Draft genome sequence of *Francisella tularensis* subsp. *holarctica* BD11-00177

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*Francisella tularensis* is a facultative intracellular bacterium in the class Gammaproteobacteria. This strain is of interest because it is the etiologic agent of tularemia and a highly virulent category A biothreat agent. Here we describe the draft genome sequence and annotation of *Francisella tularensis* subsp. *holarctica* BD11-00177, isolated from the first case of indigenous tularemia detected in The Netherlands since 1953. Whole-genome DNA sequence analysis assigned this isolate to the genomic group B.FTNF002–00, which previously has been exclusively reported from Spain, France, Italy, Switzerland and Germany. Automatic annotation of the 1,813,372 bp draft genome revealed 2,103 protein-coding and 46 RNA genes.

Abbreviations: CDC- United States Centers for Disease Control and Prevention, TNO- Dutch Organization for Applied Scientific Research, FOI- Swedish Defence Research Agency

Introduction

*Francisella tularensis* is a Gram negative, non-motile, non-spore forming, facultative intracellular bacterium appearing as short rods or coccoid forms [1]. *F. tularensis* is the etiologic agent of tularemia, a zoonotic infection also known as rabbit fever and deer-fly fever. Transmission to humans has been reported by direct contact with infected animals, arthropod bites, inhalation of contaminated dust or ingestion of contaminated food or water. This pathogen is highly infectious as it can cause infection upon inhalation of as few as 10 cells. This extremely low infectious dose makes transmission via aerosols easy, and previous attempts to weaponize this microorganism have led to its recognition as a category A biothreat agent (CDC classification) [2,3]. *F. tularensis* contains three subspecies that are infectious to humans; the highly virulent *Francisella tularensis* subsp. *tularensis*, which often causes a lethal multisystemic disease with a fatality rate of up to 30%, the less virulent *Francisella tularensis* subsp. *holarctica* and *Francisella tularensis* subsp. *mediasiatica*, which both seldom cause infectious in humans. Here we present a summary classification together with the description of the draft genome sequence and annotation of *Francisella tularensis* subsp. *holarctica* BD11-00177, that was isolated from a vesicle on the forehead of a 72-year-old male living in The Netherlands. As the patient had not been abroad for years, this was the first documented case of indigenous tularemia in The Netherlands since 1953.
Classification and features

*Francisella* is the only genus within the family *Francisellaceae* and is a member of the order *Thiotrichales* and the class *Gammaproteobacteria* [4] [Table 1]. Besides *F. tularensis*, the genus *Francisella* includes the species *Francisella halioticida*, *Francisella hispaniensis*, *Francisella noatunensis*, *Francisella novicida*, *Francisella philomiragia*, *Francisella cantonensis* and the mis-classified *Wolbachia persica* [4,17, Figure 1]. Only rare human infections with *F. hispaniensis* and *F. novicida*, and *F. philomiragia* are described, often caused after nearly drowning [18,19]. *F. tularensis* is capable of infecting hundreds of different vertebrate and invertebrate hosts [20]. The most widely distributed subspecies is *F. tularensis subsp. holarctica*, which is found throughout much of the Northern Hemisphere and is the only subspecies naturally occurring in Europe [21].

| MIGS ID | Property | Term | Evidence codea |
|--------|----------|------|----------------|
|        | Domain   | Bacteria | TAS [5] |
|        | Phylum   | Proteobacteria | TAS [6] |
|        | Class    | Gammaproteobacteria | TAS [7,8] |
|        | Order    | Thiotrichales | TAS [7,9] |
|        | Family   | Francisellaceae | TAS [7-10] |
|        | Genus    | Francisella | TAS [11-14] |
|        | Species  | *Francisella tularensis* | TAS [11,12] |
|        | Subspecies | *Francisella tularensis holarctica* | TAS [15,16] |
|        | Strain   | BD11-00177 | NAS |
|        | Gram stain | negative | TAS [1]. |
|        | Cell shape | short rods or coccoid forms | TAS [1]. |
|        | Motility | No | TAS [1]. |
|        | Sporulation | No | TAS [1]. |
|        | Temperature range | Mesophilic | TAS [1]. |
|        | Optimum temperature | 37 | IDA |
|        | Carbon source | Carbohydrates | TAS [1]. |
|        | Energy source | Chemoorganotrophic | TAS [1]. |
|        | Terminal electron receptor | Facultative anaerobe | TAS [1]. |
|        | Habitat | Host | TAS [1]. |
| MIGS-15 | Biotic relationship | Obligate host-dependent | TAS [1]. |
|        | Host name | Homo sapiens | TAS [1]. |
|        | Host taxon ID | 9606 | TAS [1]. |
|        | Host gender | Male | NAS |
|        | Pathogenicity | Pathogen | |
| MIGS-14 | Biosafety Level | 3 | TAS [2]. |
| MIGS-4  | Geographic location | The Netherlands | IDA |
| MIGS-5  | Sample collection time | October 2011 | IDA |
| MIGS-4.1 | Latitude | unknown | |
| MIGS-4.2 | Longitude | unknown | |
| MIGS-4.3 | Depth | unknown | |
| MIGS-4.4 | Altitude | unknown | |
|        | Isolate site | Human host | |
| MIGS-4.5 | Isolation source | vesicle on the forehead | IDA |

*aEvidence 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.*
Figure 1. Maximum likelihood tree illustrating the phylogenetic relationships among several members of the genus *Francisella* and members of the order *Thiotrichales* based on full-length 16S rRNA gene sequences.

**Genome sequencing information**

**Genome project history**

Strain BD11-00177 was sequenced because of its relevance to biodefense. The draft genome sequence was finished in August 2012. The GenBank accession number for the project is 177784. The genome project is listed in the Genome OnLine Database (GOLD) [22] as project Gi21611. Sequencing was carried out at the Dutch Organization for Applied Scientific Research (TNO) and the Swedish Defense Research Agency (FOI). Initial automatic annotation was performed using the DOE-JGI Microbial Annotation Pipeline (DOE-JGI MAP). Table 2 shows the project information and its association with MIGS 2.0 compliance.

**Growth conditions and DNA isolation**

For DNA preparation, strain BD11-00177 was grown on 5% sheep blood agar plates for 72 h at 35°C in the presence of 5% CO₂. DNA was extracted using the Qiamp DNA Micro Kit according to the manufacturer's guidelines (Qiagen, Westburg b.v., Leusden, The Netherlands).

**Genome sequencing and assembly**

Sequencing was performed by the Microbiology and Systems Biology group at TNO and the Division for CBRN Defence and Security at FOI using the 454 Roche GS Junior and the Illumina MiSeq platforms. The initial draft assembly yielded 95 large (>1,000 bp) and 86 small (<1,000 bp), non-redundant contigs of 1,813,372 bp by combing 75,245 Roche/454 reads at 23× coverage and 8,289,332 Illumina reads at 690× coverage by hybrid assembly through the Ray Assembler V2.1 [24].
Francisella tularensis subsp. holarctica BD11-00177

Table 2. Project information

| MIGS ID | Property              | Term                                      |
|---------|-----------------------|-------------------------------------------|
| MIGS-31 | Finishing quality     | Standard Draft                            |
| MIGS-29 | Sequencing platforms  | Illumina MiSeq, 454 Roche GS Junior       |
| MIGS-31.2| Fold coverage        | 713×                                      |
| MIGS-30 | Assemblers            | Ray Assembler V2.1                        |
| MIGS-32 | Gene calling method   | Prodigal [23]                             |
|         | GOLD ID               | Gi21611                                   |
|         | IMG Taxon ID          | 1244086                                   |
|         | NCBI PROJECT ID       | 177784                                    |
| MIGS-38 | Project relevance     | Medical, biodefence                       |

Genome annotation

Open Reading Frames (ORFs) were predicted using the Prodigal gene prediction algorithm [23] as part of the DOE-JGI Microbial Annotation Pipeline (DOE-JGI MAP) using default parameters, followed by a round of manual curation. CRISPR elements were predicted using CRT and PILERCR [25]. Predictions from both methods were concatenated. Identification of tRNAs was performed using tRNAscan. Ribosomal RNA genes (5S, 16S, 23S) are predicted using the program RNAmmer [26]. With the exception of tRNA and rRNA, all models from Rfam [27] are used to search the genome sequence. For faster detection, sequences are first compared to a database containing all the ncRNA genes in the Rfam database using BLAST, with a very loose cutoff. Subsequently, sequences that have hits to any genes belonging to an Rfam model are searched using the program INFERNAL [27].

Protein coding genes were compared to protein families (e.g., COGs, Pfam, KEGG) and the proteome of selected “core” genomes, which are publicly available, and the product names were assigned based on the results of these comparisons.

Genome properties

The genome was assembled into 95 large (>1,000 bp) contigs and includes one circular chromosome with a total size of 11,813,372 bp (32.23% GC content). A total of 2,149 genes were predicted, 2,103 of which are protein-coding genes. Of the protein coding genes, 1,592 were assigned to a putative function, with the remaining being annotated as hypothetical proteins. The properties and the statistics of the genome are summarized in Tables 3 and 4.

Table 3. Nucleotide content and gene count levels of the genome

| Attribute                   | Value   | % of total |
|-----------------------------|---------|------------|
| Genome Size (bp)            | 1,813,372| 100.00%    |
| DNA coding region (bp)      | 1,611,603| 88.87%     |
| DNA G+C content (bp)        | 584,435 | 32.23%     |
| Total genes                 | 2149    | 100.00%    |
| RNA genes                   | 46      | 2.14%      |
| Protein-coding genes        | 2103    | 97.86%     |
| Genes in paralog clusters   | 1262    | 58.72%     |
| Genes assigned to COGs      | 1584    | 73.71%     |
| Protein coding genes connected to KEGG pathways | 611 | 28.43% |
| not connected to KEGG pathways | 1492 | 69.43% |
| Genes with signal peptides  | 111     | 5.17%      |
| Genes with transmembrane helices | 573 | 26.66% |

a) The total is based either on the size of the genome in base pairs or on the total number of protein coding genes in the annotated genome.
Table 4. Number of genes associated with the 25 general COG functional categories

| Code | Value | %agea | Description |
|------|-------|-------|-------------|
| J    | 152   | 8.79  | Translation |
| A    | 1     | 0.06  | RNA processing and modification |
| K    | 65    | 3.76  | Transcription |
| L    | 198   | 11.45 | Replication, recombination and repair |
| B    | -     | -     | Chromatin structure and dynamics |
| D    | 18    | 1.04  | Cell cycle control, mitosis and meiosis |
| Y    | -     | -     | Nuclear structure |
| V    | 31    | 1.79  | Defense mechanisms |
| T    | 24    | 1.39  | Signal transduction mechanisms |
| M    | 112   | 6.47  | Cell wall/membrane biogenesis |
| N    | 19    | 1.1   | Cell motility |
| Z    | 1     | 0.06  | Cytoskeleton |
| W    | -     | -     | Extracellular structures |
| U    | 44    | 2.54  | Intracellular trafficking and secretion |
| O    | 66    | 3.82  | Posttranslational modification, protein turnover, chaperones |
| C    | 107   | 6.18  | Energy production and conversion |
| G    | 118   | 6.82  | Carbohydrate transport and metabolism |
| E    | 158   | 9.13  | Amino acid transport and metabolism |
| F    | 65    | 3.76  | Nucleotide transport and metabolism |
| H    | 96    | 5.55  | Coenzyme transport and metabolism |
| I    | 64    | 3.7   | Lipid transport and metabolism |
| P    | 71    | 4.1   | Inorganic ion transport and metabolism |
| Q    | 37    | 2.14  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 172   | 9.94  | General function prediction only |
| S    | 111   | 6.42  | Function unknown |
| -    | 565   | 26.29 | Not in COGs |

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

Comparisons with other fully sequenced genomes

Comparison of the assembled draft genome sequence of strain BD11-00177 with publicly available *F. tularensis* genome sequences revealed that it clusters in the FTNF002-00 genomic group (B.Br:FTNF002-00 and BIV:FTNF002-00) defined by the FTNF002-00 genome sequence [28-30] within the BIV clade. The presence of the 1.59 kb RD23 deletion event [31] as well as the 464 bp size of the MLVA marker FtM24 [32], both typical for the FTNF002-00 genomic group, were confirmed in silico. Notably, isolates from this genomic group had previously been exclusively reported from Spain, France, Italy, Switzerland and Germany [28,31-35].

A BLAST Ring Image Generator (BRIG) analysis comparing the *F. tularensis* subsp. *holarctica* BD11-00177 genome against the *F. tularensis* subsp. *holarctica* genomes of F92, LVS, and FTNF002-00 revealed that the BD11-00177 draft genome shows considerable resemblance to FTNF002-00 (Figure 2).

Evolutionary history of *F. tularensis* subspecies *holarctica* strain BD11-00177 was inferred using publicly available whole genome sequences. The trees in Figure 3 A and B are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the number of differences method and are in the units of the number of base differences per sequence. The overview of *Francisella* genus involved 52 public genome sequences using *Piscirickettia salmonis* as outgroup (Figure 3A). The detailed analysis involved 14 *F. tularensis* subsp. *holarctica* genome sequences using *F. tularensis* subsp. *tularensis* strain SCHU S4 as outgroup (Figure 3B) [17,30,33,36-41]. All positions containing gaps and missing data were eliminated. There were a total of 1,599,589 positions in the final dataset.
Figure 2. BRIG diagram of the *F. tularensis* subsp. *holarctica* BD11-00177, FTNF002-00 and SCHU S4 genomes using the *F. tularensis* subsp. *holarctica* FSC200 genome as a reference backbone. White regions represent absent genetic regions.
Figure 3. A) Overview of the *Francisella* genus phylogeny based on 52 public whole genome sequences. B) The phylogeny of *F. tularensis subsp. holarctica* strains based on whole genome sequences. The new isolate, BD11-00177 belongs to the FTNF002-00 genomic group inside the B.IV clade.

Conclusion

Here we have presented the draft genome of the first member of FTNF002-00 genomic group of *F. tularensis subsp. holarctica*. As more genetic information of members from this genomic group becomes available, a better understanding of the evolution and biogeography of this pathogen will be gained. This knowledge may help us to understand the epidemiology and potential expansion of the geographical distribution of this genomic group. Despite potential biases associated with discontinuous draft genomes, we would like to focus on the added value of draft bacterial genome sequencing. Taking advantage of low cost and high-throughput sequencing platforms allows us to probe the vast microbial diversity present in nature and rapidly respond to clinical outbreaks and acute biosecurity hazards. From an evolutionary ecology perspective, increased sequencing efforts allow us to characterize the biogeography of microbial taxa and differentiate between neutral and conserved genome contents.

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