Seohaeicola saemankumensis gen. nov., sp. nov., isolated from a tidal flat

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A Gram-negative, non-motile and rod-, oval- or coccoid-shaped bacterial strain, SD-15T, was isolated from a tidal flat of the Yellow Sea, Korea. The novel strain, which was phylogenetically closely related to the genera Phaeobacter, Leisingera and Marinovum, was studied using a polyphasic taxonomic approach. Strain SD-15T grew optimally at pH 7.0–8.0 and 30 °C in the presence of 2 % (w/v) NaCl. It contained Q-10 as the predominant ubiquinone and C18 : 1ω7c and 11-methyl C18 : 1ω7c as the major fatty acids. The major polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified lipid. The DNA G+C content was 63.4 mol%. Strain SD-15T exhibited the highest 16S rRNA gene sequence similarity values (95.1–96.4 %) to the type strains of species of the genus Phaeobacter, Leisingera methylhalidivorans MB2T and Marinovum algicola ATCC 51440T. Strain SD-15T could be differentiated from members of the genera Phaeobacter, Leisingera and Marinovum by differences in the contents of some fatty acids, by the absence of aminolipid and by differences in some phenotypic properties. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain SD-15T represents a new genus and novel species, for which the name Seohaeicola saemankumensis gen. nov., sp. nov. is proposed. The type strain of the type species is Seohaeicola saemankumensis SD-15T (=KCTC 22175T=CCUG 55328T).

In the course of screening micro-organisms from a tidal flat of Saemankum in the Yellow Sea, Korea, using diluted marine agar 2216 (Difco), many novel bacterial strains that belong to the class Alphaproteobacteria have been isolated. One of these isolates, strain SD-15T, which was most phylogenetically related to the genera Phaeobacter, Leisingera and Marinovum, is described in this study. The genus Phaeobacter was created by Martens et al. (2006) through the reclassification of Roseobacter gallaeciensis and the description of one novel species, Phaeobacter inhibens. Two further species of the genus have been described recently, Phaeobacter daeponensis (Yoon et al., 2007) and Phaeobacter caeruleus (Vandecandelaere et al., 2009). The genus Marinovum was created by the reclassification of Ruegeria algicola (previously Roseobacter algicola) (Martens et al., 2006). The genus Leisingera was proposed by Schaefer et al. (2002) with a single species, Leisingera methylhalidivorans. The aim of the present study was to determine the exact taxonomic position of strain SD-15T by using a polyphasic approach that included determination of phenotypic and chemotaxonomic properties and a detailed phylogenetic investigation based on 16S rRNA gene sequences.

Strain SD-15T was isolated by means of the standard dilution plating technique at 25 °C on 10 × diluted marine agar 2216 (MA; Difco). Growth on 10 × diluted MA, 5 × diluted MA, 2 × diluted MA and full strength MA was measured at 30 °C. The morphological, physiological and biochemical characteristics of strain SD-15T were investigated using routine cultivation on MA at 30 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy (TEM). Flagellation was determined by using a Philips CM-20 TEM with cells from exponentially growing cultures. For this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the 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 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 excluded from the
medium formula. Growth at various NaCl concentrations [0.5 % (w/v) and 1.0–10.0 % (w/v), in increments of 1.0 %] was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various tempera-
tures (4, 10, 15, 20, 22, 25, 28, 30, 35 and 37 and from 40 to 45 °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 and hydrolysis of casein, starch and Tween 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965).

Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were investigated as described previously (Lányi, 1987) with a modification that artificial seawater (ASW) was used for the preparation of media. The ASW contained (l−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).

For in vivo pigment-absorption spectrum analysis, strain SD-15T was cultivated aerobically in the dark at 30 °C in MB. The cultures were washed twice by centrifugation using a MOPS buffer (0.01 M MOPS/NaOH, 0.1 M KCl, 0.001 M MgCl2; pH 7.5) and disrupted by sonication with a Branson Sonifier 450. After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a spectrophotometer (DU800; Beckman Coulter).

Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs with the following concentrations; 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), tetracycline (30 μg), kanamycin (30 μg), lincomycin (15 μg), oleandomycin (15 μg), neomycin (30 μg) and carbencillin (100 μg). Utilization of various substrates for growth was tested on basal medium agar [50 mM Tris/HCl, pH 7.5; 190 mM NH4Cl, 0.33 mM K2HPO4·3H2O, 0.1 mM FeSO4·7H2O and 1.5 % Noble agar (Difco) in half-strength ASW; Baumann & Baumann, 1981] supplemented with 0.01 % yeast extract. The ASW contained (l−1 distilled water) 24 g NaCl, 5.1 g MgCl2·6H2O, 4 g Na2SO4, 1.1 g CaCl2, 0.7 g KCl, 0.2 g NaHCO3, 0.1 g KBr, 0.027 g H3BO3, 0.024 g SrCl2 and 0.003 g NaF (Lyman & Fleming, 1940). The carbon sources were added at a concentration of 0.2 % (w/v) after sterilization by filtration. Enzyme activities were determined by using the API ZYM system (bioMérieux).

Cell biomass for DNA extraction and for the analyses of isoprenoid quinones and polar lipids 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 the contamination of RNA. The 16S rRNA gene was amplified by PCR using two universal primers, 5′-GAGTTTGATCCTGCGTCAAG-3′ and 5′-AGAAAGGAGGTGATCCAG-GG-3′, as described previously (Yoon et al., 1998). Sequencing of the amplified 16S rRNA gene and phylo-
genetic analysis were performed as described by Yoon et al. (2003). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. For cellular fatty acid analysis, cell mass of strain SD-15T 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). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). The presence of phosphatidylcholine was identified by spraying with Dragendorff’s reagent (Sigma). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC.

The morphological, cultural, physiological and biochemical characteristics of strain SD-15T are given in the genus and species descriptions (see below) or are shown in Table 1. Strain SD-15T did not produce bacteriochlorophyll a (BChl a) aerobically in the dark. The sonicated in vivo cell extracts of strain SD-15T showed no absorption maximum. The almost complete 16S rRNA gene sequence of strain SD-15T determined in this study comprised 1422 nt, representing approximately 96 % of the Escherichia coli 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain SD-15T joined the clade comprising the genera Phaeobacter, Leisingera and Marinovum with a bootstrap resampling value of 80.6 % (Fig. 1). Strain SD-15T exhibited 16S rRNA gene sequence similarity values of 95.1–96.4 % to the type strains of four species of the genus Phaeobacter, 96.0 % to Leisingera methylhalalidivorans MB2T, 95.5 % to Marinovum algicola ATCC 51440T and below 95.2 % to the other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain SD-15T was ubiquinone-10 (Q-10) at a peak area ratio of approximately 94 %. The cellular fatty acid profile of strain SD-15T is shown in Table 2, together with those of phylogenetically related genera analysed previously. The major fatty acids (>10 % of total fatty acids) detected in strain SD-15T were C18:1ω7c and 11-methyl C18:1ω7c. The major polar lipids found in strain SD-15T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified lipid; minor amounts of phospholipids were also present. The DNA G+C content of strain SD-15T was 63.4 mol%.

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The phylogenetic analyses based on 16S rRNA gene sequences indicated that strain SD-15T did not fall within the radiation encompassed by a recognized genus but formed a distinct evolutionary lineage within the class Alphaproteobacteria (Fig. 1). The differences in content of some fatty acids, particularly hydroxy fatty acids and cyclo-C₁₉:₀ω₈c, distinguished strain SD-15T from the phylogenetically related genera, Phaeobacter, Leisingera and Marinovum (Table 2). Strain SD-15T differed from the genera Phaeobacter, Leisingera and Marinovum in that aminolipid was absent (Table 1). Strain SD-15T could be differentiated from the three phylogenetically related
genera Phaeobacter, Leisingera and Marinovum (Table 2).

### Table 1. Differential phenotypic characteristics of strain SD-15T and the genera Phaeobacter, Leisingera and Marinovum

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<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Colony colour</td>
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<tr>
<td>4 °C</td>
<td>+</td>
<td>v</td>
<td>+</td>
<td>−</td>
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<td>37 °C</td>
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<td>Nitrate reduction</td>
<td>+</td>
<td>v</td>
<td>−</td>
<td>−</td>
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<td>β-Galactosidase</td>
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<td>− or ND</td>
<td>ND</td>
<td>+</td>
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<td>−</td>
<td>−</td>
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<td>Starch</td>
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<td>Acetate</td>
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<td>v</td>
<td>−</td>
<td>v(+)</td>
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<td>L-Arabinose</td>
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<td>− or ND</td>
<td>−</td>
<td>+†</td>
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<td>Cellobiose</td>
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<td>v</td>
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<td>−</td>
<td>v</td>
<td>−</td>
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<td>D-Galactose</td>
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<td>−</td>
<td>v</td>
<td>−</td>
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<td>−</td>
<td>v</td>
<td>−</td>
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<td>L-Glutamate</td>
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<td>−</td>
<td>v</td>
<td>−</td>
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<td>v</td>
<td>−</td>
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<td>Malate</td>
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<td>−</td>
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<td>D-Mannose</td>
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<td>−</td>
<td>v</td>
<td>−</td>
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<td>Pyruvate</td>
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<td>+</td>
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<td>+</td>
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<td>v</td>
<td>−</td>
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<td>D-Xylose</td>
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<td>v</td>
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<td>Susceptibility to:</td>
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<td>Chloramphenicol</td>
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<td>v</td>
<td>−</td>
<td>+</td>
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<td>Gentamicin</td>
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<td>+</td>
<td>+</td>
<td>w</td>
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<td>Kanamycin</td>
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<td>+</td>
<td>+</td>
<td>w</td>
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<td>Neomycin</td>
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<td>+ or ND</td>
<td>w</td>
<td>w</td>
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<td>Novobiocin</td>
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<td>+ or ND</td>
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<td>−</td>
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<td>Penicillin G</td>
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<td>+ or ND</td>
<td>+</td>
<td>+</td>
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<td>Streptomycin</td>
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<td>+</td>
<td>+</td>
<td>−</td>
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<td>Polar lipids</td>
<td>PC, PG, PE, L, PLs</td>
<td>PC, PG, PE, Ls, AL, PL*</td>
<td>PG, PE, PL, Ls, AL</td>
<td>PC, PG, PE, L, AL, PL</td>
</tr>
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<td>DNA G + C mol%</td>
<td>63.4</td>
<td>55.7–64.9</td>
<td>60.5</td>
<td>(60)</td>
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</table>

*Data for Phaeobacter caeruleus were not determined.
†Data for the type strain of Marinovum algicola taken from Labrenz et al. (1999).
§Data for P. gallaeciensis taken from Martens et al. (2006).
genera by differences in some phenotypic properties as listed in Table 1. The phylogenetic data and differential chemotaxonomic and phenotypic properties suggest that strain SD-15T constitutes a new genus and species within the class Alphaproteobacteria, for which the name Seohaeicola saemankumensis gen. nov., sp. nov. is proposed.

Description of Seohaeicola gen. nov.

Seohaeicola [Se.o.hae.i’co.la. N.L. n. Seohaeum Seohae, the Korean name of the Yellow Sea in Korea; L. suff. -cola (from L. n. incola) a dweller, inhabitant; N.L. masc. n. Seohaeicola a dweller of the Yellow Sea in Korea].

Cells are Gram-negative, non-motile and rod-, oval- or coccoid-shaped (0.7–1.8 μm × 1.0–10.0 μm). The predominant ubiquinone is Q-10. The major fatty acids are C \(18:1\) w7c and 11-methyl C \(18:1\) w7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified lipid. The type species is Seohaeicola saemankumensis.

Description of Seohaeicola saemankumensis sp. nov.

Seohaeicola saemankumensis (sa.e.man.kum.e.n’sis. N.L. masc. adj. saemankumensis of Saemankum, region from where the organisms were isolated).

The description is as for the genus with the following additional properties. Colonies on MA are circular, raised, smooth, pale yellow in colour and 1.0–1.5 mm in diameter after 7 days incubation at 30 °C. Growth occurs at 4 and 40 °C, but not at 41 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.5, but not at pH 5.0. Optimal NaCl concentration for growth is 2 % (w/v); growth occurs in the presence of 7 % (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 8 % (w/v) NaCl. Anaerobic growth occurs on MA and on MA supplemented with nitrate. Urease-negative. Tweens 20, 40 and 60, aesculin, casein, hypoxanthine, L-tyrosine and xanthine are not hydrolysed. H2S is not produced. Benzoate, formate and salicin are not utilized as sole carbon and energy sources. Susceptible to ampicillin, cephalothin, tetracycline, carbenicillin and oleandomycin, but not to lincomycin or polymyxin B. In assays with the API ZYM system, esterase (C4), esterase lipase (C8) and leucine arylamidase are present, but alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, \(\alpha\)-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, \(x\)-galactosidase, \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, \(N\)-acetyl-\(\beta\)-glucosaminidase, \(x\)-mannosidase and \(\alpha\)-fucosidase are absent. The predominant ubiquinone is Q-10. The major fatty acids (>10 % of total fatty acids) are C \(18:1\) o7c and 11-methyl C \(18:1\) o7c. Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified lipid. Other phenotypic characteristics are given in Table 1.

The type strain, SD-15T (KCTC 22175T = CCUG 55328T), was isolated from a tidal flat of Saemankum in the Yellow Sea, Korea. The DNA G+C content of the type strain is 63.4 mol% (determined by HPLC).
Table 2. Cellular fatty acid contents (% of strain SD-15T and the genera Phaeobacter, Leisingera and Marinovum

Taxa: 1, strain SD-15T; 2, Phaeobacter (data from Yoon et al., 2007; Vandecandelaere et al., 2009); 3, Leisingera methylhalidivorans DSM 14336T (Yoon et al., 2007); 4, Marinovum algicola DSM 10251T (Yoon et al., 2007). Fatty acids that represented <0.5% in all strains, except Phaeobacter caeruleus, were omitted. —, Not detected.

<table>
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<th>Fatty acid</th>
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<td>Straight-chain fatty acids:</td>
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</tr>
<tr>
<td>C12:0</td>
<td>–</td>
<td>0.4–1.2*</td>
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<tr>
<td>C16: 0</td>
<td>8.0</td>
<td>3.7–8.6</td>
<td>3.2</td>
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<tr>
<td>C17:1ω8c</td>
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<td>–</td>
<td>*</td>
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<td>C18:1ω7c</td>
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<td>C13:0 3-0H</td>
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<td>1.7–3.2</td>
<td>1.8</td>
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<td>–*</td>
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<td>11-methyl C16:1ω7c</td>
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<td>C20:0 9ω8c</td>
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*Data for Phaeobacter caeruleus <1.0% or not detected.
†Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 contained C16:1ω7c and/or iso-C15:0 2-0H.

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References


