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Hanamia caeni gen. nov., sp. nov., a Member of the Family *Chitinophagaceae* Isolated from Activated Sludge in Korea

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Abstract

A novel Gram-stain-negative, aerobic, yellowish-pigmented, non-motile, rod-shaped bacterial strain, designated strain BO-59^T, was isolated from the activated sludge of a wastewater treatment plant in Hanam City, South Korea. Phylogenetic study based on the 16S rRNA gene sequence positioned BO-59^T in a distinct lineage in the family *Chitinophagaceae*, sharing less than 92.8% sequence similarity with members of the closely related genera *Ferruginibacter*, *Flavitalea*, *Pseudoflavitalea, Flavisolibacter, Niastella*, and *Terrimonas*. Phylogenomic- and genomic relatedness analyses revealed that strain BO-59^T is clearly distinguished from other genera in the family *Chitinophagaceae* by average nucleotide identity < 66.9%) and the genome-to-genome distance (< 29.5%) values. The strain BO-59^T contained MK-7 as the predominant quinone, and iso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, and iso-$C_{15:1}$ G as major fatty acids (> 10%). The DNA G + C content was 39.1 mol% based on genome sequence analysis. The polar lipids of strain BO-59^T were phosphatidylethanolamine, an unidentified aminophospholipid and three unidentified polar lipids. 16S rRNA gene sequence similarity, physiological, and biochemical characteristics indicated that strain BO-59^T represents a novel species of a new genus, for which the name *Hanamia caeni* gen. nov., sp. nov. is proposed. The type strain is BO-59^T (= KACC 19646^T = LMG 30865 ^{T}).

Introduction

The family *Chitinophagaceae* was described by Kämpfer et al. [1] with *Chitinophaga* as the type genus, which also indicated that the genera *Sediminibacterium*, *Lacibacter*, *Flavihumibacter*, *Flavisolibacter*, *Niabella*, *Niastella*, *Segetibacter*, *Parasegetibacter*, *Terrimonas*, *Ferruginibacter*, *Filimonas*, and others are phylogenetically reclassified in the same family. At the time of writing, the family *Chitinophagaceae* contained 46 genera (https://www.ncbi.nlm.nih.gov). The members of this family are widespread microorganisms commonly isolated from various sources, including rhizosphere soil, subtropical rainforest compost, freshwater sediment, estuarine water, and human peritoneal tumors [2–7].

During the course of a study on the diversity of bacterial communities associated with activated sludge in Hanam City, Korea, a bacterial strain, designated BO-59^T, which form yellowish-pigmented colonies, was isolated. The results of 16S rRNA gene sequence and phylogenetic analyses indicated that BO-59^T is closely related to members of the family *Chitinophagaceae* within the phylum Bacteroidetes. Based on taxonomic data obtained in this study, we established the putative taxonomic position of strain BO-59^T as the type strain of a new genus in the family *Chitinophagaceae*, for which the name *Hanamia caeni* gen. nov., sp. nov. is proposed.

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Materials and Methods

Strain Isolation

During the course of a study on cultivable aerobic bacterial strains, we screened an activated sludge sample collected from a wastewater treatment plant in Hanam City, South Korea (37° 31′ 14.5″ N, 127° 10′ 21.2″ E). The sample was processed using a standard dilution plating technique by spreading on R2A agar using sterile saline (0.85% NaCl). The isolates were purified by transferring onto an R2A agar plate and incubating at 30 °C for 3 days. A novel bacterium, designated BO-59^T, was isolated and assessed to be closely related to members in the family *Chitinophagaceae* within the phylum Bacteroidetes. Strain BO-59^T was routinely cultured on R2A agar and maintained in a glycerol suspension (20%, v/v in R2A broth), at − 80 °C.

Phylogenetic and Genomic Analysis

Genomic DNA of strain BO-59^T was obtained using a genomic DNA extraction kit (Macrogen Co. Ltd, Korea) for subsequent genome and 16S rRNA sequence analyses. The 16S rRNA gene was amplified using a set of universal bacterial primers (800R, 1492R, 27F, and 518F) [8], and the purified PCR products were sequenced commercially by Macrogen, Inc. (Seoul, South Korea). The sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR, USA) and for the purposes of phylogenetic analysis, we obtained the 16S rRNA gene sequences of related taxa from the GenBank database and EzTaxon-e server (http://www.ezbiocloud.net) [9]. Multiple alignments were performed by Clustal_X program with gaps edited using the BioEdit program [10, 11]. Neighbor joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) trees were constructed using Molecular Evolutionary Genetics Analysis 7 (MEGA X) software with bootstrap analysis based on 1,000 replications. Kimura two-parameter model was used for ML and NJ tree construction with pairwise deletion of gaps, whereas the MP tree was constructed based on the Subtree–Pruning–Regrafting heuristic method with pairwise deletion of gaps [12–16].

The draft genomic sequencing for strain BO-59^T was performed based on Illumina HiSeq X Ten analysis and the sequences were assembled using the SOAPdenovo v. 3.10.1 de novo assembler. The draft genome sequence has been submitted to the GenBank database (www.ncbi.nlm.nih.gov) and annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) [17]. The genomic DNA G + C content was determined directly from the draft genome sequence, and pairwise genome-based relatedness between strain BO-59^T and closely related strains was estimated based on the average nucleotide identity (ANI) using the ANI calculator employing the OrthoANIu algorithm [18] available from the EzBioCloud service. Digital DNA–DNA hybridization (dDDH) values were determined using the online Genome-to-Genome Distance Calculator (GGDC) based on GGDC's formula 2 (http://ggdc.dsmz.de/ggdc.php) [19]. To calculate the genus-level average amino acid identity (AAI) value of the phylum "Bacteroidetes," AAI calculation was performed using the Lab AAIrCalculator (http://lycofs01.lycoming.edu/*newman/AAI/) [20].

Phenotypic and Biochemical Characteristics

Gram staining was performed using the procedure described by Buck [21]. The optimum growth of BO-59^T was examined on R2A (BD, USA), nutrient (NA, BD), trypticase soy (TSA, BD), LB (BD), and MacConkey (BD) agars at 30 °C for 7 days. Given that BO-59^T was found to grow optimally on R2A agar, we monitored cell growth on R2A agar (BD) over a range of different temperatures between 4 and 50 °C (4, 10, 15, 18, 20, 25, 30, 35, 37, 40, 42, 45, and 50 °C). Growth was also assessed over a range of pH values from 4 to 10 (at intervals of 0.5 pH units) adjusted using the following buffers: acetate buffer for pH 4.0–5.5, phosphate buffer for pH 6.0–8.0, and Tris buffer for pH 8.5–10.0. Salt tolerance was assessed on R2A medium supplemented with 0.5% and 1% to 10% (w/v at intervals of 1% unit) NaCl after 7 days of incubation. Cell shape, size, and the presence of flagella were examined by scanning electron microscopy (Hitachi SU-3500) and Nikon light microscopy (× 1,000 magnification), after cells have been grown for 3 days at 30 °C on R2A agar medium. Motility was examined on R2A broth supplemented with 0.2% agar [22]. Catalase activity was determined as previously described [23] by assessing bubble production by cells in 3% (v/v) H_2O_2, and oxidase activity was determined using 1% (w/v) *N*,*N*,*N*,*N*,-tetramethyl-1,4-phenylenediamine reagent. An anaerobic growth test was conducted over 2 weeks using the GasPak™ EZ anaerobe pouch system (BD). Tests for the hydrolysis of starch, casein, carboxylmethyl cellulose, DNA, and Tween-60 [24, 25] were carried out after incubating for 5 days at 30 °C. Production of a flexirubin-type pigment was determined based on a reversible color shift to red, purple, or brown when yellow or orange colonies are covered with a 20% aqueous KOH solution [26]. Biochemical tests were carried out using API (API 20NE, API ID 32GN, and API ZYM) kits (BioMérieux) according to the manufacturer's instructions. API ZYM test strip was read after 5 h

of incubation at 37 °C, whereas strips for the other API kits were examined after 3 days at 30 °C.

Chemotaxonomic Analysis

Isoprenoid quinones were extracted using chloroform/methanol (2:1, v/v), evaporated under vacuum conditions, and reextracted in n-hexane/water (1:1, v/v). The crude n-hexane–quinone solution thus obtained was purified using Sep-Pak Vac cartridge silica (Waters, USA) and subsequently analyzed by HPLC as previously described [27]. Strain BO-59^T was examined for polar lipid contents as described previously [28]. For the extraction of cellular fatty acids, strain BO-59^T was cultured on R2A for 48 h at 30 °C, harvested at the exponential phase, and subjected to saponification, methylation, and extraction according to the protocol of the Sherlock Microbial Identification System (MIDI). The resulting fatty acid methyl esters were analyzed via gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package [29].

Results and Discussion

Phylogenetic and Genomic Analysis

The 16S rRNA gene sequence (1451 bp) of strain BO-59^T was determined and subjected to a comparative analysis. The novel isolate, identified as a member of the family *Chitinophagaceae*, showed 16S rRNA gene sequence similarities of less than 92.8% compared with *Ferruginibacter alkalilentus* HU1-GD23^T (92.8%) and *Ferruginibacter lapsinanis* HU1-HG42^T (92.6%) and less than 92% compared with other assessed members of the *Chitinophagaceae*. Moreover, in our phylogenetic tree reconstructions, we established that strain BO-59^T forms an independent branch with the genus *Ferruginibacter* (Fig. 1, Supplementary Fig. S1).

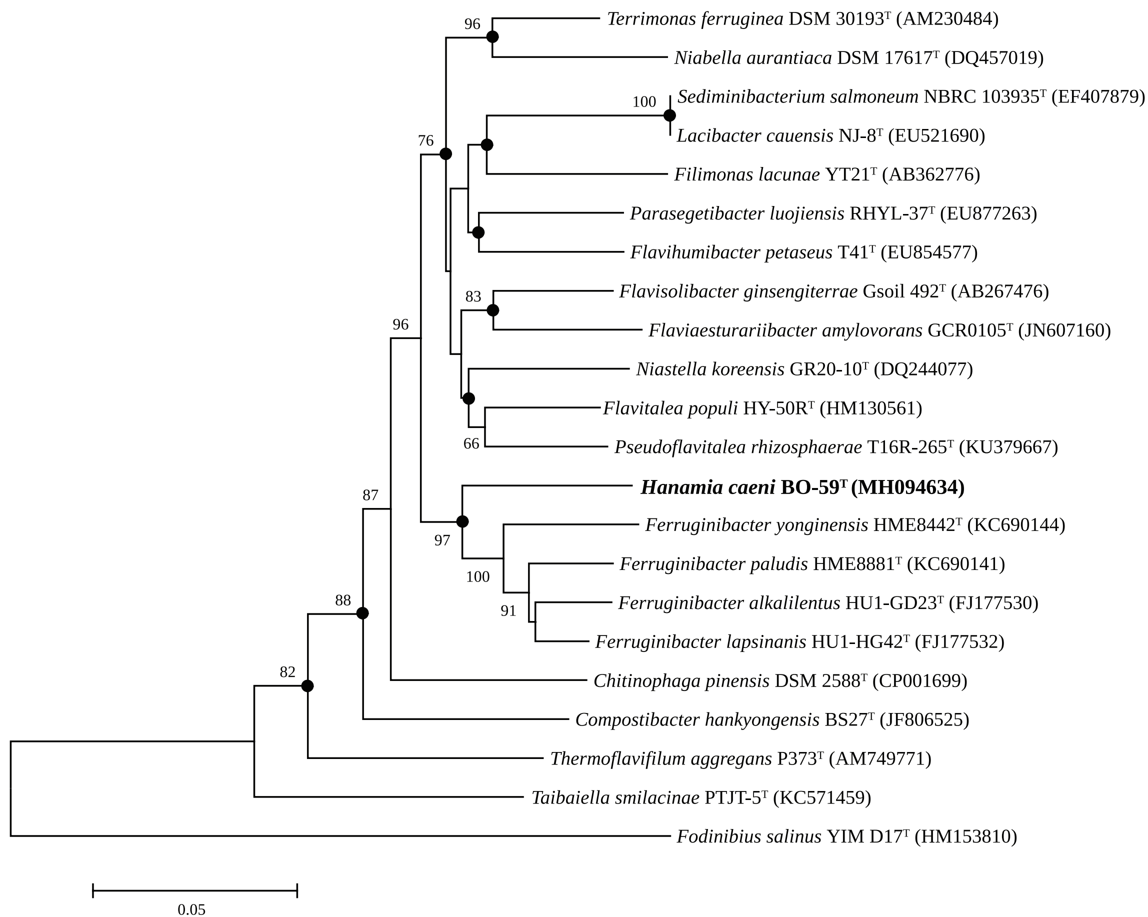

Fig. 1 Neighbour-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of BO-59^T with related species of the family *Chitinophagaceae*. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithm. Bootstrap values expressed as percentages of 1000 replications) greater than 60% are shown at the branch points. Bar, 0.050 substitutions per nucleotide position

Based on these finding, we thus propose that strain BO-59^T should be considered as a new genus with the family *Chitinophagaceae*. Furthermore, the phylogenetic tree based on the core/house-keeping gene sets in the family *Chitinophagaceae* was also reconstructed with whole-genome sequencing using automated multi-locus species tree analysis (Supplementary Fig. S2) [30].

The genome of strain BO-59^T consists of a circular chromosome of 4.96 Mb in length comprising 49 contigs with an N50 value of 275,585 bp and G + C content of 39.1 mol%. Among the 4254 predicted genes, there are 4209 protein-coding genes (CDSs), three rRNAs, 39 tRNAs, three other RNA genes, and 52 pseudogenes. In addition, two CRISPR arrays were also identified. The draft genome sequence of BO-59^T has been deposited in the DDBJ/EMBL/NCBI GenBank database under the accession number RJJR00000000. The digital DDH analysis between strain BO-59^T and related type species showed 22.2–22.9%, which were below the proposed thresholds for species delineation of 60–70%. [31]. Strain BO-59^T shared highest AAI (59.8%) with *Ferruginibacter lapsinanis* KCTC 22305 ^{T} and the AAI values among strain BO-59^T and other family *Chitinophagaceae* were 54.7–55.6%. These values are lower than the genus describing threshold AAI value of 60%, which supports the new genus proposal [20]. In addition, the average nucleotide identity values of strain BO-59^T compared with members of the genera *Flavisolibacter* and *Pseudoflavitalea* were between 66.5% and 66.9%, which indicates that strain BO-59^T is not a strain of any of the genome-sequenced species of these genera (Table 2) [32]. These findings thus tend to indicate that the isolated strain BO-59^T represents a novel genus.

Phenotypic and Biochemical Characteristics

When cultured on R2A agar for 3 days, strain BO-59^T grew as yellowish-pigmented, convex, circular colonies with entire margins. The cells were Gram-negative, aerobic, non-motile, and long rod-shaped with dimensions in the range of 3–4 × 11–45 µm (Supplementary Fig. S3). As shown in Table 1, the physiological and biochemical characteristics of BO-59^T differentiate this bacterium from related genera in the family *Chitinophagaceae* (Table 2).

Chemotaxonomic Analysis

Strain BO-59^T contains MK-7 as the only respiratory quinone. The major polar lipids were identified as phosphatidylethanolamine (PE), an unidentified aminophospholipid (APL1), and three unidentified polar lipids (PL1, PL2, and PL3). The minor polar lipids were two unidentified polar lipids (PL4 and PL5) and two unidentified aminolipids (AL1 and AL2) (Supplementary Fig. S4). Based on the polar lipid analysis, we established that strain BO-59^T has major polar lipids profile similar to that of recently designated members of the family *Chitinophagaceae* [33–36], whereas the suite of minor polar lipid distinguishes strain BO-59^T from related genera in this family *Chitinophagaceae*. The predominant cellular fatty acids profiles of strain BO-59^T mainly comprise iso-$C_{15:0}$ (48.6%), iso-$C_{17:0}$ 3OH (17.1%), and iso-$C_{15:1}$ G (10.9%), whereas the minor fatty acids are $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$ (summed feature 3, 5.1%) and other fatty acids (< 5.0%) (Supplementary Table S1). The strain BO-59^T strain can be distinguished from the related neighboring genera within the family *Chitinophagaceae* primarily by the presence of hydroxyl fatty acids. Although other members of the family *Chitinophagaceae* have similar major fatty acid profiles (iso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, and iso-$C_{15:1}$ G), the relative proportions tend to differ. Strain BO-59^T has larger relative amounts of iso-$C_{15:0}$ and iso-$C_{15:1}$ G that are 15.5% higher and 7.1% lower, respectively, than those in members of the genus *Ferruginibacter*, which is the closest neighboring taxon based on 16S rRNA gene analysis [33]. The absence of iso-$C_{16:0}$ 3OH and $C_{16:0}$ 3OH is mostly unique to the four-related strains when compared with the fatty acid profiles of representatives of related genera. Although strain BO-59^T does not produce iso-$C_{16:0}$ 3OH and $C_{16:0}$ 3OH, these fatty acids are produced by *Ferruginibacter alkalilentus* HU1-GD23^T and also detected four-related genera. Similarly, strain BO-59^T does not appear to produce $C_{15:0}$ 2OH, $C_{15:0}$ 3OH, or $C_{17:0}$ 3OH. Thus, the qualitative and quantitative differences between fatty acid profiles of representatives of each genus (Table 3) could also be used for differentiation at the genus level.

Taxonomic Conclusion

On the basis of our phylogenetic, phenotypic, and chemotaxonomic characterization of the novel isolate BO-59^T, we were unable to assign this strain BO-59^T to any of the known taxa. Certain phenotypic features (Table 1), fatty acids profiles (Supplementary Table S1), and 16S rRNA gene sequences clearly differentiate the strain BO-59^T from the phylogenetically related taxa and provide convincing evidence to indicate that this bacterium is a novel species of a novel genus in the family *Chitinophagaceae*, for which the name *Hanamia caeni* gen. nov., sp. nov. is proposed.

Description of *Hanamia* gen. nov.

Hanamia (Ha.nam'i.a. N.L. fem. n. *Hanamia*, named after the city of Hanam).

Cells are Gram-stain-negative, non-motile, aerobic, and long rods. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and $C_{15:1}$ G. The major quinone is MK-7. The major polar lipids are phosphatidylethanolamine (PE), an unidentified aminophospholipid, and three unidentified polar lipids.

Table 1 Differential characteristics of strain BO-59^{T} and type species of most closely related genera of the family *Chitinophagaceae*

Characteristic	1	2	3	4	5	6	7
Salinity range for growth (% NaCl w/v)	0–1	0–0.5[a]	0-2[b]	0–3[c]	0–3[d]	ND	0–1[f]
Temperature range	15–37	18–30[a]	15–37[b]	10–40[c]	30[d]	10–37[e]	10–37[f]
pH range	6.0–9.0	6.0–8.0[a]	5.0–8.0[b]	6.0–9.0[c]	7.0[d]	5.0–8.0[e]	ND
Oxidase	+	–[a]	–[b]	+[c]	+[d]	–[e]	+[f]
Catalase	+	+[a]	+[b]	+[c]	–[d]	–[e]	W[f]
Enzymes activity							
Nitrate reduction	–	–	–	–	–	+	–
Arginine dihydrolase	+	+	–	–	+	+	+
Urease	+	+	–	–	+	+	+
β-Glucosidase (esculin hydrolysis)	+	+	+	+	+	–	+
Protease (gelatin hydrolysis)	–	+	–	+	–	–	+
Cystine arylamidase	+	+	+	–	+	–	+
Trypsin	–	–	–	+	–	–	–
α-Chymotrypsin	+	+	–	–	+	–	–
α-Galactosidase	+	–	+	+	+	–	+
β-Galactosidase	+	+	+	+	+	–	+
β-Glucuronidase	+	–	–	–	–	–	–
α-Glucosidase	+	–	+	+	+	–	+
β-Glucosidase	+	+	+	+	+	–	+
N-Acetyl-*β*-glucosaminidase	+	–	+	+	+	–	+
α-Mannosidase	+	–	+	–	W	–	+
α-Fucosidase	+	–	–	+	W	–	–
Major fatty acid (> 10%)	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, Summed features 3	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, Summed features 3
DNA G+C contents (mol%)	39.1	39.4[a]	46.8[b]	46.2[c]	43.0[d]	45.8[e]	48.9[f]

Genera: 1, *Hanamia caeni* BO-59^{T}; 2, *Ferruginibacter alkalilentus* HU1-GD23^{T}; 3, *Flavitalea populi* HY-50R^{T}; 4, *Pseudoflavitalea rhizosphaerae* T16R-265^{T}; 5, *Flavisolibacter ginsengiterrae* Gsoil 492^{T}; 6, *Niastella koreensis* GR20-10^{T}; 7, *Terrimonas ferruginea* DSM 30193^{T}. 1, 2, 5, and 6 data were from this study, unless otherwise indicated. All strains were positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase and negative for indole production and glucose acidification

+ positive, *w* weakly positive, − negative, *ND* not mentioned

[a]Data from; a, Lim et al. [33]; b, Wang et al. [34]; c, Kim et al. [35]; d, Yoon & Im. [36]; e, Weon et al. [37]; f, Xie & Yokota [38]

Phylogenetically, the genus is affiliated to the family *Chitinophagaceae* in the phylum Bacteroidetes. The type species is *Hanamia caeni*.

Description of *Hanamia caeni* sp. nov.

Hanamia caeni (cae'ni. L. gen. n. *caeni*, of sludge).

The long non-motile rod-shaped cells (3–4 × 11–45 µm) are Gram-stain-negative, oxidase, and catalase positive, and there is an absence of the flexirubin-type pigment production. Colonies grown on R2A are yellowish-pigmented, convex, and circular with entire. Growth occurs well at temperatures between 15 and 37 °C (optimum, 25–30 °C) and pH 6.0 to 9.0 (optimum pH 7.0) without NaCl supplementation and in the presence of 1% NaCl (w/v, optimum 0%). Colonies develop on R2A, NA, and TSA agars (optimally on R2A agar), although no growth are detected on LB and MacConkey agars. Negative for the hydrolysis of casein, DNA, starch, Tween-60, and cellulose. On API ZYM strips, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidase, α- and β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities and negative for lipase (C14). On API ID 32GN and API 20NE strips, positive for arginine dihydrolase, urease, and esculin hydrolysis and negative for nitrate reduction, indole production, and glucose acidification. Utilizes the sugars such as L-arabinose, *N*-acetyl-β-glucosaminidase, D-maltose, salicin, D-melibiose, D-sorbitol, and D-sucrose, while negative for the other. The predominant quinone is MK-7.

Table 2 Genome characteristics between strain BO-59^T and other type species in the family *Chitinophagaceae*

Strain name	Genome size (Mbp)	Coding genes (CDSs)	N50 value (bp)	G+C content (mol%)	Strain BO-59^T			GenBank assembly accession number
					ANI (%)	dDDH (%)	AAI (%)	
Strain BO-59^T	4.96	4167	275,585	39.1	–	–	–	GCA_003721595
Ferruginibacter lapsinanis HU1-HG42^T	3.29	2851	3,455,339	37.0	68.5	22.2	59.2	GCA_020783315
Pseudoflavitalea rhizosphaerae T16R-265^T	6.40	5241	388,019	46.9	66.9	24.5	55.1	GCA_003991355
Flavisolibacter ginsengisoli Gsoil 492^T	4.46	4080	210,944	40.6	67.2	23.6	55.5	GCA_900129295
Niastella koreensis GR20-10^T	8.62	7044	9,033,684	44.9	67.1	24.7	55.6	GCA_000246855
Terrimonas ferruginea DSM 30193^T	4.47	3926	217,613	48.1	66.7	33.4	54.7	GCA_000425585

Table 3 Characteristics between strain BO-59^T and other related genera in the family *Chitinophagaceae*

Characteristics	1	2	3	4	5	6	7
Growth at 37 °C	+	−	v	+	v	v	+
Oxidase/catalase	+/+	+/+	v/+	+/+	v/v	−/v	+/+
Nitrate reduction	+	−	−	−	−	−	+
Fatty acids (> 10%)[a]	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH or summed feature 3[a]	iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, summed feature 3[a]
Major polar lipids	PE, APL, PL, AL	PE, APL, L / ND	PE, AL, L	PE, L	ND	ND	PE, GL, L
G+C content (mol%)	39.1	35.8–39.8	48.1–48.8	46.2–55.7	42.7–43.0	44.3–45.8	41.0–48.9

Taxa: 1, *Hanamia caeni* BO-59^T; 2, *Ferruginibacter* (four species) [33, 39, 40]; 3, *Flavitalea* (two species) [34, 41]; 4, *Pseudoflavitalea* (two species) [35]; 5, *Flavisolibacter* (two species) [36, 42]; 6, *Niastella* (two species) [37]; 7, *Terrimonas* (two species) [38, 43].

+ positive, − negative, *ND* not determined, *v* variable, *PE* phosphatidylethanolamine, *APL* unidentified aminophospholipid, *AL* aminolipid, *PL* unidentified phospholipid

[a]Summed features represent groups of two or three fatty acids that could not be separated by gas chromatography (GLC) with the MIDI system

The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and $C_{15:1}$ G. In addition to the major polar lipids phosphatidylethanolamine (PE), an unidentified aminophospholipid (APL1), and three unidentified polar lipids (PL1, PL2, and PL3), unidentified polar lipids (PL4 and PL5) and unidentified aminolipids (AL1 and AL2) are also present. The size of the strain BO-59^T draft genome was determined to be 4.96 Mbp and the DNA G+C content of the type strain is 39.1 mol%.

The type strain, BO-59^T (=KACC 19646^T =LMG 30865^T), was isolated from activated sludge in Hanam city, South Korea.

The 16S rRNA gene and draft genome sequence accession numbers are MH094634 and RJJR00000000, respectively.

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Author Contributions Conceived and designed the experiments: GMC, QL (2nd author), QL (3rd author), and WTI. Performed the experiments: GMC, MOJ, and WJC. Analyzed the data: GMC, SYK, JHW, and WTI. Wrote the paper: GMC and WTI.

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Declarations

Conflict of interest This article does not contain any studies with human participants or animals performed by any of the authors. Moreover, all authors read and approved the final manuscript. All the authors declare that they have no direct or indirect conflict of interest.

Ethical Approval It is the original work of the author. The work described has not been submitted elsewhere for publication, in whole or in part, and all authors listed carry out the data analysis and manuscript writing.

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