Chromosome and Megaplasmid Sequences of Borrelia anserina (Sakharoff 1891), the Agent of Avian Spirochetosis and Type Species of the Genus.
Chromosome and Megaplasmid Sequences of *Borrelia anserina* (Sakharoff 1891), the Agent of Avian Spirochetosis and Type Species of the Genus

Haitham Elbir, Parth Sitlani, Sven Bergström, Alan G. Barbour

Department of Molecular Biology and the Laboratory of Molecular Infection Medicine, Umeå University, Umeå, Sweden; Departments of Microbiology and Molecular Genetics and Medicine, University of California Irvine, Irvine, California, USA

**ABSTRACT** Sequences of the linear chromosome and plasmids of *Borrelia anserina*, the cause of avian spirochetosis of poultry, revealed a smaller genome than those of other *Borrelia* spp. transmitted by argasid ticks. Missing or disrupted genes included a dam methylase and those in the pathway for synthesis of phospholipids from glycerol.

*Borrelia anserina* is the globally distributed agent of avian spirochetosis, a tick-transmitted disease of poultry (1). *B. anserina* is phenotypically distinguished from other species in the relapsing fever group by a host range limited to birds and the exclusive use of *Argas* sp. soft ticks as vectors. *B. anserina* has a linear chromosome of ~900 kb and a megaplasmid, like other members of the genus, but fewer plasmids in total (2, 3).

*B. anserina* strain Es (ATCC 49835) had been isolated from a domestic chicken in California (4) and was cultivated in Barbour-Stoenner-Kelly medium. Genomic DNA was extracted with phenol-chloroform after lysis in sodium dodecyl sulfate and proteinase K. For sequencing, the single-molecule, real-time long-read approach on a Pacific Biosciences (PacBio) RS I instrument (Menlo Park, CA, USA) was combined with error-correction with short single reads from an Ion Torrent apparatus (Life Technologies, Inc., Carlsbad, CA, USA), as previously described (5, 6).

The 56,438 PacBio reads (N_{50}, 20,171 nucleotides [nt]) provide chromosome and megaplasmid coverages of 662× and 410×, respectively. These were assembled with the Hierarchical Genome Assembly Process 2 (PacBio). The 2,394,657 Ion Torrent single reads had a mean length of 148 nt, and the chromosome and megaplasmid coverages were 227× and 340×, respectively. The Assembly Cell of Genomics Workbench version 8.5 (Qiagen, Denmark) was used for short reads. Gene prediction was completed with the Prokaryotic Genome Automatic Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_prok), followed by manual annotation.

The linear chromosome comprised 906,833 bp, with a G+C content of 29.5%, and 799 protein-coding sequences, 32 tRNAs, three rRNAs (5S, 16S, and 23S), and seven pseudogenes. Gene order was generally syntenic with that of *B. hermsii* (CP00048). The maximum cumulative GC skew was at position ~453,000. The sequence length was consistent with the smaller size of the *B. anserina* chromosome by pulsed-field gel electrophoresis (3). Alignment of the strain Es sequence with the 904,790-nt sequence of strain BA2’s chromosome (CP005829) identified four transversions and 37 single-nucleotide indels.

Received 5 January 2017. Accepted 12 January 2017. Published 16 March 2017.

**Citation** Elbir H, Sitlani P, Bergström S, Barbour AG. 2017. Chromosome and megaplasmid sequences of *Borrelia anserina* (Sakharoff 1891), the agent of avian spirochetosis and type species of the genus. Genome Announc 5: e00018-17. https://doi.org/10.1128/genomeA.00018-17.

**Copyright** © 2017 Elbir et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Alan G. Barbour, abarbour@uci.edu.
The unique absence of dam methylation of B. anserina DNA, as previously reported (7), was confirmed by base analysis with PacBio’s SMRT Analysis for 6-methyladenine modification (8). Locus N187_02280 is orthologous to a methylase-coding sequence of B. hermsii but is a pseudogene with multiple frameshifts. B. anserina has a glpQ gene (9) but lacks glpA, glpF, and glpK, and glpT is partial. Thus, it can acquire glycerol-3-phosphate for phospholipid synthesis from dihydroxyacetone phosphate, but, unlike other Borreliae spp., not from environmental or salvaged glycerol (9).

Megaplasmid IpA89’s length of 89,872 bp (G+C content, 28.8%) was consistent with reported pulsed-field gel electrophoretic migrations (2, 3). The shorter length of IpA89, which otherwise was largely collinear with B. hermsii’s 183-kb megaplasmid (CP0143450), was accounted for by gene loss (e.g., for factor H-binding protein and chitobiose transport proteins) and by fewer paralogs in the gene families of megaplasmids (10).

Accession number(s). Sequences for the chromosome and megaplasmid have been deposited in the GenBank/DDBJ/EMBL database under accession numbers CP013704 and CP014325 (BioProject PRJNA311246 and BioSample SAMN04481062). Associated sequences are complete plasmids pB25 (CP014520) and cp5 (CP014521) and a plasmid fragment (CP018882).

ACKNOWLEDGMENTS

This work was supported by University of California, Irvine, intramural funds and a research gift to A.G.B. and by Swedish Research Council grant 07922 to S.B. This work was made possible, in part, through access to the Genomic High-Throughput Facility Shared Resource of the Cancer Center Support (grant CA-62203) at the University of California, Irvine.

REFERENCES