Aliilewinella sediminis gen. nov. sp. nov., isolated from the coastal sediment, and reclassification of some Lewinella species as the members of the genus Alilewinella

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Abstract

A Gram-negative, non-motile, aerobic, gliding, slender rod-shaped bacterium was isolated from the coastal sediment of Xiaoshi Island, Weihai, China. The newly isolated strain, designated W8^T, grew at 15–37°C (optimum, 30°C) and pH 6.0–8.5 (optimum, pH 7.0–7.5) in the presence of 1.0–5.0% (*w*/*v*) NaCl (optimum, 3.0%). The major cellular fatty acids were summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$), iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and summed feature 9 ($C_{16:0}$ 10-methyl/iso-$C_{17:1}\omega9c$). The sole isoprenoid quinone of the strain was MK-7. The polar lipid profile consisted of one phosphatidylethanolamine, one unidentified phospholipid, and eight unidentified polar lipids. Based on 16S rRNA gene sequence similarity, strain W8^T was found to be closely related to *Lewinella agarilytica* KCTC 12774^T (94.5%), *L. lacunae* KCTC 42187^T (94.1%) and *L. aurantiaca* SSH13^T (93.3%), belonging to the family *Lewinellaceae*. Based on the results of polyphasic taxonomic analyses and genomic analyses, strain W8^T (= KCTC 72084^T = MCCC 1H00378^T) is considered to represent a novel species within a new genus *Aliilewinella*, for which the name *Aliilewinella sediminis* gen. nov., sp. nov. is proposed. We also propose to reclassify 11 species of the genus *Lewinella* as the members of the genus *Aliilewinella*.

Introduction

The family *Lewinellaceae* belonged to the phylum *Bacteroidetes*, class *Saprospiria*, order *Saprospirales*, was first described by Hahnke et al. (2016). According to the List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/family/lewinellaceae), the family *Lewinellaceae* contained five child taxa with a validly published name, *Lewinella*, *Flavilitoribacter*, *Haliscomenobacter*, *Phaeodactylibacter* and *Portibacter*. The genus *Lewinella*, previously affiliated to the family *Saprospiraceae*, was first reported by Sly et al. (1998) and amended by Khan et al. (2007), then reclassified as the type genus of the family *Lewinellaceae* by Hahnke *et al.*(2016). At the time of writing, the genus *Lewinella* comprised 12 validly published and correct name species, *Lewinella cohaerens* (type species), *L. agarilytica*, *L. antarctica*, *L. aquimaris*, *L. aurantiaca*, *L. litorea*, *L. lutea*, *L. marina*, *L. lacunae*, *L. maritima, L. persica* and *L. xylanilytica*. Although some descriptions and modifications have been made, the reclassification of this group remains to be studied.

Members of the family *Lewinellaceae* have been isolated from various marine environments and marine organisms, including marine sediment, seawater, beach sand, and snails (Jung et al. 2016; Kang et al. 2017; Khan et al. 2007; Lee. 2007; Oh et al. 2009; Sung et al. 2015) and likely play an important role in marine *Lichina* lichen symbiosis (West et al. 2018). Furthermore, the bacteria of *Lewinella* could be central to effective COD (Chemical Oxygen Demand) and BOD (Biochemical oxygen demand) removal in wastewater (Gendaszewska and Liwarska-Bizukojc 2016; Zhou et al. 2016). Bacteria of the family *Lewinellaceae* are typically characterized as being Gram-negative, aerobic, rod-shaped or filament-shaped, and non-motile or motile by gliding. The major isoprenoid quinone is menaquinone-7 (MK-7). In this study, we characterized a representative of a novel species of the family *Lewinellaceae*, designated strain W8^T, and proposed a novel genus *Aliilewinella.*

Materials And Methods

Isolation, maintenance and cultural conditions

Strain W8^T was isolated from the coastal sediment of Xiaoshi Island, Weihai, China (37° 31' 36" N, 122° 00' 58" E) in October 2018. Using enrichment culture methods for strains from marine sediment as described by Mu et al. (2018), the sediment sample was serially diluted to 10^{-4} with sterilized seawater, and 100 µL aliquots of each dilution were spread on marine agar 2216 (MA; BD). Strain W8^T was isolated after incubation at 30 °C for 7 days, and stored at − 80 °C in sterile 15% (*v/v*) glycerol supplemented with 1% (*w/v*) NaCl. *L. cohaerens* NBRC 102661^T, and *L. agarilytica* KCTC 12774^T and *L. lacunae* KCTC 42187^T, were obtained from NITE Biological Resource Center (NBRC) and Korean Collection for Type Cultures (KCTC), respectively. These three type strains were used as the related species for physiological tests and biochemical characterizations performed in parallel.

Phylogenetic and genome sequence analyses

The genomic DNA of strain W8^T was extracted and purified using a genomic DNA extraction kit (Takara) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using the universal primers 27F and 1492R (Liu et al. 2014). Purified PCR products were cloned into a pMD18-T vector (Takara), and recombinant plasmids were used to transform *Escherichia·coli* DH5*α* cells (Trans-Gen Biotech), and sequencing was performed by GBI Co., Ltd (Qingdao, China). The 16S rRNA gene sequence of strain W8^T was submitted to the GenBank database to search for similar sequences using the BLAST algorithm (http://www.ncbi.nlm.nih.gov) and compared with sequences available from the EzTaxon-e server (http://www.ezbiocloud.net/, Yoon et al. 2017). The draft genome of strain W8^T was sequenced by Novogene, China using the Illumina HiSeq platform. All good-quality paired reads were assembled using the SOAPdenovo software (version 2.04) into several scaffolds (Li et al. 2010). The DNA G + C content of strain W8^T was calculated from the genome sequence. The genes of strain W8^T were identified by the NCBI Prokaryotic Genome Annotation Pipeline server online (Angiuoli et al. 2008) and the genes involved in metabolic pathways were analyzed by KEGG databases (Kanehisa et al. 2016).

To ascertain the phylogenetic position of strain W8^T, the phylogenetic trees based on the 16S rRNA gene, the up-to-date bacterial core gene set (UBCG, Na et al. 2018), and ribosomal protein sequences were reconstructed. The 16S rRNA gene sequence of strain W8^T was obtained by conventional Sanger sequencing and the 16S rRNA gene sequences of the related strains were downloaded from the NCBI nucleotide database. Genome sequences of the related strains were retrieved from the NCBI genome database, and UBCGs sequences were obtained by annotating genome sequences. Phylogenetic trees based on 16S rRNA gene sequences were reconstructed with neighbor-joining (NJ, Saitou and Nei 1987), maximum-likelihood (ML, Felsenstein 1981), and maximum-parsimony (MP, Fitch 1971) algorithms in the computer program MEGA version 7.0 (Kumar et al. 2016). Phylogenetic trees based on UBCGs sequences were supported by the neighbor-joining methods using the computer program MEGA version 7.0. The robustness of the phylogenetic trees was confirmed by bootstrap analysis based on 1,000 replications.

For the genome relatedness analysis, the average amino acid identity (AAI) value (http://ekhidna2.biocenter.helsinki.fi/AAI) and the percentage of conserved proteins (POCP) value (https://github.com/2015qyliang/POCP) between strain W8^T and the related species were calculated. In addition, the AAI and POCP values among available genomes of type strains of species in the genus *Lewinella* were calculated. To complement the demonstration of genetic relationships among genomes, we calculated the average nucleotide identity (ANI) using EzBioCloud's online ANI calculator(https://www.ezbiocloud.net/tools/ani, Yoon et al. 2017). The digital DNA–DNA hybridization (dDDH) values were calculated with the the Genome-to-Genome Distance Calculator (GGDC 3.0, Meier-Kolthof *et al*. 2022).

Morphological, physiological and biochemical analyses

For phenotypic tests, strain W8^T was incubated at 28 ℃ for 3 days on MA; at this stage, strain W8^T was in the late exponential growth phase. Cell morphology and size were examined by scanning electron microscopy (model Nova NanoSEM450; FEI). Motility was assessed using the hanging-drop technique, and gliding motility was determined as described by Bowman (2000). Gram staining was tested as described by Smibert and Krieg (2007). To determine the temperature conditions for growth, cells were grown on MA at 4, 10, 15, 20, 25, 28, 30, 33, 37, 40 and 45 ℃ in triplicate. Salt tolerance was tested using 5 g/L peptone, 1 g/L yeast extract, 0.1 g/L ferric citrate, and 18 g/L agar prepared using artificial seawater (3.2 g/L $MgSO_4$, 2.2 g/L $MgCl_2$, 1.2 g/L $CaCl_2$, 0.7 g/L KCl, 0.2 g/L $NaHCO_3$, and distilled water) with different NaCl concentrations (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9% as final concentration, *w/v*). The pH range for growth was tested at 28 ℃ at pH between 5.5 and 9.5 (in increments of 0.5 pH units) in modified MB containing the following buffers: MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5), and CAPSO (pH 9.0 and 9.5) at concentrations of 20 mM. The results for temperature, salt tolerance, and pH were recorded every 12 hours. The reduction of nitrate was determined using the method described by Cowan and Steel (1974). Anaerobic growth of strain W8^T was determined after incubation at 28 ℃ for 2 weeks on modified MA with or without 0.1% (*w/v*) KNO_3 in an anaerobic jar. Oxidase activity was examined using the bioMérieux oxidase reagent kit according to the manufacturer's instructions. Catalase activity was determined via bubble production in 3% (*v/v*) H_2O_2. Hydrolysis of starch, CM-cellulose, alginate, casein, DNA, and Tweens 20, 40, 60, and 80 degradation were investigated according to the methods of Dong and Cai (2001). Other physiological or biochemical characteristics were examined using the identification system according to the manufacturers' instructions, except that the cells were suspended in 3% (*w/v*) NaCl solution. All tests were carried out simultaneously with the related type strain, and they were performed in duplicate with three related species.

Chemotaxonomic characterization

For extraction of respiratory quinones, fatty acids and polar lipids, cells of strain W8^T and related species were harvested from MA after incubation at 28 ℃ for 3 days; at this stage, strains were all in the late exponential growth phase. Respiratory isoprenoid quinones were extracted from 300 mg freeze-dried cells

as described by Minnikin *et al.*(1984) and analyzed using HPLC (High Performance Liquid Chromatography) as described by Kroppenstedt *et al.*(1982). For analyses of fatty acids, cellular fatty acids were extracted, methylated, and analyzed using an Agilent 6890N gas chromatograph and identified using the TSBA40 database of the microbial identification system (1990). For polar lipid analysis, polar lipids were extracted from 50 mg of freeze-dried cell material and separated via two-dimensional silica gel TLC (plates coated with silica gel, 10*10 cm; Merck) as described by Minnikin et al. (1984). Total lipid material was detected using molybdatophosphoric acid, and the functional groups were determined using spray reagents specific to particular functional groups. Complete details were described by Tindall et al. (2007).

Results And Discussion

Phylogenetic and genome analysis

According to the BLASTn program, phylogenetic analyses of the 16S rRNA gene sequences demonstrated that the closest relatives of strain W8^T were found to be *L. lacunae* HME9359^T (94.3%), *L. agarilytica* SST-19^T (94.3%) and *L. aurantiaca* SSH13^T (93.4%), followed by the type strains of other *Lewinella* species (88.7–93.0%). In phylogenetic tree based on NJ algorithms and supported by ML, and MP algorithms, strain W8^T was included within the family *Lewinellaceae* and formed a distinct branch adjacent to members of the genus *Lewinella* (Fig. 1). Although the branching of strain W8^T was close to most members of the genus *Lewinella* in the phylogenetic tree, sequence similarity with the type species of the genus *Lewinella* (*L. cohaerens* NBRC 102661^T) were very low (88.7%).

The phylogenetic tree of strain W8^T based on UBCG sequences were constructed (Fig. 2). The overall topologies of these phylogenetic trees were somewhat different, but these strains, *L. agarilytica*, *L. persica*, *L. antarctica, L. lacunae, L. xylanilytica, L. litorea* and *L. maritima*, and strain W8^T are all clustered in one branch (named branch *Ali*), the clade had moderate bootstrap support and represented an independent lineage. Significantly, strain *L. cohaerens* was not included in the the branch *Ali*, which were separated with each other. These above phylogenetic analyses, combined with the phylogenetic tree based on 16S rRNA gene sequences, classified these 11 strains of the genus *Lewinella*, *L. agarilytica, L. lacunae, L. persica, L. aquimaris, L. marina, L. xylanilytica, L. antarctica, L. lutea, L. maritima, L. litorea*, and *L. aurantiaca*, and strain W8^T into a clade, designated the genus *Aliilewinella*.

The genome of strain W8^T was assembled into 91 contigs with a total length of 6,183,643 bp, a scaffold N50 value of 238,064 bp, and a mean coverage of 108 ×. The complete 16S rRNA gene sequence (1372 bp) was extracted from the draft genome, which shared 99.5% similarity with the sequence (1527 bp) obtained by conventional Sanger sequencing, indicating the authenticity of genome data. Genome sequencing revealed the DNA G + C content was 56.3%. Automated annotation identified 4,710 potential protein-coding sequences and 62 RNA genes. Secondary metabolite clusters were identified by antiSMASH, and the cluster with the highest known similarity was the carotenoid biosynthetic gene

cluster (28% of genes show similarity). Strain W8^T contained complete genes for assimilatory sulfate reduction. Gene homologs involved in nitrate and nitrite ammonification, as well as organic sulfur assimilation, were undetected in the genome of strain W8^T.

The ANI value between strain W8^T and *L. marina* MKG-38^T, *L. lacunae* KCTC 42187^T and *L. litorea* HSMS-39^T was 72.27%, 72.25% and 71.83%, respectively. The dDDH value between strain W8^T and *L. cohaerens* NBRC 102661^T, *L. aurantiaca* SSH13^T and *L. marina* MKG-38^T was 36.7%, 19.9% and 19.7%, respectively (Table S1). The ANI values and dDDH values were significantly lower than the threshold for species boundaries (94–96% of ANI and 70% of dDDH), indicating a low taxonomic kinship between strain W8^T and the closest relatives. These values suggest that strain W8^T represents a dissimilar genomic species. To enrich interspecies data, the ANI and dDDH values between the strain W8^T and the related type strains in the genus *Lewinella* were shown in Table S1, respectively. To further determine the genome relatedness, the AAI and POCP values between the strain W8^T and the related type strains in the genus *Lewinella* were shown in Table S2 and Table S3, respectively. The AAI values between the genomes of strain W8^T and 8 related type strains in the *Aliilewinella* group were higher than the threshold proposed to include an organism in a given genus (60.0%, Feng et al. 2021), but the AAI values between the genomes of *L. cohaerens* NBRC 102661^T and each related type strain in the *Aliilewinella* group were lower than the genus threshold of 60.0%. The POCP values between the genomes of strain W8^T and 8 related type strains in the *Aliilewinella* group were higher than the genus threshold of 50.0% (Feng et al. 2021), and the POCP values between the genomes of *L. cohaerens* NBRC 102661^T and each related type strain in the *Aliilewinella* group was lower 50.0%. Moreover, the AAI and POCP values between the two strains in the *Aliilewinella* group were all higher than the genus threshold. These data suggested that these strains in the *Aliilewinella* group belong to the same genus, but they do not belong to the same genus as *Lewinella cohaerens* NBRC 102661^T. To complement its reliability, We also calculated the dDDH values between *Lewinella cohaerens* NBRC 102661^T. and *Aliilewinella* group, which is lower than 70% for the same species (Wayne *et al*. 1987).

The 16S rRNA gene sequence similarities, these phylogenetic trees based on 16S rRNA gene, UBCG sequences and the AAI and POCP values suggested that strain W8^T represented the type species of a novel genus *Aliilewinella* in the family *Lewinellaceae*, and other 11 strains, *L. agarilytica, L. lacunae, L. persica, L. aquimaris, L. marina, L. xylanilytica, L. antarctica, L. lutea, L. maritima, L. litorea*, and *L. aurantiaca*, belonged to the same genus as strain W8^T.

Morphological, physiological and biochemical characteristics

Cells of strain W8^T were Gram-negative, non-motile, aerobic, gliding, slender rod-shaped, approximately 0.3–0.4 μm in width, and 1.5–8.0 μm in length after 3 days at 30 ℃ on MA (Fig. S1). Colonies were orange-colored, convex, and circular on MA. Strain W8^T contained typical characteristics of the family *Lewinellaceae*. The novel isolate displayed basic characteristics of members of the family *Lewinellaceae*,

e.g. rod-shaped, non-motile cells (Fig. S1). Comparison of strain W8^T with other members of the the family *Lewinellaceae* is shown in Table 1. In addition to the above, the complete physiological and biochemical characteristics that distinguish strain W8^T from the related species are summarized in Table 2. Strains W8^T could be distinguished from their closest relatives by the flagellum, use of D-fructose and enzyme activities. For instance, strain W8^T and the related type strains were all negative for glucose fermentation and indole production, but positive for *β*-galactosidase, alkaline phosphatase, leucine arylamidase, valine arylamidase, and n-acetyl-*β*-glucosaminidase. Some differences were observed. Strain W8^T, *L. cohaerens* NBRC 102661^T, and *L. agarilytica* KCTC 12774^T were positive for gelatinase activity, but *L. lacunae* KCTC 42187^T was negative for gelatinase activity. Strain W8^T has no flagellum and could not use D-fructose to produce acid, but the related species possess and can.

Table 1

Differential characteristics among strain W8^T and other members of the family *Lewinellaceae*

Characteristic	**W8^T**	**1**	**2**	**3**	**.4**
Colony colour	orange	Reddish orange	Pink	Orange	Yellow
Growth range					
Temperature (°C)	15–37	20–37	16–30	20–37	20–37
NaCl (*w/v*, %)	1.0–5.0	1–9	3–7	1–8	2–4
Oxidation of					
Fermentation/oxidation (glucose)	–	–	+	–	–
Fermentation/oxidation (sucrose)	–	–	+	–	–
Enzymic activities					
Catalase	+	–	+	+	+
Esterase lipase (C8)	–	+	+	–	+
Lipase (C14)	–	+	+	–	–
α–Chymotrypsin	–	+	+	+	+
α–Galactosidase	–	+	–	–	+
β–Galactosidase	+	+	+	+	+
β–Glucuronidase	–	+	–	–	–
α–Glucosidase	+	+	–	+	+
β–Glucosidase	+	+	–	–	+
n–Acetyl–*β*–glucosaminidase	+	+	+	+	+
α–Mannosidase	–	–	–	+	+
α–Fucosidase	–	+	–	–	–
Arginine dihydrolase	–	–	+	+	+
Urease	–	–	+	+	+
Susceptibility to:					
Kanamycin	+	–	+	+	–

Stains: 1, W8^T; 2, *Phaeodactylbacter xiamenensis* KD52^T; 3, *Haliscomenobacter hydrossis* DSM 1100^T; 4 *Portibacter lacus* KCTC 23747^T, 5 *Flavilitoribacter. nigricans* NBRC 102662^T. All data are from this study unless otherwise indicated. +, positive; –, negative. *Data from (Chen et al. 2014).

Characteristic	W8^T	1	2	3	.4
Norfloxacin	+	–	+	+	+
Rifampicin	+	–	+	+	+
Tetracycline	+	–	+	+	+
Neomycin	+	+	+	+	+
Degradation of:					
Tween 20	+	+	–	+	+
Tween 40	+	+	–	+	–
Tween 60	+	+	–	+	–
Tween 80	+	–	–	–	–
Starch	+	–	+	–	+
DNA G + C content (%)	56.3	51.0	47.8	54.3	52.9

Stains: 1, W8^T; 2, *Phaeodactylbacter xiamenensis* KD52^T; 3, *Haliscomenobacter hydrossis* DSM 1100^T; 4 *Portibacter lacus* KCTC 23747^T, 5 *Flavilitoribacter. nigricans* NBRC 102662^T. All data are from this study unless otherwise indicated. +, positive; –, negative. *Data from (Chen et al. 2014).

Table 2

Differential phenotypic and genotypic characteristics of the strain W8^T and the type strains of related species of the genus *Lewinella.*

Characteristic	**1**	**2**	**3**	**4**
Cell size (μm)	0.3–0.4 × 1.5–8.0	0.3–0.5 × 1.2–2.0[a]	0.4–0.6 × 1.5–5.0[b]	0.6–0.8 × 2.0–3.1[c]
Growth range				
Temperature (°C)	15–37	10–30	4–37	15–37
NaCl (*w/v*, %)	1.0–5.0	2.0–5.0	1.0–3.0	1.0–5.0
pH	6.0–8.5	6.0–8.0	5.0–10.0	6.0–9.0
Oxidation of				
Gentiobiose	–	–	+	+
d-Mannitol	–	+	–	+
d-Malic acid	+	–	–	+
n-Acetyl-d-Glucosamine	–	+	–	+
Inosine	–	–	+	–
d-Cellobiose	+	–	+	+
Acid production from				
Mannitol	–	+	+	–
d-Galactose	+	+	–	–
d-Fructose	–	+	+	+
Methyl-*α*-d-mannopyranoside	+	+	–	–
d-Melibiose	+	–	–	–
d-trehalose	–	+	+	–
Enzymic activities				
Cystine arylamidase	–	–	+	+
Esterase (C4)	+	–	+	+
Acid phosphatase	+	–	+	+

Stains: 1, W8^T; 2, *L. cohaerens* NBRC 102661^T; 3, *L. agarilytica* KCTC 12774^T; 4 *L. lacunae* KCTC 42187^T. All data are from this study unless otherwise indicated. +, positive; –, negative. *Data from a, (Sly et al. 1998); b, (Lee 2007); c, (Kang et al. 2017).

Characteristic	1	2	3	4
Glatinase	+	+	+	–
DNA G + C content (%)	56.3	45[a]	51.3[b]	62.0[c]

Stains: 1, W8^T; 2, *L. cohaerens* NBRC 102661^T; 3, *L. agarilytica* KCTC 12774^T; 4 *L. lacunae* KCTC 42187^T. All data are from this study unless otherwise indicated. +, positive; –, negative. *Data from a, (Sly et al. 1998); b, (Lee 2007); c, (Kang et al. 2017).

The sole isoprenoid quinone of strain W8^T was MK-7, which was consistent with the members of the family *Lewinellaceae*. The major fatty acids (> 10%) of strain W8^T are summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 20.6%), iso-C15:0 (17.3%), iso-C17:0 3-OH (11.8%), and summed feature 9 (comprising iso-$C_{16:0}$ 10-methyl and/or $C_{17:1}\omega9c$, 11.1%, Table S4). Distinguishing strain W8^T from the related species could be achieved using summed feature 9, which was not the major fatty acid of the related species. The detailed fatty acid compositions of strain W8^T and the related species can be found in Table S4. The polar lipid profile of strain W8^T contained one phosphatidylethanolamine, one unidentified phospholipid, and eight unidentified polar lipids. Further detailed polar lipid images of strain W8^T and the related are given in Fig. S2.

Based on the morphological, physiological and chemotaxonomic characteristics, as well as phylogenetic inference (Fig. 1 and Fig. 2), strain W8^T is proposed to represent a novel species of a new genus of the family *Lewinellaceae* with the name *Aliilewinella sediminis* sp. nov..

Description of Aliilewinella **gen. nov.**

Aliilewinella (A.li.i.le.win.el'la. L. pronoun alius other, another; N.L. masc. n. Lewinella a bacterial generic name; N.L. masc. n. Aliilewinella the other Lewinella).

The cells are Gram-stain-negative, aerobic, slender rod-shaped and motile by gliding. catalase and oxidase activities are positive. Typical fatty acids are feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$) and iso-$C_{15:0}$. The respiratory quinone is MK-7. The major polar lipids are one phosphatidylethanolamine, one unidentified phospholipid, and several unknown polar lipids. The type species is *Lewinella sedimins*, which belongs to the family *Lewinellaceae*, phylum *Bacteroidetes*, according to the results of polyphasic taxonomic analysis. The G + C content of the genomic DNA of the type strain of the type species is 56.3%.

Description of Aliilewinella agarilytica **comb. nov**

Lewinella agarilytica (a.ga.ri.ly'ti.ca. Malayan n. *agar* agar, gelling polysaccharides from seaweed; N.L. n. *agarum* agar; N.L. adj. *lyticus -a -um* from Gr. adj. *lutikos* dissolving; N.L. fem. adj. *agarilytica* agar-dissolving).

Basonym: *Lewinella agarilytica*

The description is as given for *Lewinella agarilytica* Lee et al. (2007).

The type strain is SST-19^T (= JBRI 2009^T = KCTC 12774^T = JCM 14216^T), which was isolated from beach sediment on the coast of Jeju Island, Republic of Korea.

Description of Aliilewinella lacunae **comb. nov**

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

Basonym: *Lewinella lacunae*

The description is as given for *Lewinella lacunae* Kang et al. (2017).

The type strain is HME9359^T (= KCTC 42187^T = CECT 8679^T), which was isolated from a lagoon in the Republic of Korea.

Description of Aliilewinella maritima **comb. nov**

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

Basonym: *Lewinella maritima*

The description is as given for *Lewinella maritima* Kang et al. (2017).

The type strain is HME9321^T (= KACC 17619^T = CECT 8419^T), which was isolated from seawater of the Yellow Sea in Shinan-Gun, Republic of Korea.

Description of Aliilewinella persica **comb. nov**

Lewinella persica [per'si.ca. L. adj. *persica* Persian (of peach), i.e. peach-coloured].

Basonym: *Lewinella persica*

The description is as given for *Lewinella persica* Khan et al. (2007).

The type strain is T-3^T (= NBRC 102663^T = NCIMB 1396^T), which was isolated from brown mud, Galway, Ireland.

Description of Aliilewinella marina **comb. nov**

Lewinella marina (ma.ri'na. L. fem. adj. *marina* of the sea, marine).

Basonym: *Lewinella marina*

The description is as given for *Lewinella marina* Khan et al. (2007).

The type strain is MKG-38^T (= NBRC 102633^T = NCIMB 14312^T), which was isolated from a marine sediment sample of Kamogawa city, Japan.

Description of Aliilewinella lutea **comb. nov**

Lewinella lutea (lu.te'a. L. fem. adj. *lutea* orange-coloured).

Basonym: *Lewinella lutea*

The description is as given for *Lewinella lutea* Khan et al. (2007).

The type strain is FYK2402M69^T (= NBRC 102634^T = NCIMB 14313^T), which was isolated from a marine snail collected from Mikurajima island, Japan.

Description of Aliilewinella aquimaris **comb. nov**

Lewinella aquimaris (a.qui.ma'ris. L. n. *aqua* water; L. gen. n. maris of the sea, marine; N.L. gen. n. *aquimaris* of seawater).

Basonym: *Lewinella aquimaris*

The description is as given for *Lewinella aquimaris* Jung et al. (2016).

The type strain is HDW-36^T (= KCTC 42719^T = CECT 8901^T), which was isolated from seawater of Hwang-do in Korea.

Description of Aliilewinella xylanilytica **comb. nov**

Lewinella xylanilytica [xy.la.ni.ly'ti.ca. N.L. neut. n. *xylanum* xylan; N.L. fem. adj. *lytica* (from Gr. masc. adj. *lytikos*) able to loose, dissolving; N.L. fem. adj. *xylanilytica* xylandissolving]. Basonym: *Lewinella xylanilytica*

The description is as given for *Lewinella xylanilytica* Sung et al. (2015).

The type strain is 13-9-B8^T (= DSM 29526^T = KCTC 32663^T), which was isolated from seawater collected at Marado in Jeju, Korea.

Description of Aliilewinella antarctica **comb. nov**

Lewinella antarctica (ant.arc'ti.ca. L. fem. adj. *antarctica* southern and, by extension, of the Antarctic, where the type strain was isolated).

Basonym: *Lewinella antarctica*

The description is as given for *Lewinella antarctica* Oh et al. (2009).

The type strain is IMCC3223^T (= KCCM 42688^T = NBRC 103142^T), which was isolated from surface seawater from Maxwell Bay, King George Island, western Antarctica.

Description of Aliilewinella aurantiaca **comb. nov**

Lewinella aurantiaca (au.ran.ti'a.ca. N.L. fem. adj. *aurantiaca*, orange-coloured).

Basonym: *Lewinella aurantiaca*

The description is as given for *Lewinella aurantiaca* Kim et al. (2020).

The type strain is SSH13^T (= KACC 21167^T = NBRC 113866^T), which was isolated from seawater collected at Sehwa Beach, Jeju-do, Republic of Korea.

Description of Aliilewinella litorea **comb. nov**

Lewinella litorea (li.to.re'a. L. fem. adj. *litorea* belonging to the seashore, coast).

Basonym: *Lewinella litorea*

The description is as given for *Lewinella litorea* Park et al. (2020).

The type strain is HSMS-39^T (= KACC 19866^T = NBRC 113585^T), which was isolated from marine sand sampled at Hongsung on the Yellow Sea, Republic of Korea.

Description of Aliilewinella sediminis **sp. nov.**

Aliilewinella sediminis (se.di' mi.nis. L. gen. n. sediminis of sediment).

The cells were Gram-negative, non-motile, aerobic, gliding, slender rod-shaped, 0.3–0.4 μm in width, and 1.5–8.0 μm in length after 3 days at 30 ℃ on MA. Colonies were orange-colored, convex and circular on MA, and 1.0–1.5 mm in diameter after 3 days of incubation at 30 ℃. Growth occurred between 15–37 ℃ (optimum 30 ℃) in the presence of 1.0–5.0% (*w/v*) NaCl (optimum 3.0%), and between pH 6.0–8.5 (optimum pH 7.0–7.5). Nitrate was not reduced. Oxidase and catalase activities were positive. The hydrolysis of gelatin, starch, CM-cellulose, casein, and Tweens 20, 40, 60, and 80 were positive, and negative for DNA and alginate. arginine dihydrolase, urease, and indole production. Acid was produced from d-xylose, d-galactose, d-glucose, inositol, arbutin, aesculin ferric citrate, d-melibiose, d-sucrose, d-melezitose, starch, glycogen, and 5-keto-potassium gluconate. The carbon sources of d-malic acid and d-cellobiose were oxidized. The activities of alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, and n-acetyl-*β*-glucosaminidase were present, but the activities of esterase lipase (C8), lipase (C14), cystine arylamidase, chymotrypsin, *α*-galactosidase, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase were absent. The major fatty acids (> 10%) of strain W8^T are summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and summed feature 9

(comprising iso-$C_{16:0}$ 10-methyl and/or $C_{17:1}\omega9c$). MK-7 was the sole respiratory quinone. The polar lipid profile consisted of one phosphatidylethanolamine, one unidentified phospholipid, and eight unidentified polar lipids. The genomic DNA G + C content of the type strain was 56.3%.

The type strain is W8^T (= KCTC 72084^T = MCCC 1H00378^T), which was isolated from the coastal sediment of Xiaoshi Island in China.

The GenBank accession number for the 16S rRNA gene sequence and the draft genome sequence of strain W8^T is MK101048.1 and NZ_SNAQ00000000.3, respectively.

Abbreviations

KCTC, The Korean Collection for Type Cultures; MCCC, The Marine Culture Collection of China; NBRC, NITE Biological Resource Center; MA, Marine agar 2216; MB, Marine broth 2216; MK-7, menaquinone-7; NCBI, National Center of Biotechnology Information; HPLC, High Performance Liquid Chromatography; TLC, Thin-layer chromatography; ANI, average Nucleotide Identify; dDDH, Digital DNA–DNA hybridization; AAI, Average Amino Acid Identity; POCP, Percentage Of Conserved Proteins

Declarations

Funding information

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Author contributions

Strain W8^T was isolated by Yan-Lin Zhong and Ya-Jing Zhang. Material preparation, experimental operation, data collection and analysis were performed by Chu-Xuan Ji, Ya-Jing Zhang, Fan Li and Yan-Lin Zhong. The manuscript was written by Ya-Jing Zhang, Chu-Xuan Ji, Ya Gong and Zong-Jun Du. Project guidance and critical revision of manuscripts were performed by Ya Gong and Zong-Jun Du. All authors read, revised and approved the final manuscript.

Conflicts of interest and ethical statements

The authors declare that there is no conflict of interest. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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Figures

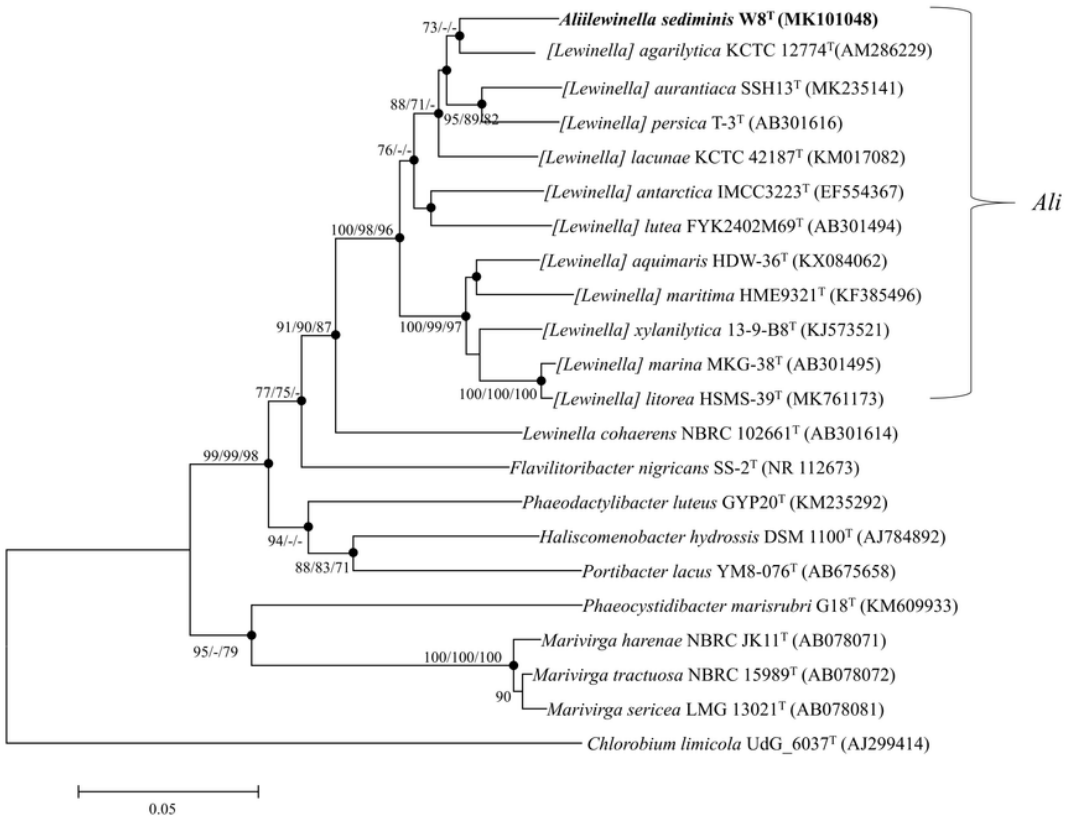

Figure 1

Phylogenetic trees based on the complete 16S rRNA gene sequences from bacterial taxa showing the taxonomic position of strain W8^T. Filled circles indicate nodes overlapping on trees reconstructed using FastTree and IQ-TREE algorithms. Numbers on nodes represent bootstrap values (FastTree/IQ-TREE) based on 1,000 replications, and bootstrap values (> 70 %) are shown at branch nodes. *Chlorobium limicola* UdG_6037^T (AJ299414) was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

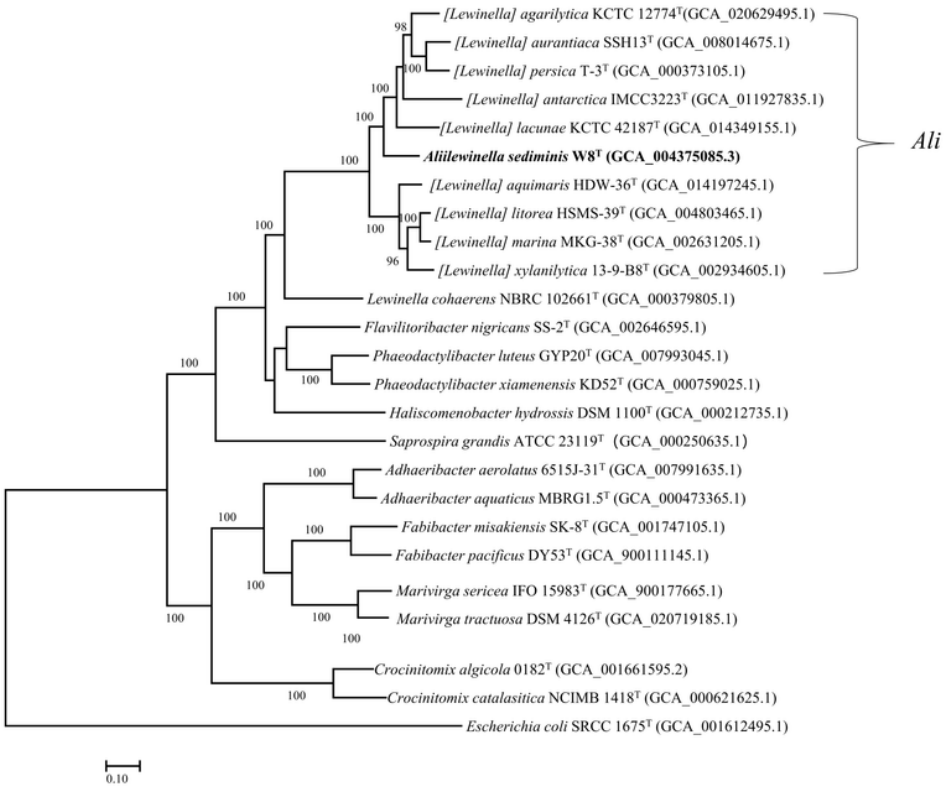

Figure 2

Phylogenomic tree showing the phylogenetic relationships between strain W8^T and closely related members of the family *Aliilewinella*. *Escherichia coli* SRCC 1675^T (GCA_001612495.1) was used as an outgroup. The POCP values between the genomes of strain W8^T and 9 related type strains in the *Aliilewinella* group were higher than the genus threshold of 50.0 %, and the POCP values between the genomes of *Lewinella cohaerens* NBRC 102661^T and each related type strain in the *Aliilewinella* group was lower 50.0 %. The complete POCP values are shown in Table S2. Bootstrap values (> 70 %) are given at the nodes. Bar, 0.1 substitutions per nucleotide position.

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