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Cochleicola gelatinilyticus gen. nov., sp. nov., Isolated from a Marine Gastropod, *Reichia luteostoma*

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A yellow, rod-shaped, non-motile, gram-negative, and strictly aerobic bacterial strain, designated LPB0005^T, was isolated from a marine gastropod, *Reichia luteostoma*. Here the genome sequence was determined, which comprised 3,395,737 bp with 2,962 protein-coding genes. The DNA G+C content was 36.3 mol%. The 16S rRNA gene sequence analysis indicated that the isolate represents a novel genus and species in the family *Flavobacteriaceae*, with relatively low sequence similarities to other closely related genera. The isolate showed chemotaxonomic properties within the range reported for the family *Flavobacteriaceae*, but possesses many physiological and biochemical characteristics that distinguished it from species in the closely related genera *Ulvibacter*, *Jejudonia*, and *Aureitalea*. Based on phylogenetic, phenotypic, and genomic analyses, strain LPB0005^T represents a novel genus and species, for which the name *Cochleicola gelatinilyticus* gen. nov., sp. nov. is proposed. The type strain is LPB0005^T (= KACC 18693^T = JCM 31218^T).

Keywords: *Cochleicola gelatinilyticus*, *Flavobacteriaceae*, novel species, taxonomy

Introduction

As part of the study on culturable bacterial diversity in Korea, a flavobacteria-like strain was isolated. The preliminary identification results, based on a partial 16S rRNA gene sequence, indicated that the new isolate is closely related to the members of the genera *Ulvibacter*, *Jejudonia*, and *Aureitalea* in the family *Flavobacteriaceae* [2, 17]. The genus *Ulvibacter* currently comprises three species: *U. litoralis* [13], *U. antarcticus* [4], and *U. marinus* [1]. *Ulvibacter litoralis* was isolated from the green alga, and *U. antarcticus* and *U. marinus* were isolated from seawater. The genus *Jejudonia* contains only one known species, *J. soesokkakensis*, and was isolated from brackish water [14]. The genus *Aureitalea* is also a monospecific genus, with *A. marina* as the type species, and was isolated from seawater [15]. All members of the genera *Ulvibacter*, *Jejudonia*, and *Aureitalea* isolated to date are of marine origin.

Current prokaryotic taxonomy is largely determined based on molecular biological data rather than phenotypic traits, in particular 16S rRNA gene sequence similarity and DNA-DNA hybridization (DDH). More recently, genome sequence-based in silico DDH is gradually replacing wet-lab-based DDH experiments. In the future, analysis of individual complete genome sequences is expected to replace a variety of time-consuming experiments. However, as analytical techniques and knowledge of bacterial genomes are advanced, accumulation of genomic data will be required for this switch. Thus, it is recommended that researchers currently investigating bacterial taxonomy produce high-quality genome sequences, especially for type strains, to facilitate future analyses.

In this study, a novel bacterial strain, designated LPB0005^T, was isolated and subjected to genomic and phenotypic investigation. The taxonomic status of the isolate was identified and its whole genome sequence was also determined.

Materials and Methods

Isolation

Strain LPB0005^T was isolated from a marine gastropod, *Reichia luteostoma*, caught near Yeongheung Island, Korea (37°15′16.1′′N; 126°29′46.5′′E). The gastropod was ground and diluted with sterilized artificial seawater (ASW; Sigma, USA) and applied to marine agar 2216 (MA; BD, USA) using a standard dilution plating method. The isolate from the gastropod was routinely cultured on MA at 25°C and preserved in a glycerol suspension (20% in DW (w/v)) at –80°C. For comparative taxonomic study, *Ulvibacter litoralis* CCUG 47093^T, *U. antarcticus* NBRC 102682^T, *U. marinus* KCTC 32322^T, and *J. soesokkakensis* KCTC 32325^T were selected as reference strains and obtained from corresponding culture collections.

Genome Sequencing

Genomic DNA was extracted from bacterial cells with a QIAamp DNA mini kit (Qiagen, Germany). The purified genomic DNA was evaluated on a 1% agarose gel to verify the absence of low molecular weight fragments and quantified using the Quant-iT PicoGreen ds DNA Assay Kit (Invitrogen, USA). A paired-end library was generated and sequenced using a Nextera DNA sample prep kit (Illumina, USA) and a Miseq_PE_300 system (Illumina). Sequencing reads were then trimmed, the adapters were removed, and subsequent de novo assembly was performed using CLC Genomics Workbench 8.0. Gene annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline [22]. The DNA G+C content of strain LPB0005^T was calculated from the genome sequence.

16S rRNA Gene Sequencing and Phylogenetic Analyses

The 16S rRNA gene was amplified using universal bacterial primers (27F, 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R, 5′-TACGGYTACCTTGTTACGACTT-3′) under the following conditions: initial denaturation at 94°C for 5 min, followed by 25 cycles of denaturation at 94°C for 40 sec, annealing at 55°C for 45 sec, and elongation at 72°C for 90 sec, with a final extension step at 72°C for 7 min. The PCR products were purified using a QIAquick PCR purification kit (Qiagen) and DNA sequencing was performed using a 3730xl DNA analyzer (ABI). Primers 27F, 518F (5′-CCAGCAGCCGCGGTAATACG-3′), 907R (5′-CCGTCAATT CMTTRAGTTT-3′), and 1492R were used for sequencing. The resultant 16S rRNA gene sequence for strain LPB0005^T was compared against 16S rRNA gene sequences of prokaryotic type strains with validly published names on the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; [5]). Multiple sequence alignments were constructed using the EzEditor program [10]. Maximum-likelihood [9] and neighbor-joining [19] trees were inferred using the MEGA6 program [20]. Genetic distance was calculated using the Jukes–Cantor distance model [11], and the trees were evaluated using bootstrap analyses with 1,000 replicates [8]. A Bayesian tree was constructed using the MrBayes 3.2 program [18], using the General Time Reversible model with gamma-distributed rate variation. The program was run for 12,000,000 generations with a sample frequency of 100 and a burn-in of 1,000,000.

Morphological, Physiological, and Biochemical Characterization

The temperature (4°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C) and NaCl concentration (0–5% (w/v) with 1% increments) ranges for growth were tested using MA and marine broth 2216 (MB; BD, USA) for up to 5 days. The pH range (pH 4–11 at increments of 0.5 pH units) for growth was determined using MB adjusted using the following buffering systems: citric acid/sodium citrate buffer (pH 4.0–5.5), KH_2PO_4/K_2HPO_4 (pH 6.0–8.5), or $NaHCO_3/Na_2CO_3$ (pH 9.0–11.0). Growth under anaerobic conditions was determined after incubation for 4 weeks in an AnaeroPack (Mitsubishi Gas Chemical Co., Japan) on MA at 25°C. Cellular morphology was investigated by transmission electron microscopy after incubation on MA agar at 25°C for 2 days. The colonial morphology, size, and color were examined using cultures grown aerobically on MA for 4 days. Gliding motility was observed by the hanging-drop method, as previously described [3]. The presence of flexirubin-type pigments was determined by flooding the cell mass taken from agar plates with 20% (w/v) KOH [2], and confirmed by examining the bathochromic shift of the absorbance spectrum (200–800 nm) of ethanol and alkaline-ethanol extracts of lysed cells [24]. The Gram stain reaction was tested using a Gram staining kit (Sigma), according to the manufacturer's instructions and the non-staining KOH method [16]. Catalase activity was determined by bubble formation in a 3% (v/v) hydrogen peroxide solution, and oxidase activity was investigated using color change in 1% (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux, France). Hydrolysis of adenine (0.5% (w/v)), agar (1.5% (w/v)), carboxymethylcellulose (CM-cellulose; 0.5% (w/v)), casein (0.5% (w/v)), chitin (0.5% (w/v)), L-tyrosine (0.5% (w/v)), hypoxanthine (0.5% (w/v)), starch (0.2% (w/v)), xanthine (0.4% (w/v)), and Tween 20, 40, 60, and 80 (1% (w/v)) was tested using MA as the basal media, according to standard protocols [23]. The DNase test was conducted with DNase test agar (BD) fortified with 2.0% (w/v) NaCl. Other enzymatic activities and carbon source utilization were determined using the API 20NE and API ZYM kits (bioMérieux, France) according to the manufacturer's instructions, with the exception that the strips were inoculated with bacterial suspensions in 2.0% (w/v) NaCl and incubated at 25°C.

Chemotaxonomy

Cells for polar lipids and isoprenoid quinone analyses were grown in MB for 2 days at 28°C, harvested, and lyophilized. The polar lipids were extracted and separated by two-dimensional TLC on silica gel 60 plates (10 × 10 cm, 0.25 mm thickness; Merck, Germany). Chloroform/methanol/water (65:25:4 (v/v)) was used in the first direction and chloroform/acetic acid/methanol/water (80:15:12:4 (v/v)) in the second direction [7]. The polar lipids were identified by spraying with molybdatophosphoric acid (for total

lipids), phosphomolybdic acid (for phospholipids), ninhydrin (for aminolipids), and naphthol/sulfuric acid reagent (for glycolipids). Isoprenoid quinones were extracted from freeze-dried cells, purified by TLC, and separated by HPLC with a reverse-phase type Zorbax ODS column using acetonitrile/isopropanol (65:35 (v/v)) as the mobile phase at a flow rate of 1.5 ml/min [6, 12]. Cells for fatty acids analysis were grown on MA for 4 days at 25°C. Extraction of the fatty acid methyl esters and their gas chromatographic separation were performed using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the TSBA6 database.

Results and Discussion

Cells of strain LPB0005^T were rod-shaped with rounded ends, with approximate dimensions of 0.3–0.7 × 1.0–2.3 μm (Fig. S1). Strain LPB0005^T did not grow under microaerophilic or anaerobic conditions. Flexirubin-type pigments were not detected, unlike in the closely related species *U. litoralis* CCUG 47093^T and *U. antarcticus* NBRC 102682^T. The cellular pigment showed a UV-visible spectrum with absorption maxima at 452 and 478 nm. The results of other morphological, cultural, physiological, and biochemical analyses are presented in the species description and in Table 1.

The 16S rRNA gene sequence analyses showed that strain LPB0005^T is closely related to members of the genus *Ulvibacter*. The strain exhibited the highest sequence similarity to *U. antarcticus* NBRC 102682^T (96.4%), followed by *U. litoralis* CCUG 47093^T (95.5%), *U. marinus* KCTC 32322^T (95.1%), *J. seosokkakensis* (94.9%), and *Aequorivita viscosa* (94.0%). Although the highest sequence similarity was observed between strain LPB0005^T and *U. antarcticus*, the assignment of this strain to the genus *Ulvibacter* was not strongly supported by the phylogenetic trees. In the phylogenetic trees, strain LPB0005^T was not clustered tightly with any known bacterial species (Fig. 1). In the neighbor-joining tree, strain LPB0005^T was equidistant from the genera *Ulvibacter* and *Jejudonia*, branching between the two genera. In the maximum-likelihood tree, strain LPB0005^T formed a cluster with *J. soesokkakensis*, but the relationship was unstable. In the Bayesian tree, strain LPB0005^T formed a distinct phyletic line within the family *Flavobacteriaceae*, distinct from the other three genera *Ulvibacter*, *Jejudonia*, and *Aureitalea*. In all phylogenetic trees inferred in this study, the boundary between the two monospecific genera *Jejudonia* and *Aureitalea* and the genus *Ulvibacter* was unclear. Thus, the taxonomic relationship of the three genera and the demarcation of the genus *Ulvibacter* should be evaluated with more members of these genera that have been identified. At this point, it can reasonably be concluded that strain LPB0005^T represents a novel genus, as it shows no solid relationship to any known bacterial genus.

Table 1. Major phenotypic characteristics that differentiate strain LPB0005^T from closely related species.

Characteristic	1	2	3	4	5
Growth range					
Temperature (°C)[a]	4–35	4–36	3–25	4–37	10–30
NaCl (%)[a]	1–5	1–3	1–6	0.5–6	1–5
pH[a]	5.5–8.0	6.0–10	5.5–10	6.0–8.5	7.0–7.5
Gliding motility	-	+	-	-	-
Nitrate reduction	-	+	-	-	-[a]
Flexirubin-type pigments	-	+	+	-[d]	-[a]
Decomposition of					
Casein	+	+[b]	-	+	-[e]
CM-Cellulose	-	-	-	-[d]	-
Gelatin	+	+	+	-	+
L-Tyrosine	+	-	+	+	+
Starch	+	+	+	-[d]	-
DNA	-	+	-	-	-
Tween 40	+	+	+[c]	+[d]	+
Tween 80	+	+[b]	+	+[d]	-
Enzyme activity					
Esterase (C4)	+	+	+	+	-
Esterase lipase (C8)	+	+	+	+[d]	+
Trypsin	-	-	-	+	-
α-Chymotrypsin	+	+	-	-[d]	+[e]
Naphthol-AS-BI-phosphohydrolase	+	+	+	-	+[e]
DNA G+C content (mol%)[a]	36.3	36.7–38.0	37.0	38.1	39.9

Strains: 1, Strain LPB0005^T; 2, *U. litoralis* KCTC 12104^T; 3, *U. antarcticus* NBRC 102682^T; 4, *U. marinus* KCTC 32322^T; 5, *J. soesokkakensis* KCTC 32325^T. Data are from this study unless otherwise indicated. All strains were positive for the following characteristics: catalase, oxidase, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, and acid phosphatase activities; hydrolysis of Tween 20, Tween 40, Tween 60, and Tween 80. All strains were negative for the following characteristics: production of H_2S or indoles; decomposition of agar, chitin, esculin, hypoxanthine, or xanthine; assimilation of carbohydrates in the API 20NE kit; urease, arginine dihydrolase, lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase activities. Abbreviations: +, positive; -, negative.

[a]Data from previous reports [1, 4, 13, 14].

[b]Data differ from previous report [13].

[c]Data differ from previous report [4].

[d]Data differ from previous report [1].

[e]Data differ from previous report [14].

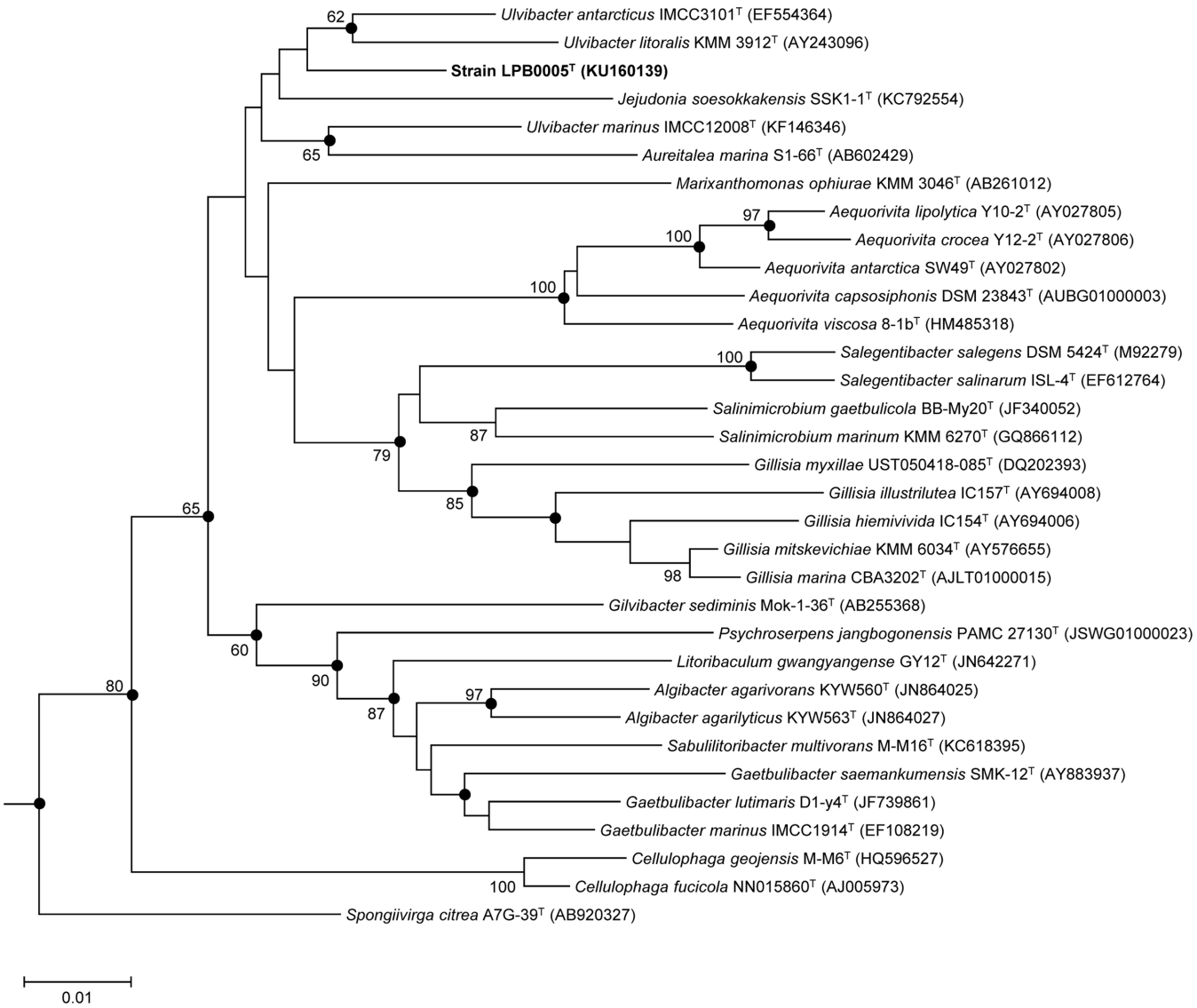

Fig. 1. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain LPB0005^T and other related species.

Aquimarina addita JC2680^T (HQ596527) was used as an outgroup. The numbers at the nodes are given as percentages and represent the level of bootstrap support (>50%) based on 1,000 resampled data sets. Circles indicate that the corresponding nodes (groupings) were also recovered in the maximum-likelihood and Bayesian trees. Scale bar, 0.01 nt substitutions per position.

The major cellular fatty acids identified in strain LPB0005^T were iso-$C_{15:0}$ (20.3%), iso-$C_{15:1}$ G (14.6%), iso-$C_{16:0}$ 3-OH (10.6%), and iso-$C_{17:0}$ 3-OH (15.0%), similar to the four reference strains (Table 2). The polar lipids were composed of phosphatidylethanolamine, three unidentified aminolipids, two unidentified phospholipids, and seven unidentified polar lipids, similar to those of other members of the genera *Ulvibacter* and *Jejudonia* (Fig. S2). The isoprenoid quinone was identified as menaquinone-6 (MK-6), which is the major quinone typical of *Flavobacteriaceae*.

The genome of strain LPB0005^T comprised 3,395,737 bp, and was composed of 216 contigs with 132× coverage. Of the 3,047 predicted genes, 2,962 were protein-coding genes and 38 were RNA genes (3 rRNA genes and 35 tRNA genes). The G+C content of the test strain was 36.3 mol%, similar to other species in the genus *Ulvibacter* (36.7–38.1 mol%) [13]. The majority of the protein-coding genes (1,696 genes, 57.26%) were assigned for putative functions,

Table 2. Cellular fatty acid composition of strain LPB0005^T and closely related strains.

Fatty acids (%)	1	2	3	4	5
$C_{13:0}$	ND	ND	ND	ND	1.1
$C_{16:0}$	2.0	0.5	0.7	0.6	0.5
Iso-$C_{14:0}$	1.4	2.2	2.2	3.4	5.1
Iso-$C_{15:0}$	20.3	20.9	19.8	17.8	17.6
Iso-$C_{15:1}$ G	14.6	13.0	4.8	10.2	5.8
Iso-$C_{16:0}$	5.3	8.0	15.8	5.2	13.6
Iso-$C_{16:1}$ G	3.0	5.9	7.8	4.3	3.4
Anteiso-$C_{15:0}$	6.4	2.7	2.2	6.8	13.8
Anteiso-$C_{15:1}$ A	1.2	1.1	ND	1.4	0.2
$C_{16:1}$ ω5*c*	1.7	ND	ND	ND	ND
$C_{17:1}$ ω6*c*	1.4	1.5	2.0	1.9	2.1
$C_{20:4}$ ω6,9,12,15*c*	ND	ND	ND	6.5	ND
$C_{15:0}$ 2-OH	1.2	1.5	1.7	1.6	1.5
$C_{17:0}$ 2-OH	4.3	2.6	4.1	3.9	6.4
Iso-$C_{15:0}$ 3-OH	2.4	3.0	2.8	3.0	1.2
Iso-$C_{16:0}$ 3-OH	10.6	8.4	7.3	16.0	13.0
Iso-$C_{17:0}$ 3-OH	15.0	17.2	16.0	8.4	4.7
Summed features[a]					
3	6.9	3.8	4.3	5.3	6.7
9	ND	4.5	3.6	ND	ND

Strains: 1, Strain LPB0005^T; 2, *U. litoralis* KCTC 12104^T; 3, *U. antarcticus* NBRC 102682^T; 4, *U. marinus* KCTC 32322^T; 5, *J. soesokkakensis* KCTC 32325^T. All data are from this study. Fatty acids with >1% abundance are shown. ND, not detected.

[a]Summed features represent groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 3 comprised $C_{16:1}$ω6*c* and/or $C_{16:1}$ω7*c*; Summed feature 9 comprised $C_{16:0}$ 10-methyl and/or iso-$C_{17:1}$ω9*c*.

Table 3. Genome properties of strain LPB0005^T.

Attribute	Value
Genome size (bp)	3,395,737
G+C content (%)	36.3
Total genes	3,047
Protein-coding genes	2,962
Pseudogenes	43
tRNAs	35
rRNAs	3

whereas the remaining genes were annotated as hypothetical proteins (1,266 genes, 42.74%). The properties of the genome and the results of the relevant statistical analyses are summarized in Table 3.

The DNA G+C content, and the isoprenoid quinone, fatty acid, and polar lipid profiles of strain LPB0005^T supported assignment of the strain to the family *Flavobacteriaceae*. However, its distinct phylogenetic placement within the family and the low levels of 16S rRNA gene sequence similarities to other species [21] implied that strain LPB0005^T represents a novel genus and species. A number of phenotypic characteristics, including growth ranges, the absence of flexirubin-type pigments, and differences in enzymatic properties clearly differentiated strain LPB0005^T from closely related species in the genera *Ulvibacter* and *Jejudonia*. The polyphasic data presented here conclusively demonstrate that the test strain merits classification as a novel genus and species within the family *Flavobacteriaceae*. The name *Cochleicola gelatinilyticus* gen. nov., sp. nov. is therefore proposed for this isolate, with strain LPB0005^T as the type strain.

Description of *Cochleicola* gen. nov.

Cochleicola (Co.ch.le.i´co.la. L. fem. n. *cochlea* snail; L. masc. suff. *–cola*, a dweller, inhabitant of; N.L. masc. n. *Cochleicola* inhabitant of snail)

Gram-reaction-negative and strictly aerobic. Does not produce flexirubin-type pigments or spores. The isoprenoid quinone detected is MK-6. Major polar lipids are phosphatidylethanolamine, three unidentified aminolipids, two unidentified phospholipids, and seven unidentified polar lipids. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{16:0}$ 3-OH, and iso-$C_{17:0}$ 3-OH. The DNA G+C content is 36.3 mol%.

The type species is *Cochleicola gelatinilyticus*. A member of the family *Flavobacteriaceae* in the phylum Bacteroidetes.

Description of *Cochleicola gelatinilyticus* sp. nov.

Cochleicola gelatinilyticus (ge.la.ti.ni.ly´ti.cus. N.L. n. *gelatium*, gelatin; Gr. adj. *lutikos*, able to dissolve; N.L. adj. *lyticus* dissolving; N.L. masc. adj. *gelatinilyticus* gelatin-dissolving)

Gram-reaction-negative, catalase- and oxidase-positive and strictly aerobic. Cells are non-motile, rods with rounded ends, approximately 0.3–0.7 × 1.0–2.3 μm. Colonies are yellow, convex, shiny, viscous, circular with entire margins, and approximately 1.0 mm in diameter after incubation on MA at 25°C for 3 days. Cells do not glide on agar plates. Cells do not produce flexirubin-type pigments or spores. Grows at 4–35°C (optimum, 25°C), pH 5.5–8.0 (optimum, pH 7.0), and 1.0–5.0% NaCl (optimum 2.0%). Hydrolyzes adenine, casein, gelatin, L-tyrosine, starch, Tween 20, Tween 40, Tween 60, and Tween 80, but not colloidal chitin, CM-cellulose, DNA, esculin, hypoxanthine, or xanthine. Does not produce H_2S or indole. Does not reduce nitrates to

nitrites or nitrogen. Negative for glucose fermentation. Positive for activities of alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for arginine dihydrolase, urease, β-galactosidase, esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase. Does not assimilate any carbon source provided in the API 20NE kit as a sole carbon source. The isoprenoid quinone is MK-6. Major polar lipids are phosphatidylethanolamine, three unidentified aminolipids, two unidentified phospholipids, and seven unidentified polar lipids. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{16:0}$ 3-OH, and iso-$C_{17:0}$ 3-OH. The DNA G+C content is 36.3 mol%.

The type strain is LPB0005^T (= KACC 18693^T = JCM 31218^T), isolated from a marine gastropod, *Reichia luteostoma*, caught near Yeongheung Island, Korea.

GenBank Accession Numbers

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the genome sequence of *Cochleicola gelatinilyticus* LPB0005^T are KU160139 and LRX00000000, respectively.

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References

1. Baek K, Jo H, Choi A, Kang I, Cho JC. 2014. *Ulvibacter marinus* sp. nov., isolated from coastal seawater. *Int. J. Syst. Evol. Microbiol.* **64:** 2041-2046.
2. Bernardet JF, Nakagawa Y, Holmes B. 2002. Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int. J. Syst. Evol. Microbiol.* **52:** 1049-1070.
3. Bowman JP. 2000. Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int. J. Syst. Evol. Microbiol.* **50:** 1861-1868.
4. Choi TH, Lee HK, Lee K, Cho JC. 2007. *Ulvibacter antarcticus* sp. nov., isolated from Antarctic coastal seawater. *Int. J. Syst. Evol. Microbiol.* **57:** 2922-2925.
5. Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW. 2007. EzTaxon: a Web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **57:** 2259-2261.
6. da Costa MS, Albuquerque L, Nobre MF, Wait R. 2011. The extraction and identification of respiratory lipoquinones of prokaryotes and their use in taxonomy. *Methods Microbiol.* **38:** 197-206.
7. da Costa MS, Albuquerque L, Nobre MF, Wait R. 2011. The identification of polar lipids in prokaryotes. *Methods Microbiol.* **38:** 165-181.
8. Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783-791.
9. Felsenstein J. 1993, posting date. PHYLIP (phylogenetic inference package) version 3.5c. Department of Genetics, University of Washington. [Online.]
10. Jeon YS, Lee K, Park SC, Kim BS, Cho YJ, Ha SM, Chun J. 2014. EzEditor: a versatile sequence alignment editor for both rRNA- and protein-coding genes. *Int. J. Syst. Evol. Microbiol.* **64:** 689-691.
11. Jukes TH, Cantor CR. 1969. Evolution of protein molecules, pp. 21-132. *In* Munro HN (ed.). *Mammalian Protein Metabolism.* Academic Press, New York.
12. Minnikin DE, Odonnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2:** 233-241.
13. Nedashkovskaya OI, Kim SB, Han SK, Rhee MS, Lysenko AM, Falsen E, *et al.* 2004. *Ulvibacter litoralis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the green alga *Ulva fenestrata*. *Int. J. Syst. Evol. Microbiol.* **54:** 119-123.
14. Park S, Lee JS, Lee KC, Yoon JH. 2013. *Jejudonia soesokkakensis* gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from the junction between the ocean and a freshwater spring, and emended description of the genus *Aureitalea* Park *et al*. 2012. *Antonie Van Leeuwenhoek* **104:** 139-147.
15. Park S, Yoshizawa S, Inomata K, Kogure K, Yokota A. 2012. *Aureitalea marina* gen. nov., sp. nov., a member of the family *Flavobacteriaceae*, isolated from seawater. *Int. J. Syst. Evol. Microbiol.* **62:** 912-916.
16. Powers EM. 1995. Efficacy of the Ryu nonstaining KOH technique for rapidly determining Gram reactions of food-borne and waterborne bacteria and yeasts. *Appl. Environ. Microbiol.* **61:** 3756-3758.
17. Reichenbach H. 1989. Order 1. *Cytophagales* Leadbetter 1974, 99^{AL}, pp. 2011-2013. *In* Staley JT, Bryant MP, Pfenning N, Holt JG (eds.). *Bergey's Manual of Systematic Bacteriology*. The Williams & Wilkins Co., Baltimore.

18. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, *et al.* 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61:** 539-542.
19. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406-425.
20. Sohpal VK, Dey A, Singh A. 2010. MEGA biocentric software for sequence and phylogenetic analysis: a review. *Int. J. Bioinform. Res. Appl.* **6:** 230-240.
21. Stackebrandt E, Goebel BM. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44:** 846-849.
22. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciufo S, Li WJ. 2013. Prokaryotic genome annotation pipeline. *In* Beck J, Benson D, Coleman J, Hoeppner M, Johnson M, Maglott D, *et al.* (eds.). *The NCBI Handbook*. National Center for Biotechnology Information, Bethesda, MD.
23. Tindell BJ, Sikorski J, Smibert RA, Krieg NR. 2007. Phenotypic characterization and the principles of comparative systematics, pp. 330-393. *In* Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM, Snyder LR (eds.). *Methods for General and Molercular Microbiology*. American Soceity for Microbiology, Washington, DC.
24. Weeks OB. 1981. Preliminary studies of the pigments of *Flavobacterium breve* NCTC 11099 and *Flavobacterium odoratum* NCTC 11036, pp. 108-114. *In* Reichenbach H, Weeks OB (eds.). *The Flavobacterium-Cytophaga Group*. Gesellschaft fur Biotechnologische Forschung, Weinheim.