

Complete Genome Sequence of *Rickettsia heilongjiangensis*, an Emerging Tick-Transmitted Human Pathogen

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***Rickettsia heilongjiangensis* is an emerging tick-transmitted human pathogen causing far-Eastern spotted fever. Here we report the complete sequence and the main features of the genome of *R. heilongjiangensis* (strain 054).**

Rickettsia heilongjiangensis was first isolated from *Dysmicoccus sylvarum* ticks in the Heilongjiang province of China in 1983 and was classified as one of the *Rickettsia japonica* subgroup of spotted fever group rickettsiae (5). The disease caused by *R. heilongjiangensis* has been named far-Eastern spotted fever (FESF), and the disease has been diagnosed in patients in Northeastern China (11), Siberia, far-eastern Russia (7–9), and Japan (1), suggesting that FESF is an important emerging tick-borne infectious disease in these areas. Our previous study in the BALB/c mouse model revealed that *R. heilongjiangensis* established disseminated intracellular infection in mice and caused pathological lesions and inflammatory cytokine expression in major organs, similar to what is observed in human spotted fever (4). The complete genome sequence of *R. heilongjiangensis* will help us to gain an insight into the pathogenicity mechanisms of the emergent pathogen.

To characterize the genome of *R. heilongjiangensis* strain 054 (ATCC VR-1524), the genomic DNA was isolated from the bacteria cultivated in Vero cells. Whole-genome sequencing of this organism was performed with a combined strategy involving Solexa and Roche/454. Using an Illumina Solexa GA IIX, 1,634,610 paired-end reads (500-bp insert) were generated, and the raw reads were assembled into five large scaffolds to reach a depth of 116.4-fold mean coverage by using Velvet 1.0.18 (12), SOAPdenovo63mer (6), and Abyss 1.2.6 ($k = 60$) (10). Based on this assembly, the interscaffold and intrascaffold gaps were closed by local assembly and by sequencing PCR products using an ABI 3730 sequencer. For sequence validation and reassurance, 436,817 reads from Roche/454 were mapped to the complete genome, reaching a depth of 130.4-fold mean coverage. The complete genome of *R. heilongjiangensis* 054 contains a circular 1,278,471-bp chromosome with a G+C content of 32.32%.

The annotation was performed by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP). A total

of 1,297 predicted protein coding genes, one copy each of the 5S, 16S, and 23S rRNA genes, 33 predicted tRNA genes, 5 pseudogenes, and 73 potential frameshifts were identified in the genome. The genome encodes the rickettsial major surface proteins, including outer membrane protein A, outer membrane protein B, and DsbA. A total of 14 proteins are involved in a type IV secretion system.

To compare genome sequences according to one annotation method, the RAST (Rapid Annotation using Subsystem Technology) server (2) was used to annotate genomes of *R. heilongjiangensis* 054, *R. rickettsii* Sheila Smith, and *R. prowazekii* Madrid E. Mauve was employed to perform the comparison (3). The results showed that a total of 677 common genes were identified in all three rickettsial genomes and that 420 genes are unique to 054. In addition, 371 genes are shared by 054 and Sheila Smith, 4 genes are shared by 054 and Madrid E, and 8 genes shared by Sheila Smith and Madrid E are absent from 054. Compared with Sheila Smith, 054 contains 74 insertions and 70 deletions bigger than 100 bp.

Nucleotide sequence accession number. The genome sequence and annotation information are accessible in the GenBank database with accession number CP002912.

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