Non-contiguous finished genome sequence and description of the gliding bacterium *Flavobacterium seoulense* sp. nov.

Su-Kyoung Shin¹, Heemoon Goo², Yong-Joon Cho³, Soonsung Kwon⁴, Dongeun Yong⁴ and Hana Yi¹,²,⁵*

**Abstract**

*Flavobacterium seoulense* strain EM1321ᵀ is the type strain of *Flavobacterium seoulense* sp. nov., a proposed novel species within the genus *Flavobacterium*. This strain is a Gram-reaction-negative, aerobic, rod-shaped bacterium isolated from stream water in Bukhansan National Park, Seoul. This organism is motile by gliding. Here, we describe the features of *Flavobacterium seoulense* EM1321ᵀ, together with its genome sequence and annotation. The genome comprised 3,792,640 bp, with 3,230 protein-coding genes and 52 RNA genes.

**Keywords:** Flavobacterium, Gliding motility, Aerobic, Flavobacteriaceae

**Introduction**

*Flavobacterium* is the type genus of the family *Flavobacteriaceae* in the phylum Bacteroidetes. *Flavobacterium* was proposed by Bergey et al. [1,2] and the description was emended by Bernardet et al. [3]. *Flavobacterium* species have been isolated from various environments, including seawater, freshwater, river sediments, and soil [4-8]. Members of the genus *Flavobacterium* are Gram-negative, rod-shaped, yellow-pigmented, aerobic bacteria. At the time of writing, about 118 *Flavobacterium* species with validly published names have been described [9]; however, the genomes of only 14 type strains in this genus have been sequenced.

*Flavobacterium seoulense* sp. nov. strain EM1321ᵀ (= KACC 18114ᵀ = JCM 30145ᵀ) was isolated from stream water in Bukhansan National Park, Seoul, Korea. Here, we present a summary classification and the features of *Flavobacterium seoulense* EM1321ᵀ as well as its genome sequence and annotation.

**Classification and features**

Based on its 16S rRNA gene phylogeny and phenotypic characteristics, strain EM1321ᵀ was classified as a member of the genus *Flavobacterium* (Table 1). Preliminary sequence-based identification using the 16S RNA gene sequences in the EzTaxon database [10] indicated that strain EM1321ᵀ was most closely related to *F. granuli* Kw05ᵀ (GenBank accession no. AB180738) with a sequence similarity of 96.54%. This value was lower than the 98.7% 16S rRNA gene sequence similarity as a threshold recommended by Stackebrandtia and Ebers [11] to delineate a new species without carrying out DNA-DNA hybridization. Subsequent phylogenetic analysis was performed using the 16S rRNA gene sequences of strain EM1321ᵀ and related species. Sequences were aligned according to the bacterial rRNA secondary structure model using the jPHYDIT [12]. Phylogenetic trees were constructed using neighbor-joining (NJ) and maximum-likelihood (ML) methods implemented in MEGA version 5 [13]. The resultant tree topologies were evaluated by bootstrap analyses with 1,000 random samplings. Strain EM1321ᵀ formed a monophyletic clade together with *Flavobacterium soli* [5] in both the NJ and ML trees; however, the clustering was not supported by the bootstrap analysis (Figure 1). *Flavobacterium nitratireducens* [8] was further recovered as a sister group of the monophyletic clade in the ML tree only. Based on these phylogenetic trees, *F. soli* KACC 17417ᵀ and *F. nitratireducens* were proposed as the type strains of the newly proposed species.
JCM 17678T were selected as reference strains and were obtained from the corresponding culture collections for comparative study. Strain EM1321T was Gram-reaction negative. Cells of strain EM1321T were rod shaped with rounded ends and motile by gliding. The cells were 1.0–1.5 μm × 0.3–0.5 μm in size (Figure 2). No flagellum was observed. The colonies were yellow in color and translucent on R2A agar medium. Growth occurred aerobically at 4–35°C, and optimal growth was observed at 30°C. The cells grew in 0–4% (w/v) NaCl. Strain EM1321T exhibited catalase and oxidase activities. Physiological and biochemical properties were tested by using the API 20NE, API 50CH, and API ZYM systems (BioMérieux). In the API ZYM system, enzyme activity was detected for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, and valine arylamidase (Table 2). No activity was detected for lipase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, cystine arylamidase, α-mannosidase, and α-fucosidase. In the API 20NE system, positive reactions were observed for nitrate reduction and negative reactions were observed for indole production, glucose fermentation, arginine dihydrolase, urease activity, and aesculin and gelatin hydrolysis. The strain assimilated d-glucose and l-arabinose, but not d-mannitol, d-mannose, d-maltose, potassium gluconate, N-acetylglucosamine, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. Acid was produced from l-arabinose, d-xylose, d-galactose, d-glucose, d-fructose, d-mannose, and d-lactose (API 50CH).

Matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) MS protein analysis was carried out as previously described [24]. Deposits were done from 12 isolated colonies for each strain (strain EM1321T and reference strains). Measurements were

| MIGS ID | Property | Term |
|---------|----------|------|
|         | Current classification | Domain Bacteria |
|         | Phylum Bacteroidetes    | TAS [15] |
|         | Order Flavobacterales   | TAS [16,17] |
|         | Family Flavobacteriaceae| TAS [17,18] |
|         | Genus Flavobacterium    | TAS [3,19-21] |
|         | Species F. seoulense    | IDA |
|         | Strain EM1321T           | IDA |
| MIGS-6  | Gram stain              | Negative |
| MIGS-6.3| Cell shape               | Rod-shaped |
| MIGS-22 | Motility                 | Gliding |
| MIGS-15 | Sporulation              | Non-sporulating |
| MIGS-14 | Temperature range        | 4–35°C |
| MIGS-5  | Optimum temperature      | 30°C |
|         | Habitats                 | Freshwater |
|         | Salinity                 | 0–4% |
|         | Oxygen requirement        | Aerobic |
|         | Carbon source            | d-glucose, l-arabinose |
| MIGS-15 | Biotic relationship       | Free-living |
| MIGS-14 | Pathogenicity             | Non-pathogenic |
| MIGS-4  | Geographic location       | Seoul, South Korea |
| MIGS-5  | Sample collection time    | September 2013 |
| MIGS-4.1| Latitude                 | 37°36′52″N |
| MIGS-4.2| Longitude                | 126°59′19″E |
|         | Isolation                | Stream water |

Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [23]. If the evidence is IDA, the property was directly observed by one of the authors.

cens JCM 17678T were selected as reference strains and were obtained from the corresponding culture collections for comparative study.

Strain EM1321T was Gram-reaction negative. Cells of strain EM1321T were rod shaped with rounded ends and motile by gliding. The cells were 1.0–1.5 μm × 0.3–0.5 μm in size (Figure 2). No flagellum was observed. The colonies were yellow in color and translucent on R2A agar medium. Growth occurred aerobically at 4–35°C, and optimal growth was observed at 30°C. The cells grew in 0–4% (w/v) NaCl. Strain EM1321T exhibited catalase and oxidase activities. Physiological and biochemical properties were tested by using the API 20NE, API 50CH, and API ZYM systems (BioMérieux). In the API ZYM system, enzyme activity was detected for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, and valine arylamidase (Table 2). No activity was detected for lipase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, cystine arylamidase, α-mannosidase, and α-fucosidase. In the API 20NE system, positive reactions were observed for nitrate reduction and negative reactions were observed for indole production, glucose fermentation, arginine dihydrolase, urease activity, and aesculin and gelatin hydrolysis. The strain assimilated d-glucose and l-arabinose, but not d-mannitol, d-mannose, d-maltose, potassium gluconate, N-acetylglucosamine, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. Acid was produced from l-arabinose, d-xylose, d-galactose, d-glucose, d-fructose, d-mannose, and d-lactose (API 50CH).

Matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) MS protein analysis was carried out as previously described [24]. Deposits were done from 12 isolated colonies for each strain (strain EM1321T and reference strains). Measurements were
made with a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). Spectra were recorded in the positive linear mode for the mass range of 2,000 to 20,000 Da (parameter settings: ion source 1 (IS1), 20 kV; IS2, 18.5 kV; lens, 7 kV). The time of acquisition was between 30 seconds and 1 minute per spot. The twelve EM1321\textsuperscript{T} spectra were imported into the MALDI BioTyper software (version 2.0; Bruker) and analyzed by standard pattern matching (with default parameter settings) against 4,613 bacterial spectra including eight Flavobacterium species, used as reference data, in the BioTyper database. For strain EM1321\textsuperscript{T} spectrum (Figure 3), no significant score was obtained, suggesting that our isolate was not a member of the eight known species in the database. Spectrum differences with the two closely related Flavobacterium species are shown in Figure 4.

**Genome sequencing information**

**Genome project history**

*Flavobacterium seoulense* EM1321\textsuperscript{T} was selected for genome sequencing based on its phylogenetic position and its 16S rRNA similarity to other members of the genus Flavobacterium. The genome sequence was deposited in GenBank under accession number JNCA00000000.1. A summary of the project and the Minimum Information about a Genome Sequence (MIGS) [14] are shown in Table 3.

**Growth conditions and DNA isolation**

*Flavobacterium seoulense* EM1321\textsuperscript{T} was cultured aerobically on R2A agar medium at 30°C. Genomic DNA was extracted using the QIAamp DNA mini kit (Qiagen).

**Genome sequencing and assembly**

The genome of strain EM1321\textsuperscript{T} was sequenced at Chun-Lab, Inc. by using an Illumina MiSeq PE 300 system.
with 2 × 300 paired-end reads. The Illumina platform provided 166× coverage (for a total of 3,792,640 sequencing reads) of the genome. CLC Genomics Workbench (ver. 6.5.1) was used for sequence assembly and quality assessment. The final draft assembly contained 56 contigs.

**Genome annotation**
The genes in the assembled genome were predicted with Rapid Annotation using Subsystem Technology (RAST) server databases [25] and the gene-caller GLIMMER 3.02 [26]. The predicted ORFs were annotated by searching clusters of orthologous groups (COGs) [11] using the

| Characteristic                | F. seoulense EM1321ᵀ | F. soli KACC 17417ᵀ | F. nitratireducens JCM 17678ᵀ |
|------------------------------|----------------------|----------------------|-------------------------------|
| Cell length (μm)             | 1.0–1.5              | 1.0–3.0¹              | 1.0–1.5²                      |
| Oxygen requirement           | Aerobic              | Aerobic²              | Aerobic³                      |
| Gram stain                   | -                    | -                    | -                             |
| Salt requirement             | 0–4%                 | 0–2%²                | 0–1%³                         |
| Motility                     | +                    | 4                   | 4                             |
| Spore formation              | -                    | -                    | -                             |
| **Production of**            |                      |                      |                               |
| Alkaline phosphatase         | +                    | +                    | +                             |
| Acid phosphatase             | +                    | +                    | +                             |
| Catalase                     | +                    | +                    | +                             |
| Oxidase                      | +                    | +                    | +                             |
| Nitrate reductase            | +                    | -                    | +                             |
| Urease                       | -                    | -                    | +                             |
| α-Galactosidase              | -                    | -                    | +                             |
| β-Galactosidase              | +                    | -                    | -                             |
| β-Glucuronidase              | -                    | -                    | -                             |
| α-Glucosidase                | -                    | -                    | +                             |
| β-Glucosidase                | -                    | 4                   | -                             |
| N-Acetyl-β-glucosaminidase   | -                    | -                    | +                             |
| Indole                       | -                    | -                    | -                             |
| Esterase                     | +                    | +                    | +                             |
| Esterase lipase              | +                    | +                    | +                             |
| Naphthol-AS-BI-phosphohydrolase | +                 | +                    | +                             |
| Leucine arylamidase          | +                    | -                    | +                             |
| Cystine arylamidase          | -                    | -                    | +                             |
| Valine arylamidase           | +                    | 4                   | +                             |
| **Utilization of**           |                      |                      |                               |
| d-glucose                    | +                    | -                   | +                             |
| l-arabinose                  | +                    | -                   | -                             |
| d-mannose                    | -                    | -                   | +                             |
| d-mannitol                   | -                    | -                   | -                             |
| d-maltose                    | -                    | -                   | +                             |
| G + C content (mol%)         | 33.25                | 36.9⁴               | 36.3⁵                        |
| **Habitat**                  | Freshwater           | Soil⁶               | Seawater⁷                     |

¹: positive result, ²: negative result.
³Data from Yoon et al. [5].
⁴Data from Nupur et al. [8].
⁵Data incongruent with a previous study [5].

Table 2 Phenotypic characteristics of *Flavobacterium seoulense* EM1321ᵀ and phylogenetically related *Flavobacterium* species.
Figure 3 Reference mass spectrum from *Flavobacterium seoulense* EM1321<sup>T</sup>. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

Figure 4 Gel view comparing the *Flavobacterium seoulense* EM1321<sup>T</sup> spectrum with those of other members in the genus *Flavobacterium*. The gel view displays the raw spectra of all loaded spectrum files arranged in a pseudo-gel-like look. The x-axis records the m/z value. Peak intensity is shown as a gray-scale scheme code. The color bar and the right y-axis indicate the relation between the color of a peak and peak intensity in arbitrary units.
SEED database [27]. RNAmmer 1.2 [28] and tRNAscan-SE 1.23 [29] were used to identify rRNA genes and tRNA genes, respectively. CRISPR repeats were examined using CRISPR recognition tool (CRT) [30]. CLgenomics™ 1.06 (ChunLab) was used to visualize the genomic features.

**Genome properties**

The genome comprised a circular chromosome with a length of 3,792,640 bp and 33.25% G + C content (Figure 5 and Table 4). It is composed of 56 contigs. Of the 3,282 predicted genes, 3,230 were protein-coding genes and 52 were RNA genes (2 rRNA genes and 50 tRNA genes). The sequencing coverage of rRNA operon (673×) indicated that 4 copies of rRNA operons are exist in this genome. The majority of the protein-coding genes (2,054 genes, 62.58%) were assigned putative functions, while the remaining genes were annotated as hypothetical proteins (1,176 genes, 35.83%). The properties of and statistics for the genome are summarized in Table 4. The distribution of genes into COG functional categories is presented in Table 5 and Figure 5.

**Conclusions**

Based on the results from phylogenetic and phenotypic analyses, we formally propose the creation of the new species *Flavobacterium seoulense* sp. nov. for strain EM1321^T_.

**Description of Flavobacterium seoulense** sp. nov.

*Flavobacterium seoulense* (seo.ul.en’se. N.L. neut. adj., named after Seoul, Korea, the geographical origin of the type strain).

Aerobic, Gram-reaction negative. Cells are rod shaped and motile by gliding. Does not have a flagellum. The colonies are yellow in color and translucent on R2A agar medium. Grows at 4–35°C, with optimum growth at 30°C and in 0–4% (w/v) NaCl. Catalase- and oxidase-positive.

Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, and valine arylamidase. Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease activity, and aesculin and gelatin

| Table 3 Genome sequencing project information |
|-----------------------------------------------|
| MIGS ID | Property | Term |
| MIGS-31 | Finishing quality | High-quality draft |
| MIGS-28 | Libraries used | One paired-end Illumina library |
| MIGS-29 | Sequencing platforms | Illumina MiSeq |
| MIGS-31.2 | Fold coverage | 166x |
| MIGS-30 | Assemblers | CLCbio CLC Genomics Workbench, version 6.5.1 |
| MIGS-32 | Gene calling method | Glimmer 3.0 |
| Genbank ID | | | |
| Genbank Date of Release | | 2014/05/27 |
| BIOPROJECT | | PRJNA248341 |
| Project relevance | | Environmental, Biotechnological |
| MIGS-13 | Source Material Identifier | KACC 18114, JCM 30145 |

| Table 4 Genome statistics |
|---------------------------|
| Attribute | Value | % of total* |
| Genomic size (bp) | 3,792,640 | 100 |
| DNA coding region (bp) | 3,386,688 | 89.30 |
| G + C content (bp) | 1,261,070 | 33.25 |
| Total genes | 3,282 | 100 |
| RNA genes | 52 | 1.58 |
| rRNA operons | 4 | - |
| Protein-coding genes | 3,230 | 98.42 |
| Pseudo genes | 45 | 1.37 |
| Genes with function prediction | 2,054 | 62.58 |
| Genes assigned to COGs | 2,281 | 69.50 |
| Genes assigned Pfam domains | 1,997 | 60.85 |
| Genes with signal peptides | 119 | 3.63 |
| Genes with transmembrane helices | 682 | 20.78 |
| CRISPR repeats | 0 | - |

*The total is based on either the size of the genome in base pairs or the total number of protein-coding genes in the annotated genome.*
Table 5 Number of genes associated with the 25 general COG functional categories

| Code | Value | %  | Description                                                                 |
|------|-------|----|------------------------------------------------------------------------------|
| J    | 157   | 4.86 | Translation                                                                  |
| A    | 1     | 0.03 | RNA processing and modification                                              |
| K    | 148   | 4.58 | Transcription                                                                |
| L    | 123   | 3.81 | Replication, recombination, and repair                                       |
| B    | 0     | 0.00 | Chromatin structure and dynamics                                             |
| D    | 23    | 0.71 | Cell cycle control, mitosis, and meiosis                                     |
| Y    | 0     | 0.00 | Nuclear structure                                                             |
| V    | 40    | 1.24 | Defense mechanisms                                                           |
| T    | 121   | 3.75 | Signal transduction mechanisms                                               |
| M    | 220   | 6.81 | Cell wall/membrane biogenesis                                                |
| N    | 18    | 0.56 | Cell motility                                                                |
| Z    | 0     | 0.00 | Cytoskeleton                                                                 |
| W    | 0     | 0.00 | Extracellular structures                                                     |
| U    | 45    | 1.39 | Intracellular trafficking and secretion                                      |
| O    | 81    | 2.51 | Posttranslational modification, protein turnover, and chaperones             |
| C    | 122   | 3.78 | Energy production and conversion                                             |
| G    | 207   | 6.41 | Carbohydrate transport and metabolism                                         |
| E    | 170   | 5.26 | Amino acid transport and metabolism                                          |
| F    | 62    | 1.92 | Nucleotide transport and metabolism                                          |
| H    | 127   | 3.93 | Coenzyme transport and metabolism                                            |
| I    | 93    | 2.88 | Lipid transport and metabolism                                               |
| P    | 170   | 5.26 | Inorganic ion transport and metabolism                                        |
| Q    | 42    | 1.30 | Secondary metabolites biosynthesis, transport, and catabolism                |
| R    | 318   | 9.85 | General function prediction only                                             |
| S    | 196   | 6.07 | Function unknown                                                             |
| -    | 949   | 29.38| Not in COGs                                                                  |

The total is based on the total number of protein coding genes in the annotated genome.

The authors declare that they have no competing interests.

Authors’ contributions
SS drafted the manuscript, performed laboratory experiments, and analyzed the data. HG cultured samples and performed the electron micrograph and phylogenetic analysis. YC, SK and DY sequenced, assembled, and annotated the genome. HY organized the study and drafted the manuscript. All authors read and approved the final manuscript.

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