**Lutimaribacter saemankumensis** gen. nov., sp. nov., isolated from a tidal flat of the Yellow Sea

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A Gram-negative, non-motile, rod-shaped bacterial strain, designated SMK-117\(^{T}\), belonging to the *Alphaproteobacteria*, was isolated from a tidal flat of the Yellow Sea, Korea, and was subjected to a polyphasic taxonomic study. Strain SMK-117\(^{T}\) grew optimally at pH 7.0–8.0 and 30 °C in the presence of 2 % (w/v) NaCl. Neighbour-joining and maximum-likelihood phylogenetic trees based on 16S rRNA gene sequences showed that strain SMK-117\(^{T}\) clustered with *Maritimibacter alkalophilus* HTCC2654\(^{T}\), with which it exhibited a sequence similarity of 95.3 %. Strain SMK-117\(^{T}\) contained Q-10 as the predominant ubiquinone and C\(_{18:1}\) \(\omega 7\)c and 11-methyl C\(_{18:1}\) \(\omega 7\)c as the major fatty acids. The major polar lipids were phosphatidylcholine, phosphatidylethanolamine, an unidentified aminolipid and two unidentified phospholipids. The DNA G + C content was 63.5 mol%. Strain SMK-117\(^{T}\) was differentiated from members of the genera *Maritimibacter* and *Oceanicola* on the basis of differences in the fatty acid and polar lipid profiles. The phenotypic, chemotaxonomic and phylogenetic data indicated that strain SMK-117\(^{T}\) represents a novel genus and species, for which the name *Lutimaribacter saemankumensis* gen. nov., sp. nov. is proposed. The type strain of *Lutimaribacter saemankumensis* is SMK-117\(^{T}\) (=KCTC 22244\(^{T}\) =CCUG 55760\(^{T}\)).

A tidal flat is a part of a coast that is covered with seawater during the flood tide and left covered with mud or slime at ebb tide. In recent years, bacteria belonging to the *Proteobacteria* have been isolated from a variety of tidal flats on the west coast of the Korean peninsula (Yi et al., 2003, 2004; Yoon et al., 2004, 2005, 2007b; Baik et al., 2005; Jung et al., 2006, 2007). In this study, we describe a bacterial strain, designated SMK-117\(^{T}\), isolated from a tidal flat sediment at Saemankum (Yellow Sea, Korea). The aim of the present work was to determine the exact taxonomic position of strain SMK-117\(^{T}\) by using a polyphasic characterization that included determination of its phenotypic and chemotaxonomic properties and a detailed phylogenetic investigation based on its 16S rRNA gene sequence.

Strain SMK-117\(^{T}\) was isolated from a tidal flat sediment by means of the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). *Maritimibacter alkalophilus* KCCM 42376\(^{T}\) was obtained from the Korean Culture Center of Microorganisms (Seoul, Republic of Korea), *Oceanicola nanhaiensis* LMG 23508\(^{T}\) and *Oceanicola marinus* LMG 23705\(^{T}\) were obtained from the Laboratorium voor Microbiologie (Universiteit Gent, Ghent, Belgium) and *Oceanicola batsensis* KCTC 12145\(^{T}\) and *Oceanicola granulosus* KCTC 12143\(^{T}\) were obtained from the Korean Collection for Type Cultures (Taejon, Republic of Korea). The morphological, physiological and biochemical characteristics of strain SMK-117\(^{T}\) were investigated using routine cultivation on MA at 30 °C. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was investigated by using transmission electron microscopy on cells (from an exponentially growing culture) negatively stained with 1 % (w/v) phosphotungstic acid (see Supplementary Fig. S1, available in IJSEM Online). Grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was omitted. Growth at various NaCl concentrations (0.5 %, w/v, and 1.0–13.0 %, w/v, in increments of 1.0 %) was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various temperatures (4, 10, 15, 20, 25, 28, 44, 45, 46 and...
47 °C and from 30 to 40 °C in 1 °C increments) was measured on MA. The pH range for growth was determined in MB that was adjusted to various pH values (pH 4.5–9.5, in increments of 0.5 pH units). Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch and Tween 20, 40, 60 and 80, hypoxanthine, tyrosine and xanthine was tested on MA, using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of ascuscin, gelatin and urea and nitrate reduction were investigated as described previously (Lányi, 1987) but with the modification that artificial seawater was used for the preparation of media. The artificial seawater contained (1− distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns et al., 2001). H₂S production was tested as described previously (Bruns et al., 2001). Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs containing the following: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg) and tetracycline (30 µg). Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Baumann & Baumann (1981), using supplementation with 0.01 % (w/v) yeast extract. The carbon sources were added at a concentration of 0.2 % (w/v) after sterilization by filtration. Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux). For spectral analysis of in vivo pigment absorption, strain SMK-117ᵀ was cultivated aerobically in the dark at 30 °C in MB. The culture was washed twice using centrifugation with a MOPS buffer (MOPS/NaOH, 0.01 M; KCl, 0.1 M; MgCl₂, 0.001 M; pH 7.5) and disrupted by means of sonication (450 sonifier; Branson). After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer.

Cell biomass of strain SMK-117ᵀ for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown in MB at 30 °C. Cell biomass of M. alkalophilus KCCM 42376ᵀ for polar lipid analysis was obtained from cultures grown in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon et al. (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination by RNA. The 16S rRNA gene was amplified using a PCR with two universal primers (5’-GAGTTTGATCCTGCTCAAGCG-3’ and 5’- AGAAGGAGGTGATCCAGCC-3’) as described previously (Yoon et al., 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon et al. (2003). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC. Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and were identified by means of two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). The presence of phosphatidycholine was tested by spraying Dragendorff’s reagent (Sigma). For cellular fatty acid analysis, cell mass of strain SMK-117ᵀ, M. alkalophilus KCCM 42376ᵀ, O. nanhaiensis LMG 23508ᵀ, O. marinus LMG 23705ᵀ, O. botswenendi KCTC 12145ᵀ and O. granulosus KCTC 12143ᵀ was harvested from MA plates after cultivation for 3 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by means of reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain SMK-117ᵀ were given in the species description (see below) or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain SMK-117ᵀ determined in this study comprised 1420 nt. After gaps at the 5’ and 3’ ends of the alignment had been omitted, the remaining 1383 positions were used in the phylogenetic analysis. In the phylogenetic tree based on the neighbour-joining algorithm, strain SMK-117ᵀ joined the lineage comprising M. alkalophilus at a bootstrap support value of 72.2 % (Fig. 1). This topology was also found in the tree constructed using the maximum-likelihood algorithm (Fig. 1). Strain SMK-117ᵀ exhibited 16S RNA gene sequence similarity of 95.3 % with respect to M. alkalophilus HTCC2654ᵀ, 94.3–95.5 % with respect to the type strains of Oceanicola species and <94.6 % with respect to the other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain SMK-117ᵀ was Q-10, at a peak area ratio of approximately 94 %. The cellular fatty acid profile of strain SMK-117ᵀ is shown in Table 2, together with those of the type strains of M. alkalophilus and Oceanicola species analysed in this study. The major fatty acids in strain SMK-117ᵀ were C₁₈:₁ω7c (68.7 %) and 11-methyl C₁₈:₁ω7c (12.3 %) and the major polar lipids were phosphatidylcholine, phosphatidylethanolamine, an unidentified aminolipid and two unidentified phospholipids. The DNA G+C content of strain SMK-117ᵀ was 63.5 mol%. The phylogenetic trees constructed using the neighbour-joining and maximum-likelihood algorithms indicated that strain SMK-117ᵀ clusters with the type strain of M. alkalophilus (Fig. 1). However, strain SMK-117ᵀ was distinguishable from M. alkalophilus KCCM 42376ᵀ on the basis of differences in the cellular fatty acid and polar lipid profiles (Table 2). The fatty acids C₁₆:₀ and C₁₈:₀ 2-OH were minor components in strain SMK-117ᵀ, whereas they were major components in M. alkalophilus KCCM 42376ᵀ (Table 2). An unidentified aminolipid was detected
Table 1. Differential phenotypic characteristics of strain SMK-117<sup>T</sup> and members of some phylogenetically related genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td><strong>Cell shape</strong></td>
<td>Rod</td>
<td>Short rod</td>
<td>Short rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Coccus or rod</td>
<td>Rod</td>
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<tr>
<td><strong>Motility</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td><strong>Pigmentation</strong></td>
<td>Pale yellow</td>
<td>Beige</td>
<td>Yellow</td>
<td>Pink</td>
<td>–</td>
<td>–</td>
<td>Red</td>
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<td><strong>Bacteriochlorophyll a</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>+</td>
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<td><strong>Growth at/with:</strong></td>
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<td>4 °C</td>
<td>–</td>
<td>–</td>
<td>v&lt;sup&gt;++&lt;/sup&gt;</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>40 °C</td>
<td>+</td>
<td>–</td>
<td>v&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>pH 10–12</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>–</td>
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<tr>
<td>10 % (w/v) NaCl</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td><strong>Catalase</strong></td>
<td>+</td>
<td>+</td>
<td>v&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td><strong>Nitrate reduction</strong></td>
<td>–</td>
<td>–</td>
<td>v&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Acid production from glucose</strong></td>
<td>–</td>
<td>–</td>
<td>or ND†</td>
<td>–</td>
<td>–</td>
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<td><strong>Hydrolysis of:</strong></td>
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<tr>
<td>Aesculin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
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<td>Gelatin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
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<td>ND</td>
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<td>Alkaline phosphatase (API ZYM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Polar lipids§</td>
<td>PC, PG, PE, AL, PLs</td>
<td>PC, PG, PE, AL, PLs</td>
<td>PC, PG, PE</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>63.5</td>
<td>61.7</td>
<td>64.7–71.5</td>
<td>59.7–64.4</td>
<td>64.5</td>
<td>59.7</td>
<td>67</td>
</tr>
</tbody>
</table>

*Variable results reported as follows: a, all species positive except O. nanhaiensis; b, all species positive except O. marinus; c, all species positive except O. granulosus; d, all species negative except O. nanhaiensis (weakly positive); e, positive for O. granulosus and O. marinus, negative for O. batsensis, no data available for O. nanhaiensis.
†No data available for O. marinus.
§AL, Unidentified aminolipid; DPG, diphosphatidylglycerol; NP, unidentified non-polar lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid.

In strain SMK-117<sup>T</sup>, but it was not detected in M. alkaliphilus (Lee et al., 2007). An unidentified non-polar lipid was present as a major constituent in M. alkaliphilus, whereas it was absent in strain SMK-117<sup>T</sup> (Lee et al., 2007), the same polar lipid profile for M. alkaliphilus KCCM 42376<sup>T</sup> was also observed in this study. Strain SMK-117<sup>T</sup> was distinguished from type strains of Oceanicola in strain SMK-117<sup>T</sup>, but it was not detected in M. alkaliphilus (Lee et al., 2007). An unidentified non-polar lipid was present as a major constituent in M. alkaliphilus, whereas it was absent in strain SMK-117<sup>T</sup> (Lee et al., 2007), the same polar lipid profile for M. alkaliphilus KCCM 42376<sup>T</sup> was also observed in this study. Strain SMK-117<sup>T</sup> was distinguished from type strains of Oceanicola species in terms of some fatty acids, i.e. C<sub>16:0</sub>, 11-methyl C<sub>18:1ω7c</sub>, C<sub>19:0</sub> cyclo ω8c or C<sub>18:1ω7c</sub> (Table 2). Strain SMK-117<sup>T</sup> is also distinguishable from some phylogenetically related genera through differences in some phenotypic properties (listed in Table 1). On the basis of phylogenetic data and the differential chemotaxonomic and phenotypic properties, strain SMK-117<sup>T</sup> represents a novel genus and species within the Alphaproteobacteria, for which the name Lutimaribacter saemankumensis gen. nov., sp. nov. is proposed.

**Description of Lutimaribacter saemankumensis sp. nov.**

Lutimaribacter saemankumensis (sae.man.kum.en’sis. N.L. masc. adj. saemankumensis from Saemankum, the location on the Yellow Sea in Korea where the type strain was isolated).

Has the following characteristics in addition to those described for the genus. Cells are 0.4–0.8 μm wide and 1.0 to at least 10.0 μm long and non-motile. Colonies on MA are circular, slightly convex, smooth, glistening, pale yellow in colour and 1.0–1.5 mm in diameter after 7 days incubation at 30 °C. Growth occurs at 10 (weakly) and 45 °C, but not at 4 or 46 °C. The optimal pH for growth is...
between 7.0 and 8.0; growth occurs at pH 5.5, but not at pH 5.0. Growth occurs in the presence of 11% (w/v) NaCl, but not in the absence of NaCl or in the presence of 12% (w/v) NaCl. Anaerobic growth occurs on MA and on MA supplemented with nitrate. Tweens 20, 40, 60 and 80 are hydrolysed, but casein, hypoxanthine, xanthine, starch and L-tyrosine are not. H2S is not produced. In assays with the API ZYM system, esterase (C4), esterase lipase (C8) and leucine arylamidase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. D-Mannose is utilized and citrate is weakly utilized, but L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, sucrose, trehalose, D-xylene, acetate, benzoate, formate, L-malate, pyruvate, salicyl, succinate and L-glutamate are not utilized. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, oleandomycin, penicillin G, polymyxin B and streptomycin, but not to lincomycin or tetracycline. The major polar lipids are phosphatidylcholine, phosphatidylethanolamine, an unidentified aminolipid and two unidentified phospholipids. The DNA G+C content of the type strain is 63.5 mol% (determined using HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, SMK-117T (=KCTC 22244T =CCUG 55760T), was isolated from sediment from a tidal flat at Saemankum (Yellow Sea, Korea).

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References


