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Lewinella maritima sp. nov., and *Lewinella lacunae* sp. nov., novel bacteria from marine environments

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Abstract

Two Gram-staining-negative, rod-shaped, aerobic, marine bacteria, designated HME9321^T and HME9359^T, were isolated from seawater and lagoon water samples in the Republic of Korea. Phylogenetic analysis of the 16S rRNA gene sequences of the two strains revealed that they belonged to the genus *Lewinella* within the family *Saprospiraceae*. The 16S rRNA gene sequence similarity of strain HME9321^T showed highest similarities with *Lewinella aquimaris* HDW-36^T (95.2 %), *Lewinella marina* MKG-38^T (94.7 %) and *Lewinella xylanilytica* 13–9-B8^T (94.0 %). Strain HME9359^T had highest sequence similarities with *Lewinella agarilytica* SST-19^T (94.7 %), *Lewinella persica* T-3^T (94.1 %) and *Lewinella antarctica* IMCC3223^T (93.3 %). The predominant fatty acids of strain HME9321^T were summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), iso-$C_{15:0}$ and summed feature 9 (comprising iso-$C_{16:0}$ 10-methyl and/or $C_{17:1}\omega9c$) while those of strain HME9359^T were summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and iso-$C_{15:0}$. The major isoprenoid quinone of both strains was MK-7. Strain HME9321^T contained the polar lipids, phosphatidylethanolamine, one unidentified aminolipid, one unidentified phospholipid and nine unidentified polar lipids, while strain HME9359^T contained phosphatidylethanolamine, one unidentified phospholipid and nine unidentified polar lipids. The DNA G+C contents of strains HME9321^T and HME9359^T were 58.7 and 62.0 mol%, respectively. Based on the results of the phenotypic, genotypic, chemotaxonomic and phylogenetic investigation, two novel species, *Lewinella maritima* sp. nov. and *Lewinella lacunae* sp. nov. are proposed. The type strains are HME9321^T (=KACC 17619^T=CECT 8419^T) and HME9359^T (=KCTC 42187^T=CECT 8679^T), respectively.

The genus *Lewinella*, together with the genera *Saprospira* [1], *Haliscomenobacter* [2], *Aureispira* [3], *Phaeodactylibacter* [4] and *Membranicola* [5], belong to the family *Saprospiraceae*. Default members are likely important in the breakdown of complex organic compounds in the environment. Such a role is demonstrated *in situ* for activated sludge wastewater treatment systems where these organisms are frequently observed in abundance [6]. The genus *Lewinella* was proposed by Sly *et al.* [7] with the description of three species: *Lewinella cohaerens*, *L. persica* and *L. nigricans*. The descriprion of the genus was subsequently emended by Khan *et al.* [8]. The members of the genus *Lewinella* are generally characterized as being Gram-staining-negative, aerobic, flexirubin-negative and chemoheterotrophic rods or filaments [9]. The genus *Lewinella* currently contains nine species, *L. cohaerens*, *L. persica*, *L. nigricans*, *L. agarilytica*, *L. marina*, *L. lutea*, *L. antarctica*, *L. xylanilytica* and *L. aquimaris*, all of which were isolated from marine environments, including seawater, beach sand and brown mud [7–12]. In the study, the taxonomic position of strains HME9321^T and HME9359^T were investigated using a polyphasic approach that included phenotypic, phylogenetic and chemotaxonomic characteristics.

Strain HME9321^T was isolated from seawater of the Yellow Sea located on Shinan-gun in Jeollanam-do (35° 01′ 28″ N 126° 10′ 13″ E) and HME9359^T was isolated from a lagoon water sample (Hyang-ho) located on the East Sea coast in Gangwon-do (37° 54′ 49″ N 128° 48′ 36″ E), Republic of Korea, respectively. Both samples were serially diluted with 3 % NaCl (w/v) solution and spread on Marine agar 2216 (MA; Difco). Plates were incubated at 30 °C for 7 days and orange coloured strains HME9321^T and HME9359^T were selected. The two isolates were purified by repeated streaking on agar plates. Both strains were preserved at −80 °C in distilled water supplemented with 20 % (v/v) glycerol and by lyophilization. *Lewinella agarilytica* KCTC 12774^T, *Lewinella lutea* KACC 15205^T, *Lewinella aquimaris* KCTC 42719^T, *Lewinella marina* KACC 15206^T, *Lewinella xylanilytica* KCTC 32663^T, *Lewinella cohaerens* KACC 14388^T and *Lewinella nigricans* KACC 14389^T were obtained from the Korean Agricultural Culture Collection (KACC) and

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The GenBank accession number for the 16S rRNA gene sequence of strain HME9321^T is KF385496 and strain HME9359^T is KM017082.
One supplementary figure and one supplementary table are available with the online Supplementary Material.

Korean Collection for Type Cultures (KCTC). The seven type strains were used as reference strains for phenotypic tests and fatty acids analysis. The reference strains were grown in parallel with strains HME9321^T and HME9359^T under the same conditions.

The 16S rRNA gene sequences were obtained using universal sequencing primers (27F, 518R, 785F and 1492R) [13], sequenced using an ABI 3730XL automatic DNA sequencer (Applied Biosystems). Identification of phylogenetic neighbours and gene sequence similarity were accomplished by using EzBioCloud tool [14] and the BLAST program of GenBank (http://www.ncbi.nlm.nih.gov/). Sequence alignment was carried out using the SILVA Incremental Aligner [15]. Phylogenetic trees were reconstructed by three different methods in MEGA 6 [16]. A neighbour-joining tree [17] was inferred using the Jukes-Cantor model. The maximum-likelihood tree [18] was obtained using the Tamura-Nei (TN93) model as the nucleotide substitution model. The nearest-neighbour-interchange (NNI) was used as the maximum-likelihood heuristic method. The TN93+G+I model was determined to be the best fit. The maximum-parsimony [19] tree was analysed with the subtree-pruning-regrafting (SPR) searching method. The bootstrap analysis was performed with 1000 replicates [20].

The sequence of strain HME9321^T showed highest similarities with *Lewinella aquimaris* HDW-36^T (95.2 %), *Lewinella marina* MKG-38^T (94.7 %) and *Lewinella xylanilytica* 13-9-B8^T (94.0 %), while strain HME9359^T showed highest sequence similarities with *Lewinella agarilytica* SST-19^T (94.7 %), *Lewinella persica* T-3^T (94.1 %) and *Lewinella antarctica* IMCC3223^T (93.3 %). The sequence similarity between strains HME9321^T and HME9359^T was 90.9 %. The phylogenetic tree showing the position of both strains within the genus *Lewinella* is presented in Fig. 1. This was also supported by the maximum-likelihood and maximum parsimony trees. In the neighbour-joining tree, strains HME9321^T and HME9359^T clustered with *L. aquimaris* HDW-36^T and *L. agarilytica* SST-19^T, respectively. The overall topologies of the neighbour-joining tree were almost the same as that of the maximum-likelihood tree (data not shown). This phylogenetic inference, together with the 97 % similarity cut-off [21, 22] among strains HME9321^T, HME9359^T and related *Lewinella* species, suggested that strains HME9321^T and HME9359^T represent two novel species of the genus *Lewinella*.

The genomic DNA of strains HME9321^T and HME9321^T was extracted using a Bacteria Genomic DNA prep kit (Biofact). DNA G+C contents were determined by the fluorimetric thermal denaturation method [23]. Analysis of the fluorimetric method was performed using SYBR Green 1 (SG 1, Invitrogen) and real-time PCR thermocycler (Bio-Rad). Thermal denaturation was performed with 50 µl reaction mixture containing 0.1× standard saline citrate, SYBR Green I (Invitrogen) at a dillution of 1 : 100 000 and approximately 2.5 µg DNA from the isolate and the calibration references. The strains for calibration reference were used according to Joung *et al.* [24]. The thermal cycler conditions consisted of a ramp from 25 to 99 °C at 1.2 °C min^{-1}. Fluorescent DNA melting curves were generated in triplicate. The DNA G+C content of strains HME9321^T and HME9321^T was calculated using a linear regression analysis of the melting temperature (T_m) against calibration reference strains. The DNA G+C content of HME9321^T was 58.7 mol%, whereas that of strain HME9359^T was slightly higher (62.0 mol%) than the other *Lewinella* strains, which range between 45.0 and 61.0 mol% [8].

Cell morphology and presence or absence of flagella were studied by transmission electron microscopy (LIBRA120; Carl Zeiss). Colonial properties were also examined by the naked eye. Motility was determined using semi-solid marine agar containing 0.3 % (w/v) agar, after incubation at 30 °C for 7 days. Gliding motility was also tested using the hanging drop method [25]. Gram staining was performed according to the method of Hucker [26]. Catalase activity was determined by adding a 3 % (v/v) H_2O_2 solution to colonies on solid medium. Oxidase activity was determined by a chromogenic reaction through the oxidation of 1 % (w/v) *N*,*N*,*N*′,*N*-tetramethyl-*p*-phenylenediamine dihydrochloride solution (Sigma). The presence of flexirubin-type pigments was investigated using the bathochromatic shift test with 20 % (w/v) KOH solution [27]. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37 and 42 °C) and pH values (pH 4.0–10.0 at intervals of 1.0 pH unit) were examined on MA and MB media for 10 days. The pH values of 4.0–6.0, 7.0–8.0 and 9.0–10.0 were obtained by sodium acetate/acetic acid, Tris/HCl and Na_2CO_3 buffers, respectively. Growth in the absence of NaCl and in the presence of 0, 0.5 and 1–15.0 % (w/v) NaCl (at increments of 1 % intervals) was investigated at 30 °C in MB prepared according to the formula of the Difco medium, except that NaCl was excluded. Growth under anaerobic conditions was determined on MA at 30 °C for 5 weeks by using the GasPak EZ Anaerobic container System (BD). Growth was tested on nutrient (NA), marine 2216 (MA), tryptic soy (TSA), R2A, blood and MacConkey agars (all Difco). DNase activity was assessed on DNase test agar (Difco). Hydrolysis of casein [3 % skimmed milk (Difco), w/v], carboxymethylcellulose [1 % CM-cellulose (Sigma), w/v], cellulose [1 % filter paper (Whatman # 1), w/v], dextrin [1 % dextrin (Sigma), w/v] and starch [1 % soluble starch (Sigma), w/v] was tested using 1/5 strength MA agar as the basal medium and evaluated after 3 days of incubation at 30 °C. Carbon source utilization was tested using the Biolog GN2 MicroPlates, and physiological and biochemical characterization was performed using the API test system (API ZYM and API 20NE, bioMérieux) according to the manufacturer's instructions except that the cells were suspended in 3 % (w/v) NaCl solution. Susceptibility to antibiotics was tested on MA agar plates at 30 °C for 4 days using discs containing the following antibiotics (µg per disc, Liofilchem): ampicillin (10), chloramphenicol (30), erythromycin (15), gentamycin (10), kanamycin (30), penicillin G (10 IU), rifampicin (30), streptomycin (10), tetracycline (30) and vancomycin (30). Susceptibility to antibiotics was

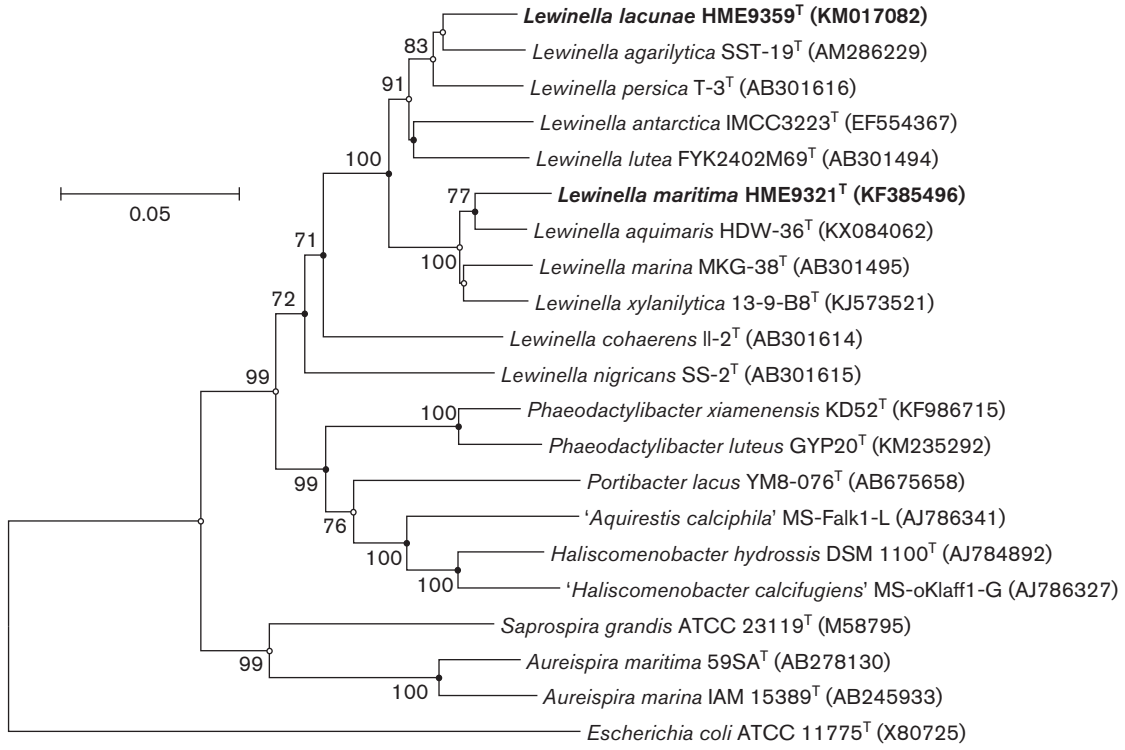

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of strains HME9321^T and HME9359^T with species of the genus *Lewinella* and representatives of the other genera in the family *Saprospiraceae*. Bootstrap percentages (based on 1000 replications) >70 % are shown at nodes. Filled and open circles indicate nodes recovered by all three treeing methods or with two treeing methods, respectively. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

determined as described by the Clinical and Laboratory Standards Institute [28].

The characteristics that differentiate strains HME9321^T, HME9359^T, as well as the type strains of the closely related species of *Lewinella*, are summarized in Table 1 and S1 (available in the online Supplementary Material). Strains HME9321^T and HME9359^T could be distinguished from their closest relatives by the temperature, pH and NaCl range for growth, nitrate reduction, hydrolysis of gelatin and enzyme activities.

The whole-cell fatty acid compositions of strains HME9321^T, HME9359^T and seven reference strains (*L. agarilytica* KCTC 12774^T, *L. lutea* KACC 15205^T, *L. aquimaris* KCTC 42719^T, *L. marina* KACC 15206^T, *L. xylanilytica* KCTC 32663^T, *L. cohaerens* KACC 14388^T, *L. nigricans* KACC 14389^T) were determined on cultures reaching the mid-exponential stage of growth according to the quadrants-streak method [29]. Fatty acid methyl esters were prepared, separated by GC (7890A GC system; Agilent) and identified according to the standard protocol of the Microbial Identification System (MIDI; Sherlock Version 6.1; RTSBA6 database). The fatty acid compositions of the isolates are shown in Table 2. The major cellular fatty acids (>10 % of the total fatty acids) of strain HME9321^T were summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), iso-$C_{15:0}$ and summed feature 9 (comprising iso-$C_{16:0}$ 10-methyl and/or $C_{17:1}\omega9c$), while those of strain HME9359^T were summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and iso-$C_{15:0}$. The overall fatty acid profiles of strains HME9321^T and HME9359^T were similar to published *Lewinella* species, although there were differences in the proportions of some fatty acids, particularly iso-$C_{17:0}$ 3-OH and summed feature 9 (comprising iso-$C_{16:0}$ 10-methyl and/or $C_{17:1}\omega9c$).

Isoprenoid quinones were extracted and purified according to the method of Minnikin *et al.* [30] and analysed by using HPLC (1100 system; Agilent) equipped with a reversed-phase column as described by Collins [31]. The predominant quinone of strains HME9321^T and HME9359^T was MK-7, a characteristically predominant respiratory quinone of the family *Saprospiraceae* [4].

For polar lipid analysis, cell biomass of strains HME9321^T and HME9359^T was harvested from MA after incubation at 30 °C for 3 days and lyophilized. Polar lipids were extracted and analysed by two-dimensional TLC (plates coated with silica gel, 10×10 cm; Merck) in solvent systems described by

Table 1. Characteristics differentiating strains HME9321^T, HME9359^T and the type strains of related species of the genus *Lewinella*

Strains: 1, HME9321^T; 2, HME9359^T; 3, *L. agarilytica* KCTC 12774^T; 4, *L. lutea* KACC 15205^T; 5, *L. aquimaris* KCTC 42719^T; 6, *L. marina* KACC 15206^T; 7, *L. xylanilytica* KCTC 32663^T; 8, *L. cohaerens* KACC 14388^T; 9. *L. nigricans* KACC 14389^T. All strains were positive for oxidase and catalase activities, aesculin hydrolysis, β-galactosidase (PNPG test) but negative for glucose fermentation, arginine dihydrolase, urease and gelatinase (API 20NE). Enzyme activities for alkaline phosphatase and leucine arylamidase were positive in nine strains but esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cysteine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase (API ZYM). +, Positive; –, negative.

Characteristics	1	2	3	4	5	6	7	8	9
Growth at/in:									
10 °C	–	+	+	+	+	+	+	–	–
37 °C	+	+	–	+	–	+	–	–	+
pH 6	–	+	+	–	+	–	+	+	+
pH 9	–	+	+	–	–	–	+	+	+
0.5 % NaCl (w/v)	–	+	–	–	+	+	+	–	–
5 % NaCl (w/v)	–	+	+	+	–	+	+	+	+
Nitrate reduction	–	–	–	–	–	–	+	–	–
Hydrolysis of gelatin	–	+	+	+	–	–	–	+	+
Enzyme activity (API ZYM)									
Esterase (C4)	–	+	+	–	+	+	+	–	–
Esterase lipase (C8)	–	–	–	–	+	–	+	–	–
Valine arylamidase	+	+	+	+	+	+	+	+	–
Cystine arylamidase	+	+	+	+	+	+	+	–	–
Trypsin	+	+	+	+	–	+	+	+	+
Acid phosphatase	–	+	+	–	–	+	–	–	+
Naphthol-AS-BI-phosphohydrolase	–	+	+	–	–	–	–	–	+
α-Glucosidase	+	+	+	–	+	+	–	–	–
β-Glucosidase	–	+	+	–	+	–	–	+	–
N-Acetyl-β-glucosaminidase	+	+	+	+	+	+	+	+	+
DNA G+C content (mol%)*	58.7	62.0	51.3[a]	56[b]	60.9[c]	61[d]	59.1[e]	45[d]	53[d]

*Data from: a, Lee [10]; b, Khan *et al.* [8]; c, Jung *et al.* [12]; d, Sly *et al.* [7]; e, Sung *et al.* [11].

Minnikin *et al.* [30]. Total polar lipids were visualized using 10 % ethanolic molybdophosphoric acid (Sigma). Aminolipids and phospholipids were determined using ninhydrin reagent (Sigma) and molybdenum blue reagent (Sigma), respectively, and glycolipids were identified with α-naphthol. The polar lipid profile of strain HME9321^T contained phosphatidylethanolamine, one unidentified aminolipid, one unidentified phospholipid and nine unidentified polar lipids (Fig. S1a). The corresponding profile of strain HME9359^T contained phosphatidylethanolamine, one unidentified phospholipid and nine unidentified polar lipids (Fig. S1b). The presence of the polar lipids, phosphatidylethanolamine and one unidentified phospholipid, are consistent with other species of the genus *Lewinella* [11, 12]. However, there were differences in the one unidentified aminolipid and several unidentified polar lipids.

On the basis of the phylogenetic relationship and the phenotypic and chemotaxonomic traits, strains HME9321^T and HME9359^T should be assigned to the genus *Lewinella* as the type strains of two novel species, for which the names *Lewinella maritima* sp. nov. and *Lewinella lacunae* sp. nov. are proposed, respectively.

DESCRIPTION OF *LEWINELLA MARITIMA* SP. NOV.

Lewinella maritima (ma.ri′ti.ma. L. fem. adj. *maritima* of the marine environment).

Cells are Gram-staining-negative, non-motile, aerobic, rods, approximately 2.0–3.2 µm long and 0.8–1.0 µm wide after 3 days at 30 °C on MA agar. Colonies are orange coloured, smooth, convex and circular with entire margins on MA agar, and approximately 0.6 mm in diameter after 3 days at 30 °C. Growth occurs at 10–37 °C (optimum, 30 °C), at pH 7–8 (optimum, pH 7.0) and with 1–4 % NaCl (optimum 2 % NaCl w/v). Oxidase- and catalase- activities are positive. Flexirubin-type pigments are not produced. Growth is not observed on R2A, NA, blood, TSA or MacConkey agars. CM-cellulose is hydrolysed. Casein (skimmed milk), DNA, starch, dextrin and cellulose (filter paper) are not hydrolysed. Susceptible to ampicillin, chloramphenicol, rifampicin, streptomycin, tetracycline and vancomycin, but resistant to erythromycin, gentamicin, kanamycin and penicillin G. In the API 20NE test, positive for aesculin hydrolysis and β-galactosidase activity (PNPG test). In the API ZYM system, alkaline phosphatase, leucine arylamidase,

Table 2. Cellular fatty acid composition (%) of strains HME9321^T, HME9359 ^{T} and the type strains of related species of the genus *Lewinella*

Strains: 1, HME9321^T; 2, HME9359^T; 3, *L. agarilytica* KCTC 12774^T; 4, *L. lutea* KACC 15205^T; 5, *L. aquimaris* KCTC 42719^T; 6, *L. marina* KACC 15206^T; 7, *L. xylanilytica* KCTC 32663^T; 8, *L. cohaerens* KACC 14388^T; 9. *L. nigricans* KACC 14389^T. Fatty acids amounting to <1 % of the total fatty acids in all strains are not shown. TR, traces (<1.0 %); –, not detected. All data from this study.

Fatty acids	1	2	3	4	5	6	7	8	9
Straight chain									
$C_{14:0}$	–	TR	TR	TR	1.4	TR	–	TR	TR
$C_{16:0}$	5.1	4.4	7.7	4.2	10.2	7.0	2.3	1.5	6.0
$C_{17:0}$	–	TR	TR	–	TR	1.5	–	1.4	1.8
Branched									
iso-$C_{14:0}$	–	1.0	–	–	–	–	–	1.5	4.2
iso-$C_{15:0}$	21.0	20.1	16.4	30.6	22.1	23.8	20.1	36.5	28.3
iso-$C_{16:0}$	–	–	TR	TR	–	–	–	3.7	2.2
iso-$C_{17:0}$	2.7	1.0	1.0	1.9	3.2	7.5	–	3.2	2.7
Unsaturated									
$C_{15:1}\omega6c$	2.7	2.8	4.1	3.2	3.4	1.9	22.8	–	–
$C_{16:1}\omega11c$	–	–	–	–	–	–	–	–	1.4
$C_{16:1}\omega5c$	–	–	TR	–	–	–	–	1.2	–
$C_{17:1}\omega6c$	1.3	3.7	3.5	1.2	3.9	1.1	–	–	–
iso-$C_{15:1}$ F	–	TR	5.4	2.2	1.0	1.0	2.3	26.1	6.2
iso-$C_{16:1}$ G	–	–	–	TR	–	TR	–	1.9	–
Hydroxy									
$C_{15:0}$ 3-OH	–	TR	TR	–	–	–	2.0	–	TR
$C_{16:0}$ 3-OH	–	1.4	3.2	TR	TR	TR	–	TR	TR
$C_{17:0}$ 3-OH	TR	TR	–	–	1.0	TR	–	1.7	–
iso-$C_{15:0}$ 3-OH	4.0	2.3	3.6	3.0	4.6	2.4	8.5	4.6	3.5
iso-$C_{17:0}$ 3-OH	9.8	5.3	–	6.6	7.6	10.0	10.8	9.4	23.9
Summed features*									
2	TR	TR	1.8	TR	1.4	–	–	TR	TR
3	35.0	43.6	42.7	32.4	27.5	21.0	25.7	TR	9.9
8	–	1.2	TR	TR	–	TR	–	–	–
9	15.8	6.7	2.7	9.7	10.2	19.2	5.5	1.2	2.5

*As indicated by Montero-Calasanz *et al.* [32], summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately. Summed feature 2 was listed as $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; summed feature 3 was listed as $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8 was listed as $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; summed feature 9 was listed as iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

valine arylamidase, cystine arylamidase, trypsin, α-glucosidase and *N*-acetyl-β-glucosaminidase activities are present; esterase (C4), esterase lipase (C8), lipase (C14), α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase activities are absent. In the GN2 Biolog MicroPlate, positive for *m*-inositol, D-mannitol, raffinose, γ-hydroxybutyric acid, propionic acid, glucuronamide, D-alanine, L-alanine, L-asparagine, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid, D, L-carnitine and α-D-glucose-1-phosphate. The other substrates are not utilized. The major cellular fatty acids (>10 %) are summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), iso-$C_{15:0}$ and summed feature 9 (comprising iso-$C_{16:0}$ 10-methyl and/or $C_{17:1}\omega9c$). The predominant menaquinone is MK-7. The major polar lipids are phosphatidylethanolamine, one unidentified aminolipid, one unidentified phospholipid and nine unidentified polar lipids.

The type strain, HME9321^T (=KACC 17619^T=CECT 8419^T), was isolated from seawater of the Yellow Sea in Shinan-Gun, Republic of Korea. The DNA G+C content of the type strain is 58.7 mol%.

DESCRIPTION OF *LEWINELLA LACUNAE* SP. NOV.

Lewinella lacunae (la.cu′nae. L. gen. n. *lacunae* a pool of water, of a pond).

Cells are Gram-staining-negative, non-motile, aerobic, rods, approximately 2.0–3.1 µm long and 0.6–0.8 µm wide after 3 days at 30 °C on MA agar. Colonies are orange coloured, smooth, convex and circular with entire margins on MA

agar, and approximately 0.9 mm in diameter after 3 days at 30 °C. Growth occurs at 15–37 °C (optimum, 30 °C), at pH 6–9 (optimum, pH 7.0) and with 0.5–5 % NaCl (optimum 2 % w/v NaCl). Oxidase- and catalase- activities are positive. Flexirubin-type pigments are not produced. Growth is not observed on R2A, NA, blood, TSA or MacConkey agars. Starch and dextrin are hydrolysed. Casein (skimmed milk), DNA, CM-cellulose and cellulose (filter paper) are not hydrolysed. Susceptible to ampicillin, chloramphenicol, penicillin G, rifampicin and vancomycin, but resistant to erythromycin, gentamicin, kanamycin, streptomycin and tetracycline. In the API 20NE test, positive for aesculin hydrolysis, gelatinase and β-galactosidase activity (PNPG test). In the API ZYM system, alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase activities are present; esterase lipase (C8), lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities are absent. In the GN2 Biolog MicroPlate, positive for α-cyclodextrin, gentiobiose, D-mannitol and D-psicose. The other substrates are not utilized. The major cellular fatty acids (>10 %) are summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or $C_{16:1}\omega6c$) and iso-$C_{15:0}$. The predominant menaquinone is MK-7. The major polar lipids are phosphatidylethanolamine, one unidentified phospholipid and nine unidentified polar lipids.

The type strain, HME9359^T (=KCTC 42187^T=CECT 8679^T), was isolated from a lagoon in the Republic of Korea. The DNA G+C content of the type strain is 62.0 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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