Utilization of:</td>
<td></td>
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<tr>
<td>Sucrose, melibiose</td>
<td>w</td>
<td>–</td>
<td>+</td>
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<tr>
<td>l- Amygdalin</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Enzyme activities (API ZYM)</td>
<td></td>
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<tr>
<td>Esterase (C4)</td>
<td>w</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipase (C14), phosphohydrolase, x-galactosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>57.2</td>
<td>53.3*</td>
<td>48.6*</td>
</tr>
</tbody>
</table>

*Data from: a, Iwaki et al. (2014); b, Iwaki et al. (2012).
The cellular fatty acids profile was determined commercially by using the MIDI/Hewlett Packard Microbial Identification System (MIS; Sasser, 1990) with Sherlock version 6.2 and RTSet6 database according to the manufacturer’s instructions, on cells grown on MA at 25 °C and harvested at late exponential growth phase (cultivation for 2 days).

The dominant fatty acids of strain MEBiC06469T were C₁₇:₁ω8c (30.9 %), summed feature 3 (comprised of C₁₆:₁ω6c and/or C₁₆:₁ω7c, 20.4 %), summed feature 8 (comprised of C₁₈:₁ω7c and/or C₁₈:₁ω6c, 9.5 %), C₁₆:₀ (9.0 %), C₁₅:₀ω8c (5.3 %), and C₁₁:₀ 3-OH (5.2 %) (Table S2). Those of Pseudomaricurvus alkaniphilicus KCTC 32386T were C₁₇:₁ω8c (22.5 %), summed feature 3 (22.2 %), summed feature 8 (9.4 %), C₁₀:₀ 3-OH (8.3 %), C₁₆:₀ (5.4 %), and C₁₁:₀ 3-OH (5.1 %). A severe disagreement for C₁₅:₀ between this study and previous data (Iwaki et al., 2014) on Pseudomaricurvus alkaniphilicus KCTC 32386T was observed. Considering the results of neighboring species, the previous data seemed a mistake. The major cellular fatty acids of M. nonylphenolicus KU41ET were summed feature 3 (29.9 %), summed feature 8 (20.9 %), C₁₆:₀ (17.2 %), C₁₀:₀ 3-OH (10.4 %), C₁₀:₀ (6.0 %), and C₁₇:₁ω8c (6.0 %). The major fatty acid components of strain MEBiC06469T and Pseudomaricurvus alkaniphilicus were similar but showed differences in the proportion. M. nonylphenolicus KU41ET showed clear differences in the proportion of summed feature 8, C₁₆:₀ and C₁₇:₁ω8c (Table S2).

To investigate hydrocarbon degradation, a 20 µl aliquot of strain MEBiC06469T cultured in MB was inoculated into 20 ml MM2 broth (Sohn et al., 2004) supplemented with 0.1 % (v/v) nonylphenol (Sigma) or hexadecane (Sigma) and incubated at 25 °C on a rotary shaker at 120 r.p.m. for 19 days. The optical density was then measured at 600 nm using a spectrophotometer and remained hydrocarbons in the culture broth were extracted, concentrated and quantified with a gas chromatograph system (Saturn 2000Varian) and eicosane as the standard compound according to the procedure described in Choi et al. (2002). Strain MEBiC06469T showed good growth with hexadecane and nonylphenol, however, the concentration of these compounds did not change after culture.

The results of the 16S rRNA gene sequence similarity and phylogenetic analysis suggests that strain MEBiC06469T should be affiliated into the genus Pseudomaricurvus (Fig. 1). Additionally, strain MEBiC06469T shared common features with Pseudomaricurvus alkaniphilicus KU41ET; both were mesophilic, aerobic, Gram-reaction-negative rods, and slightly alkalinophilic. The two strains commonly produce acid- and alkaline-phosphatases, esterase lipase (C₉), leucine arylamidase and naphthol-AS-BI phosphohydrolase. Both strains possess PE, DPG, an unidentified APL and an unidentified lipid as common major polar lipids (Fig. S2), Q-8 as the predominant quinone, and C₁₇:₁ω8c and summed feature 3 as predominant fatty acids. However, strain MEBiC06469T could be differentiated from Pseudomaricurvus alkaniphilicus KU41ET by following characteristics: requirement of sodium ions for growth, presence of PG as one of the major polar lipids, catalase activity, nitrate reduction, hydrolysis of aesculin and gelatin, and utilization of some carbohydrates (Table 1). Therefore, strain MEBiC06469T should be classified as representing a novel species of the genus Pseudomaricurvus for which the name Pseudomaricurvus alcaniphilus sp. nov. is proposed. Emended descriptions of the genus Pseudomaricurvus, Pseudomaricurvus alkaniphilicus and Maricurvus nonylphenolicus are also provided.

**Description of Pseudomaricurvus alcaniphilus sp. nov.**

Pseudomaricurvus alcaniphilus (al.ca.ni’phi.lus. N.L. n. alcanum aliphatic hydrocarbon; Gr. masc. adj. philos loving; N.L. masc. adj. alcaniphilus alcane-loving).

Cells are aerobic, Gram-reaction-negative rods, 1.4–2.5 μm in length and 0.6–1.0 μm in diameter. Colonies are ivory-coloured, opaque, convex and uniformly circular on marine agar 2216 at 25 °C. Growth occurs between 16 and 38 °C (optimum 32 °C), at pH 6.0–9.0 (optimum pH 7.5), and in the presence of 0.0–8.0 % (w/v) NaCl (optimum 1.5 %). Catalase-positive but oxidase-negative. β-Glucosidase, protease and β-galactosidase activities are positive. Produces acetoin and gelatinase, and ferments amylodalin, melibiose and sucrose. Acid phosphatase, alkaline phosphatase, esterase (C₄), esterase lipase (C₉), lipase (C₁₄), leucine arylamidase, valine arylamidase, naphthol-AS-BI phosphohydrolase and α-galactosidase activities are present, but cystine arylamidase, trypsin, z-chymotrypsin, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Oxidizes z-cycloexdrin, dextrin, Tween 40, Tween 80, L-arabinose, L-lactose, methyl β-D-glucoside, D-psicose, D-sorbitol, acetic acid, formic acid, D-gluconic acid, p-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, D-alanine, L-alanine, L-phenylalanine, L-proline, 2,3-butanediol, and glucose 6-phosphate. The major fatty acids are C₁₆:₀, C₁₅:₀ 108cG, C₁₇:₁ω8c, C₁₁:₀ 3-OH, summed feature 3 (C₁₆:₀ω6c/C₁₆:₁ω7c, summed feature 8 (C₁₈:₁ω7c/C₁₈:₁ω6c). The predominant polar lipids are PE, PG, DPG, one unidentified lipid, one unidentified APL and one unidentified AL.

The type strain, MEBiC06469T (=KCCM 42976T=JCM 18313T), was isolated from a tidal flat sediment of the Taean County, Korea. The DNA G+C content of the type strain is 57.2 mol%.

**Emended description of the genus Pseudomaricurvus Iwaki et al. 2014**

The description given by Iwaki et al. (2014) could be emended as follows. Cells are Gram-negative, aerobic rods. Chemo-organotrophic, mesophilic and moderately...
alkaliphilic. Cell growth accelerated by sodium ions and some strains require it for growth. The predominant or only respiratory quinone is Q-8. The range of the DNA G+C content is 53–57 mol%. Major polar lipids are PE, DPG, an unidentified lipid and an unidentified APL. The predominant fatty acids are C_{17:1} \, \Delta \, 9c \, \text{and summed feature 3 (C}_{16:1} \, \Delta \, 7c \, \text{and/or C}_{16:1} \, \Delta \, 7c)\text{.}

The type species is *Pseudomaricurvus alkylphenolicus*.

**Emended description of *Pseudomaricurvus alkylphenolicus* Iwaki et al. 2014**

The description is based on that given by Iwaki et al. (2014) with the following amendments. Alkaline phosphatase and naphthol-AS-BI phosphohydrolase activities are present, but lipase (C_{14}) and valine arylamidase activities are absent. Oxidase-positive but catalase-negative. The polar lipid profile contains PE, DPG, three unidentified lipids, two unidentified PL, an unidentified AL and two unidentified APL.

**Emended description of *Maricurvus nonylphenolicus* Iwaki et al. 2012**

The description is based on that given by Iwaki et al. (2012) and emended as follows. Gelatin is not hydrolysed but nitrate is reduced to nitrite. Ferments sucrose and melibiose and produces acetoin. Activities of lipase (C_{14}) and valine arylamidase are negative, but acid phosphatase is positive. The predominant polar lipids are PE, PG, DPG, one unidentified phospholipid, two unidentified APL and one unidentified AL. The dominant fatty acids are summed feature 3 (comprising C_{16:1} \, \Delta \, 6c \, \text{and/or C}_{16:1} \, \Delta \, 7c), summed feature 8 (comprising C_{18:1} \, \Delta \, 7c) , C_{16:0} \text{and C}_{10:0} \, 3\text{-OH}.

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**References**


