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PROKARYOTES

Chromosome and Large Linear Plasmid Sequences of a *Borrelia miyamotoi* Strain Isolated from *Ixodes pacificus* Ticks from California

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ABSTRACT *Borrelia miyamotoi,* a relapsing fever group spirochete, is an emerging tick-borne pathogen. It has been identified in ixodid ticks across the Northern Hemisphere, including the West Coast of the United States. We describe the chromosome and large linear plasmid sequence of a *B. miyamotoi* isolate cultured from a California field-collected *Ixodes pacificus* tick.

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Borrelia miyamotoi falls within the relapsing fever *Borrelia* group and has been described in ixodid ticks throughout the Northern Hemisphere (1). *B. miyamotoi* has been detected in and cultured from *Ixodes scapularis* ticks from the northeastern United States, where *B. miyamotoi* disease has been described in humans (2–4). While molecular evidence of *B. miyamotoi* in *I. scapularis* and *Ixodes pacificus* ticks from the Upper Midwest and California, respectively, has been published (5–7), culture isolates and genomic sequencing are lacking.

The *B. miyamotoi* genome comprises a large linear chromosome (~1 Mb) and multiple linear and circular plasmids (4, 8). In this report, we describe the culture isolation, DNA sequencing, and assembly of the chromosome and a large linear plasmid of a West Coast *B. miyamotoi* strain CA17-2241 isolated from a field-collected *I. pacificus* tick.

Ixodes pacificus ticks were collected from Olompali State Park in Marin County, CA, in December 2015. Adult females were fed on rabbits, allowed to oviposit, and tested for *Borrelia* spp. by PCR. A female with evidence of *B. miyamotoi* was identified, and larvae from its egg clutch were tested again for *Borrelia* spp. Remaining larvae were fed on naive CD1 mice and allowed to molt. Subsequent nymphs were fed on SCID mice, and blood from infected mice was transferred to ISE6 tick cell cultures (9, 10). Spirochete replication was verified by dark-field microscopy and PCR. *B. miyamotoi* spirochetes were harvested by removing supernatant from tissue culture flasks and allowing any residual ISE6 cells to settle overnight at 34°C. Spirochetes were further enriched by centrifugation of the supernatant at 6,500 × *g* for 8 minutes. The pellet was washed 2× with phosphate-buffered saline (PBS). *B. miyamotoi* DNA was extracted using the QIAamp DNA kit (Qiagen). DNA libraries were prepared using the NexteraXT library kit (Illumina) and sequenced on the MiSeq platform using the 600-cycle V3 reagent kit (Illumina). Paired-end reads were assembled using SPAdes 3.9.0 according to the recommended parameters (11). Two long contigs of lengths 905,840 and 73,089 bp were generated from the assembly with 642× and 783× coverage, respectively, that corresponded to the large linear chromosome and large linear plasmid lp72 from *B. miyamotoi* strain CT13-2396 (4). These were the only reliably assembled contigs due to the highly repetitive nature of the other relapsing fever group *Borrelia* plasmids

(4, 12, 13). Annotation was performed using the Prokaryotic Genome Annotation Pipeline (14).

The *B. miyamotoi* CA17-2241 linear chromosome and lp72 plasmid had average GC content percentages of 28.6% and 26.8%, respectively. The CA17-2241 chromosome encodes 819 predicted protein-coding open reading frames, 3 rRNAs, 31 tRNAs, and 29 predicted pseudogenes. The lp72 plasmid encodes 66 predicted protein-coding open reading frames, 3 predicted pseudogenes, and no rRNAs or tRNAs. *In silico* analysis of the 8-gene multilocus sequence type (MLST) for *Borrelia* species revealed 100% nucleotide identity to the Sonom53 strain detected in an *I. pacificus* tick from California (15). The *B. miyamotoi* CA17-2241 chromosome demonstrated 98.56% average nucleotide identity to the chromosomes of the two previously sequenced *B. miyamotoi* strains from Connecticut (4, 8).

Accession number(s). The chromosome and large linear plasmid sequences of the *B. miyamotoi* CA17-2241 chromosome and lp72 plasmid are available in GenBank under the accession numbers CP021872 and CP021873, respectively.

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