Seohaecicola 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-15^T, 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-15^T 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 C_{18:1}ω7c and 11-methyl C_{18:1}ω7c 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-15^T exhibited the highest 16S rRNA gene sequence similarity values (95.1–96.4 %) to the type strains of species of the genus *Phaeobacter*, *Leisingera methylohalidivorans* MB2^T and *Marinovum algicola* ATCC 51440^T. Strain SD-15^T 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-15^T represents a new genus and novel species, for which the name *Seohaecicola saemankumensis* gen. nov., sp. nov. is proposed. The type strain of the type species is *Seohaecicola saemankumensis* SD-15^T (=KCTC 22175^T=CCUG 55328^T).

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-15^T, 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* (Vandecastelaere *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 methylohalidivorans*. The aim of the present study was to determine the exact taxonomic position of strain SD-15^T 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-15^T 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-15^T 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

Abbreviations: ASW, artificial seawater; BChl a, bacteriochlorophyll a; TEM, transmission electron microscope.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SD-15^T is EU221274.

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 temperatures (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 Tweens 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-15^T 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 carbenicillin (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, 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'-GAGTTTGAT-CCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAG-CC-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. For cellular fatty acid analysis, cell mass of strain SD-15^T 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-15^T are given in the genus and species descriptions (see below) or are shown in Table 1. Strain SD-15^T did not produce bacteriochlorophyll *a* (BChl *a*) aerobically in the dark. The sonicated *in vivo* cell extracts of strain SD-15^T showed no absorption maximum. The almost complete 16S rRNA gene sequence of strain SD-15^T 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-15^T joined the clade comprising the genera *Phaeobacter*, *Leisingera* and *Marinovum* with a bootstrap resampling value of 80.6 % (Fig. 1). Strain SD-15^T 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 methylohalidivorans* MB2^T, 95.5 % to *Marinovum algicola* ATCC 51440^T and below 95.2 % to the other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain SD-15^T was ubiquinone-10 (Q-10) at a peak area ratio of approximately 94 %. The cellular fatty acid profile of strain SD-15^T 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-15^T were C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. The major polar lipids found in strain SD-15^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified lipid; minor amounts of phospholipids were also present. The DNA G + C content of strain SD-15^T was 63.4 mol%.

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

Taxa: 1, strain SD-15^T; 2, *Phaeobacter* (data from Ruiz-Ponte *et al.*, 1998; Martens *et al.*, 2006; Yoon *et al.*, 2007; Vandecandelaere *et al.*, 2009); 3, *Leisingera* (Schaefer *et al.*, 2002; Martens *et al.*, 2006; Yoon *et al.*, 2007); 4, *Marinovum* (Lafay *et al.*, 1995; Ruiz-Ponte *et al.*, 1998; Martens *et al.*, 2006; Yoon *et al.*, 2007). +, Positive; –, negative; w, weakly positive; ND, not determined or not described; v, variable reaction; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL, unidentified phospholipid; AL, aminolipid; L, unidentified lipid. Data in parentheses are for the type strain. All taxa are positive for catalase and oxidase activities. All taxa are negative for Gram-staining, hydrolysis of Tween 80 and presence of bacteriochlorophyll *a**.

Characteristic	1	2	3	4
Motility	–	+	+	+
Colony colour	Pale yellow	No pigment, brown or blue	No pigment	Beige/pinkish beige
Growth at:				
4 °C	+	v	+	–
37 °C	+	v	–	+
Nitrate reduction	+	v	–	–
β-Galactosidase	–	– or ND	ND	+
Hydrolysis of:				
Gelatin	–	–	–	+
Starch	–	–	+	w
Utilization of:				
Acetate	+	v	–	v(+)†
L-Arabinose	–	– or ND	–	+
Cellobiose	–	v	–	–
Citrate	–	v	–	+
D-Fructose	–	v	–	+
D-Galactose	–	v	–	–
D-Glucose	–	v	–	+
L-Glutamate	–	v	–	+
L-Malate	w	v	–	+
Maltose	–	v	–	+
D-Mannose	w	v	–	–
Pyruvate	–	+	–	+
Succinate	w	+	–	–
Sucrose	–	v	–	+
Trehalose	–	v	–	+
D-Xylose	–	v	–	–
Susceptibility to:				
Chloramphenicol	+	v	+	+
Gentamicin	+	+	+	w
Kanamycin	+	+	w	w
Neomycin	+	+	w	–
Novobiocin	+	–	–	–
Penicillin G	+	+	+	+
Streptomycin	+	+	+	–
Polar lipids	PC, PG, PE, L, PLs	PC, PG, PE, Ls, AL, PL*	PG, PE, PL, Ls, AL	PC, PG, PE, L, AL, PL
DNA G + C mol%	63.4	55.7–64.9	60.5	(60)

*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).

The phylogenetic analyses based on 16S rRNA gene sequences indicated that strain SD-15^T 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

C_{19:0ω8c}, distinguished strain SD-15^T from the phylogenetically related genera, *Phaeobacter*, *Leisingera* and *Marinovum* (Table 2). Strain SD-15^T differed from the genera *Phaeobacter*, *Leisingera* and *Marinovum* in that aminolipid was absent (Table 1). Strain SD-15^T could be differentiated from the three phylogenetically related

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain SD-15^T and other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

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-15^T constitutes a new genus and species within the class *Alphaproteobacteria*, for which the name *Seohaecicola saemankumensis* gen. nov., sp. nov. is proposed.

Description of *Seohaecicola* gen. nov.

Seohaecicola [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. *Seohaecicola* a dweller of the Yellow Sea in Korea].

Cells are Gram-negative, non-motile and rod-, oval- or coccoid-shaped (0.7–1.8 × 1.0–10.0 µm). The predominant ubiquinone is Q-10. The major fatty acids are C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified lipid. The type species is *Seohaecicola saemankumensis*.

Description of *Seohaecicola saemankumensis* sp. nov.

Seohaecicola saemankumensis (sae.man.kum.en'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, 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. The predominant ubiquinone is Q-10. The major fatty acids (>10% of total fatty acids) are C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified lipid. Other phenotypic characteristics are given in Table 1.

The type strain, SD-15^T (=KCTC 22175^T=CCUG 55328^T), 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-15^T and the genera *Phaeobacter*, *Leisingera* and *Marinovum*

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

Fatty acid	1	2	3	4
Straight-chain fatty acids:				
C _{12:0}	—	0.4–1.2*	0.6	—
C _{16:0}	8.0	3.7–8.6	3.2	2.5
C _{17:0}	—	0.2–0.6*	0.1	0.3
C _{18:0}	—	0.9–2.4	0.5	2.5
Unsaturated fatty acids:				
C _{17:1} ω8c	—	—*	0.2	0.8
C _{18:1} ω7c	68.0	57.7–82.3	77.5	81.0
Hydroxy fatty acids:				
C _{10:0} 3-OH	—	1.7–3.2	1.8	1.1
C _{12:0} 3-OH	—	1.6–2.6	2.1	1.2
C _{16:0} 2-OH	—	2.0–5.6	4.7	—
C _{18:1} 2-OH	—	—*	0.6	—
11-methyl C _{18:1} ω7c	12.9	0.9–16.6	4.7	9.6
Cyclo C _{19:0} ω8c	5.4	—*	—	—
Summed feature 3†	—	0.2–0.5*	0.5	0.2
Unknown fatty acid (ECL 11.799)	5.7	2.3–3.3	3.1	—

*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 C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

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