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GENOME SEQUENCES

Draft Genome Sequence of the Strain *Francisella tularensis* subsp. *mediasiatica* 240, Isolated in Kazakhstan

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ABSTRACT *Francisella tularensis* subsp. *mediasiatica* is the least studied among the four *F. tularensis* subspecies. We present here the genome data of *F. tularensis* subsp. *mediasiatica* 240, isolated in the southern region of Kazakhstan.

Tularemia is a zoonotic natural focal infection caused by *Francisella tularensis*. Currently, four subspecies of *F. tularensis* are recognized, differing in virulence and geographical distribution. *F. tularensis* subsp. *tularensis* (type A) is common in North America. It is the most virulent subspecies for humans. The two subtypes A.I and A.II also differ in virulence (1). *F. tularensis* subsp. *holarctica* is the second most virulent for humans and is distributed in the Northern Hemisphere (2). *F. tularensis* subsp. *novicida*, described in North America and Australia, causes sporadic opportunistic infections in immunosuppressed patients (3, 4). *F. tularensis* subsp. *mediasiatica* remains the least-studied subspecies. For a long time, it was assumed that its distribution area was limited to Central Asia (Kazakhstan and Turkmenistan), but it was recently recovered in southern Siberia (5). No human infection caused by *F. tularensis* subsp. *mediasiatica* has been reported so far. Experiments on model animals indicate a virulence of *F. tularensis* subsp. *mediasiatica* intermediate between that of *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* (5).

The genetic diversity of *F. tularensis* subsp. *mediasiatica* is poorly known. In this article, we present the genome sequence of strain *F. tularensis* subsp. *mediasiatica* 240, isolated in 1982 from ticks in the southern region of Kazakhstan. The strain was isolated using direct plating of homogenized sample onto coagulated chicken egg yolk. The inoculations were kept under aerobic conditions at 37°C for 120 h, and typical colonies were subjected to reseeding and further typing. After identification, the strain was stored in a lyophilized state. Before the study, the lyophilized strain was suspended in 0.9% NaCl, plated onto petri dishes with FT agar including vitamins and mineral additives (FBIS SRCAMB, Obolensk, Russia) (5, 6), and cultured under aerobic conditions at 37°C for 72 h. A single colony was subcultured on a petri dish with FT agar and incubated at 37°C for 72 h. The bacterial mass was collected and suspended in 0.9% NaCl. The bacterial suspension was inactivated by adding a thimerosal solution (T5125, Sigma-Aldrich) to a concentration of 0.01% and incubated at 56°C for 30 min.

DNA was isolated using a DNA minikit (Qiagen, Hilden, Germany). Preparation of the sequencing libraries was carried out using the Nextera XT DNA library prep kit (Illumina, San Diego, CA, USA). Sequencing was performed using the MiSeq system with the MiSeq reagent kit v3 (600 cycles, 2 × 300 bp). In total, 780,074 sequencing reads were obtained. The reads were trimmed using Seqtk v1.3 (7) up to a quality (Q) value of Q30 and *de novo* assembled with Skesa v2.3.0 (8) (all software was used

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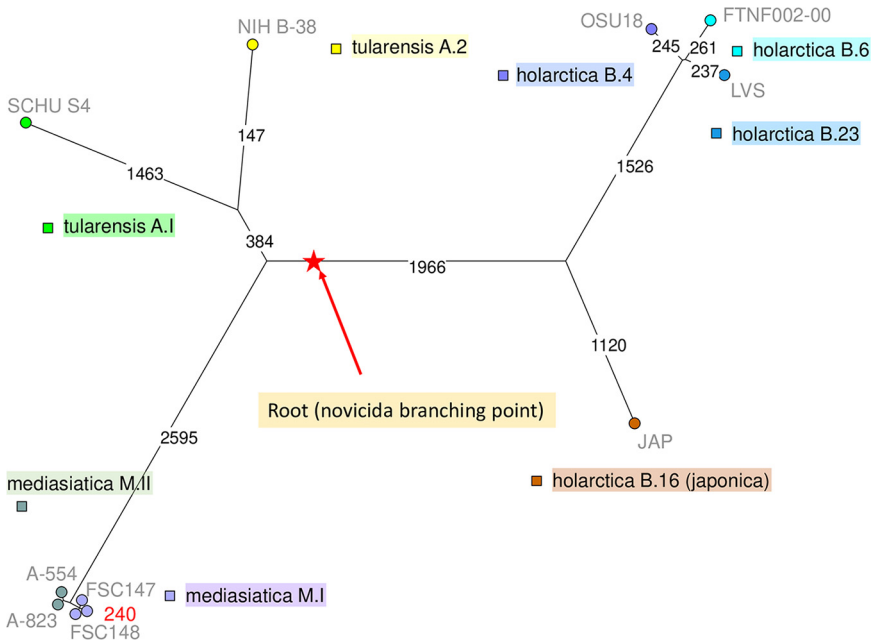

FIG 1 Maximum parsimony tree of whole-genome single nucleotide polymorphism (SNP) data. Whole-genome sequencing (WGS) data from all available *F. tularensis* subsp. *mediasiatica* strains, including Kazakhstan strain 240 (indicated in red), and from selected strains representing the main sublineages of *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holartica* were mapped onto reference genome SCHU S4 (assembly accession no. GCA_000008985), as previously described (5). In total, 10,953 SNPs were called; the tree size is 11,027 bp (homoplasia, 0.67%). Branch length of the longer branches is indicated. Branches of 70 and 44 SNPs lead to lineages M.I and M.II (Siberian strains), respectively. The red star indicates the branching point toward *F. tularensis* subsp. *novicida*, which can be considered an outgroup with respect to the three other subspecies.

with default parameters except when stated otherwise). Assembly quality assessment was performed using QUAST v5.0.2 software (9). The draft genome assembly totaled 1,791,721 bp, with 75 contigs, an average coverage of 71×, an N_{50} value of 35,408 bp, and a GC content of 32.33%. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP v4.11) (10, 11). Totals of 265 pseudogenes and 1,525 genes were predicted, of which 1,483 are protein coding genes and 42 are RNA coding genes. The genetic diversity described within *F. tularensis* subsp. *mediasiatica* is very limited compared to the genetic diversity reported in the other *F. tularensis* subspecies (Fig. 1). Strain 240 belongs to *F. tularensis* subsp. *mediasiatica* subtype M.I (Fig. 1).

Data availability. This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession no. JABWGW000000000. The version described in this paper is the first version, JABWGW010000000. The raw data from BioProject PRJNA639508 were submitted to the NCBI SRA under experiment accession no. SRR12015651.

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