High quality draft genomic sequence of *Flavobacterium enshiense* DK69\(^T\) and comparison among *Flavobacterium* genomes

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**Abstract**

*Flavobacterium enshiense* DK69\(^T\) is a Gram-negative, aerobic, rod-shaped, non-motile and non-flagellated bacterium that belongs to the family *Flavobacteriaceae* in the phylum *Bacteroidetes*. The high quality draft genome of strain DK69\(^T\) was obtained and has a 3,375,260 bp genome size with a G + C content of 37.7 mol % and 2848 protein coding genes. In addition, we sequenced five more genomes of *Flavobacterium* type strains and performed a comparative genomic analysis among 12 *Flavobacterium* genomes. The results show some specific genes within the fish pathogenic *Flavobacterium* strains which provide information for further analysis the pathogenicity.

**Keywords:** *Flavobacterium*, *Flavobacterium enshiense*, Comparative genomics, Genome sequence, Pathogenicity

**Introduction**

*Flavobacterium enshiense* DK69\(^T\) (= CCTCC AB2011 144\(^T\) = KCTC 23775\(^T\)) is a type strain that belongs to the genus *Flavobacterium* of the family *Flavobacteriaceae* [1]. In recent years, members of *Flavobacterium* were identified and widely distributed in soil, fresh water, marine water, sediment, microbial mat, and glaciers [2–5]. Some *Flavobacterium* strains are fish pathogens including *Flavobacterium columnare* ATCC 49512\(^T\) causing columnaris disease [6], *Flavobacterium psychrophilum* JIP02/86\(^T\) causing cold-water disease [7] and *Flavobacterium branchiophilum* FL-15\(^T\) causing bacterial gill disease [8].

The common characters of *Flavobacterium* strains are Gram-negative, non-spore-forming, yellow-pigmented, rod-shaped, aerobic and with a low DNA G + C content (30–41 mol %) [2–12]. The *Flavobacterium* strains contained iso-C\(_{15:0}\) as the major fatty acid, phosphatidylethanolamine as the major polar lipid and menaquinone-6 as the major respiratory quinone [9–12].

In order to provide genome information of *Flavobacterium* species, we sequenced six *Flavobacterium* strains including *F. enshiense* DK69\(^T\) [1], *Flavobacterium beibuense* F44-8\(^T\) [13], *Flavobacterium cauense* R2A-7\(^T\) [14], *Flavobacterium rivuli* WB 3.3-2\(^T\) [15], *Flavobacterium subsaxonicum* WB 4.1-42\(^T\) [15] and *Flavobacterium suncheonense* GH29-5\(^T\) [2]. In this study, we compared 12 genomes including the six strains that we sequenced and other six available *Flavobacterium* genomes in the NCBI, *Flavobacterium indicum* GPTSA100-9\(^T\) [16], *Flavobacterium frigoris* PSI\(^T\) [17], *Flavobacterium* sp. F52 [18], *Flavobacterium columnare* ATCC 49512\(^T\), *Flavobacterium psychrophilum* JIP02/86\(^T\) and *Flavobacterium branchiophilum* FL-15\(^T\). Here, we present the description of the non-contiguous finished genomic sequencing of *F. enshiense* DK69\(^T\) and the comparative genome analysis of the 12 *Flavobacterium* genomes.

**Organism information**

**Classification and features**

*F. enshiense* DK69\(^T\) is a Gram-negative, strictly aerobic, yellow-pigmented rod shaped bacterium isolated from soil collected at a pharmaceutical company in Enshi, Hubei province, China. The total soil C, N, P, S and Fe

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Fig. 1 A NJ phylogenetic tree of the strains within family Flavobacteriaceae based on 16S rRNA gene sequence comparisons. GenBank accession numbers are shown in parentheses. The sequences were aligned using CLUSTALX, and the phylogenetic tree was obtained using MEGA 6 [19] software of neighbor-joining method [39], with the bootstrap values of 500 replicates. *represents the strains sequenced by us.

Fig. 2 A NJ phylogenetic tree of the strains within family Flavobacteriaceae based on core-protein sequence comparisons. GenBank accession numbers are shown in parentheses. *represents the strains sequenced by us.
concentrations were 39.83, 3.34, 0.68, 0.36, 33.80 g kg$^{-1}$, respectively, and the pH was 6.97 [1]. A neighbor-
joining phylogenetic tree based on the 16S rRNA gene
sequences was built using MEGA 6 [19] and showed
that strain DK69$^T$ was clustered within a branch con-
taining other species in the genus *Flavobacterium*
(Fig. 1). In addition, the sequence of *F. enshiense*
DK69$^T$ was compared with other sequenced strains of
the family *Flavobacteriaceae* use BioLinux [20], and a
total of 24 core protein sequences were obtained with
50 % identity and E-value exponent of $e^{-10}$. A phylo-
genetic tree based on the 24 core protein sequences
of the core genome (Fig. 2) is similar to the 16S
rRNA gene based tree.

The colonies of *F. enshiense* DK69$^T$ are smooth with
regular edges, circular, yellowish and about 1 mm in
diameter after grown on R2A agar at 28 °C for 48 h.

### Table 1 Classification and general features of *F. enshiense* DK69$^T$ according to the MIGS recommendations [21]

| MIGS ID | Property                | Term                                      | Evidence code |
|---------|-------------------------|-------------------------------------------|---------------|
| MIGS-6  | Classification          | Domain *Bacteria*                         | TAS [22]      |
|         |                         | Phylum *Bacteroidetes*                    | TAS [23]      |
|         |                         | Class *Flavobacteria*                     | TAS [24]      |
|         |                         | Order *Flavobacteriales*                  | TAS [24]      |
|         |                         | Family *Flavobacteriaceae*               | TAS [25]      |
|         |                         | Genus *Flavobacterium*                   | TAS [5, 26]   |
|         |                         | Species *Flavobacterium enshiense*       | TAS [1]       |
|         |                         | Type strain: DK69$^T$ (=CCTCC AB 2011144$^T$ = KCTC 23775$^T$) | TAS [1]       |
|         | Gram stain              | negative                                  | TAS [1]       |
|         | Cell shape              | Rod                                       | TAS [1]       |
|         | Motility                | non-motile                                | TAS [1]       |
|         | Sporulation             | non-sporulating                           | TAS [1]       |
|         | Temperature range       | 4-32 °C                                   | TAS [1]       |
|         | Optimum temperature     | 28 °C                                     | TAS [1]       |
|         | pH range; Optimum       | 6.0-8.0; 7.0                              | TAS [1]       |
|         | Carbon source           | casein, gelatin, egg yolk, tyrosine, sucrose, D-mannitol | TAS [1]       |
|         | Habitat                 | soil                                      | TAS [1]       |
| MIGS-6.3| Salinity                | 0 % NaCl (w/v)                            | TAS [1]       |
| MIGS-22 | Oxygen requirement      | aerobic                                   | TAS [1]       |
| MIGS-15 | Biotic relationship     | free-living                               | NAS           |
| MIGS-14 | Pathogenicity           | non-pathogen                              | NAS           |
| MIGS-4  | Geographic location     | Enshi city, Hubei Province, China         | TAS [1]       |
| MIGS-5  | Sample collection       | 2010                                      | TAS [1]       |
| MIGS-4.1| Latitude                | not reported                              |               |
| MIGS-4.2| Longitude               | not reported                              |               |
| MIGS-4.4| Attitude                | not reported                              |               |

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 [27]
Growth occurs at 4–32 °C, pH 6.0–8.0 on R2A and TSA, but not on NA or LB media, and NaCl is not required [1]. Cells are non-flagellated, non-sporo-forming, non-motile, rod-shaped (Fig. 3). Oxidase- and catalase- positive. The DNA G + C content is 34.4 mol% [1]. The general description of this strain is shown in Table 1.

Chemotaxonomic data
The major cellular fatty acids of F. enshiense DK69T were iso-C15:0, iso-C17:1ω9c, C15:0, iso-C17:0 3-OH and iso-C15:0 3-OH. The major polar lipids were phosphatidylethanolamine, one unidentified aminolipid and one unidentified lipid. F. enshiense DK69T contained menaquinone 6 as the major quinone [1].

Genome sequencing information
Genome project history
Genome of F. enshiense DK69T was sequenced by Majorbio Bio-pharm Technology Co., Ltd, Shanghai, China. The high-quality draft genome sequence was deposited in the National Center for Biotechnology Information. Contigs less than 200 bp were not included. The GenBank accession number is JRLZ00000000. The summary of the genome sequencing project information is shown in Table 2.

Growth conditions and genomic DNA preparation
F. enshiense DK69T was grown on R2A medium at 28 °C for 2 d with 160 rpm shaking. Cells in late-log-phase growth were harvested and lysed by EDTA, lysozyme, and detergent treatment, followed by proteinase K and RNase digestion. The DNA was extracted and purified using the QiAamp kit according to the manufacturer’s instruction (Qiagen, Germany). The quantity of DNA was measured by the NanoDrop Spectrophotometer to ensure that the DNA concentration is greater than 20 ng/μl, then 5 μg of DNA was sent to Majorbio (Shanghai, China) for sequencing.

Genome sequencing and assembly
The Illumina Hiseq2000 with the Paired-End library strategy was used to determine the whole-genome sequence of F. enshiense DK69T. TruSeq DNA Sample Preparation Kits are used to prepare DNA libraries with insert sizes of 300–500 bp for single, paired-end, and multiplexed sequencing. The protocol used 1 μg of DNA sheared by either sonication or nebulization [28]. The genome raw data of F. enshiense DK69T generated 8,329,997 x 2 reads totaling 1,682,659,394 bp data with an average coverage of 498.4 x. Then SOAPdenovo v1.05 [29] was used to perform the following steps to assemble the sequencing data: (1) removing the adapter sequences in the reads; (2) cutting the 5’ end bases without clear A, T, C and G; (3) trimming the quality read scores lower than 20; (4) removing the reads containing more than 10 % Ns; (5) removing the reads which the length were less than 25 bp. A total of 8,217,761 x 2 high quality reads totaling 1,645,393,073 bp data with an average coverage 487.4 × was generated. The assembled sequence contained 67 scaffolds with a genome size of 3.38 Mbp.

Genome annotation
The annotation of the genomic sequences was completed using the NCBI Prokaryotic Genome Annotation Pipeline

| Table 2 Project information of F. enshiense DK69T |
|-----------------------------------------------|
| **MIGS ID** | **Property** | **Term** |
| MIGS 31 | Finishing quality | High-quality draft |
| MIGS-28 | Libraries used | Illumina Paired-End library (300 bp insert size) |
| MIGS 29 | Sequencing platforms | Illumina Hiseq2000 |
| MIGS 31.2 | Fold coverage | 487.4 x |
| MIGS 30 | Assemblers | SOAPdenovo v1.05 |
| MIGS 32 | Gene calling method | GeneMarkS+ |
| | Locus Tag | Q767 |
| | Genbank ID | JRLZ00000000 |
| | Genbank Date of Release | October 28, 2014 |
| | BIOPROJECT | PRJNA221771 |
| | Project relevance | Genome comparison |
| MIGS 13 | Source Material Identifier | DK69T |

| Attribute | Value | % of Total* |
|-----------|-------|-------------|
| Genome size (bp) | 3,375,260 | 100.00 |
| DNA coding (bp) | 2,808,588 | 83.21 |
| DNA G + C (bp) | 1,273,385 | 37.73 |
| DNA scaffolds | 67 | - |
| Total genes | 3054 | 100.00 |
| Protein coding genes | 2848 | 93.25 |
| RNA genes | 50 | 1.64 |
| Pseudo genes | 156 | 44.67 |
| Genes in internal clusters | 1113 | 3908 |
| Genes with function prediction | 1649 | 57.90 |
| Genes assigned to COGs | 1718 | 60.32 |
| Genes with Pfam domains | 2495 | 87.61 |
| Genes with signal peptides | 735 | 25.81 |
| Genes with transmembrane helices | 651 | 22.86 |
| 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
which was combined using Best-placed reference protein set and the gene caller GeneMarkS++. SignalP [30] and SOSUI [31] were used to predict signal peptides and transmembrane helices. The predicted CDSs were also used to search against the Pfam protein family database [32]. The GenBank database [33] and the COG databases [34] BLASTP search were used to predict protein sequences.

**Genome properties**

The genome statistics are provided in Table 3 and Fig. 4. After genome annotation, the genome of *F. enshiense* DK69T was found to have a total length of 3,375,260 bp, a G + C content of 1,273,385 bp (37.7 mol %) and 74 contigs. From a total of 3,054 genes predicted, 2,848 genes are protein-coding genes, 50 are RNA genes, 57.9 % are assigned with putative functions and the remaining are annotated as hypothetical proteins or proteins of unknown functions. The distribution of genes into COGs functional categories is shown in Table 4.

**Insights from the genome sequences**

**Profiles of metabolic network and pathway**

The metabolic network and pathways of *F. enshiense* DK69T (Fig. 5) were predicted using the Kyoto Encyclopedia of Genes and Genomes [35]. The metabolic network showed that *F. enshiense* DK69T possesses glycolysis, TCA cycle and pentose phosphate pathway.
Table 4 Number of genes in *F. enshiense* DK69 associated with general COG functional categories

| Code | Value | % age | Description                                           |
|------|-------|-------|-------------------------------------------------------|
| J    | 142   | 4.99  | Translation, ribosomal structure and biogenesis       |
| A    | 0     | 0.00  | RNA processing and modification                       |
| K    | 76    | 2.67  | Transcription                                         |
| L    | 93    | 3.27  | Replication, recombination and repair                  |
| B    | 1     | 0.04  | Chromatin structure and dynamics                       |
| D    | 20    | 0.70  | Cell cycle control, Cell division, chromosome partitioning |
| V    | 56    | 1.97  | Defense mechanisms                                    |
| T    | 67    | 2.35  | Signal transduction mechanisms                        |
| M    | 176   | 6.18  | Cell wall/membrane biogenesis                         |
| N    | 4     | 0.14  | Cell motility                                         |
| U    | 29    | 1.02  | Intracellular trafficking and secretion               |
| O    | 75    | 2.63  | Posttranslational modification, protein turnover, chaperones |
| C    | 100   | 3.51  | Energy production and conversion                      |
| G    | 54    | 1.90  | Carbohydrate transport and metabolism                 |
| E    | 158   | 5.55  | Amino acid transport and metabolism                   |
| F    | 60    | 2.11  | Nucleotide transport and metabolism                   |
| H    | 108   | 3.79  | Coenzyme transport and metabolism                     |
| I    | 69    | 2.42  | Lipid transport and metabolism                        |
| P    | 81    | 2.84  | Inorganic ion transport and metabolism                |
| Q    | 39    | 1.37  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 192   | 6.74  | General function prediction only                      |
| S    | 118   | 4.14  | Function unknown                                      |
| -    | 1130  | 39.68 | Not in COGs                                           |

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

Fig. 5 Metabolic network and pathways of *Flavobacterium enshiense* DK69 as predicted using KEGG [35]. Green lines indicate pathways that are possessed by this strain.
pathways and could utilize casein, tyrosine, sucrose and D-mannitol. The genome analysis results are in agreement with the phenotypes [1].

**Comparison of the 12 Flavobacterium genomes**

The genomic information of the 12 Flavobacterium genomes are summarized in Table 5. OrthoMCL [36] analysis was performed to identify the set of orthologs among the 12 Flavobacterium genomes. F. enshiense DK69\(^T\) shared 1,190 genes with the other 11 Flavobacterium strains, and had 437 strain-specific genes which may contribute to the species-specific features (Fig. 6).

Three of the 12 Flavobacterium strains are fish pathogenic bacteria [6–8]. Using OrthoMCL [36] analysis, a total of ten proteins we found to be unique in the three fish-pathogenic species. Three of the putative proteins were reported to be related to the pathogenicity of pathogenic bacteria including polysaccharide deacetylase [37], ABC transporter ATPase and ABC transporter permease [38] (Table 6).

**Conclusions**

The genomic results of F. enshiense DK69\(^T\) and related strains reveled useful information. (1) The genome based phylogenetic analysis results is in agreement with the 16S rRNA gene based one; (2) The genomic data are correlated with some phenotypes of strain DK69\(^T\); (3) Compared to the three fish pathogenic Flavobacterium

| Strains             | Size (Mp) | G + C % | Total genes | CDSs | Contigs | References |
|---------------------|-----------|---------|-------------|------|---------|------------|
| F. enshiense DK69\(^T\) | 3.4       | 37.7 %  | 3,054       | 2,848| 74      | This study |
| F. beibuense F44-8\(^T\) | 3.8       | 37.7 %  | 3,460       | 3,264| 61      | This study |
| F. cauense R2A-7\(^T\) | 3.1       | 38.2 %  | 2,910       | 2,723| 61      | This study |
| F. rivuli WB 3.3-2\(^T\) | 4.5       | 39.6 %  | 3,975       | 3,691| 63      | This study |
| F. subsaxonicum WB 4.1-42\(^T\) | 4.6     | 41.6 %  | 4,052       | 3,785| 80      | This study |
| F. suncheonense GH29-5\(^T\) | 2.9       | 40.5 %  | 2,769       | 2,594| 105     | This study |
| F. frigoris PS1\(^T\) | 3.9       | 34.4 %  | 3,640       | 3,590| 52      | [17]       |
| Flavobacterium sp. F52 | 5.3       | 34.4 %  | 4,601       | 4,549| 54      | [18]       |
| F. indicum GPTSA100-9\(^T\) | 3.0       | 31.4 %  | 2,787       | 2,671| 1       | [16]       |
| F. columnare ATCC 49512\(^T\) | 3.2      | 31.5 %  | 2,731       | 2,642| 1       | [6]        |
| F. psychrophilum JIP02/86\(^T\) | 2.9     | 32.5 %  | 2,556       | 2,446| 1       | [7]        |
| F. branchiophilum FL-15\(^T\) | 3.6      | 32.9 %  | 3,087       | 2,872| 1       | [8]        |

**Table 5** General features of the twelve Flavobacterium genomes

![Fig. 6](image_url) A venn diagram indicates the twelve genomes of Flavobacterium analyzed by OrthoMCL [36] illustrate the number of the unique proteins and the common proteins among them.
no pathogenic related genes was detected in the environmental strain DK69 which indicated its non-pathogenicity; and (4) Some specific genes were found within the three fish pathogenic Flavobacterium strains which provides information for further analysis the pathogenicity.

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
ZZ performed genome analysis the data and wrote the draft manuscript. CC and HD helped to analyze the data. GW organized the study and revised the manuscript. ML performed the comparative genomics analysis and revised the manuscript. All authors read and approved the final manuscript.

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| Strains                        | Accession            | Putative protein                                                                 |
|-------------------------------|----------------------|---------------------------------------------------------------------------------|
| *F. branchiophilum* FL-15 T    | WP_014083310.1        | SNF2_N, HepA, PLN03142                                                         |
| *F. columnare* ATCC 49512 T   | WP_014165166.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011962959.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014083635.1        | hypothetical protein                                                             |
| *F. columnare* ATCC 49512 T   | WP_014164281.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011962863.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014082960.1        | Hexameric tyrosine-coordinated heme protein                                       |
| *F. columnare* ATCC 49512 T   | WP_014165359.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011963152.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014084059.1        | polysaccharide deacetylase                                                        |
| *F. columnare* ATCC 49512 T   | WP_014165336.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011963745.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014084057.1        | membrane protein                                                                  |
| *F. columnare* ATCC 49512 T   | WP_014165338.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011963747.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014084692.1        | PepSY-associated TM helix                                                         |
| *F. columnare* ATCC 49512 T   | WP_014166184.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011963892.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014082991.1        | S-adenosylmethionine protein                                                      |
| *F. columnare* ATCC 49512 T   | WP_014164416.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011963983.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014082768.1        | ABC transporter permease                                                          |
| *F. columnare* ATCC 49512 T   | WP_014165791.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011964188.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014082767.1        | ABC transporter ATPase                                                           |
| *F. columnare* ATCC 49512 T   | WP_014165790.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011964189.1        |                                                                                 |
| *F. branchiophilum* FL-15 T    | WP_014083276.1        | Transposase                                                                      |
| *F. columnare* ATCC 49512 T   | WP_014165862.1        |                                                                                 |
| *F. psychrophilum* JIP02/86 T | WP_011964284.1        |                                                                                 |
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