Title

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

Journal

Microbiology Resource Announcements, 5(11)

ISSN

2169-8287

Authors

Elbir, Haitham

Sitlani, Parth

Bergström, Sven

et al.

Publication Date

2017-03-16

DOI

10.1128/genomea.00018-17

AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

PROKARYOTES

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

Haitham Elbir,[a] Parth Sitlani,[b] Sven Bergström,[a,b] Alan G. Barbour[b]

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

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.

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.

Address correspondence to Alan G. Barbour, abarbour@uci.edu.

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.

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 *Borreliaceae* spp., not from environmental or salvaged glycerol (9).

Megaplasmid lpA89'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 lpA89, 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

1. McNeil E, Hinshaw WR, Kissling RE. 1949. A study of *Borrelia anserina* infection (spirochetosis) in turkeys. J Bacteriol 57:191–206.
2. Marconi RT, Samuels DS, Schwan TG, Garon CF. 1993. Identification of a protein in several *Borrelia* species which is related to OspC of the Lyme disease spirochetes. J Clin Microbiol 31:2577–2583.
3. Ferdows MS, Serwer P, Griess GA, Norris SJ, Barbour AG. 1996. Conversion of a linear to a circular plasmid in the relapsing fever agent *Borrelia hermsii*. J Bacteriol 178:793–800. https://doi.org/10.1128/jb.178.3.793-800.1996.
4. DaMassa AJ, Adler HE. 1979. Avian spirochetosis: natural transmission by *Argas* (*Persicargas*) *sanchezi* (*Ixodoidea*: *Argasidae*) and existence of different serologic and immunologic types of *Borrelia anserina* in the United States. Am J Vet Res 40:154–157.
5. Barbour AG. 2016. Multiple and diverse *vsp* and *vlp* sequences in *Borrelia miyamotoi*, a hard tick-borne zoonotic pathogen. PLoS One 11: e0146283. https://doi.org/10.1371/journal.pone.0146283.
6. Hue F, Ghalyanchi Langeroudi A, Barbour AG. 2013. Chromosome sequence of *Borrelia miyamotoi*, an uncultivable tick-borne agent of human infection. Genome Announc 1(5):e00713-13. https://doi.org/10.1128/genomeA.00713-13.
7. Hughes CA, Johnson RC. 1990. Methylated DNA in *Borrelia* species. J Bacteriol 172:6602–6604. https://doi.org/10.1128/jb.172.11.6602-6604.1990.
8. Davis BM, Chao MC, Waldor MK. 2013. Entering the era of bacterial epigenomics with single molecule real time DNA sequencing. Curr Opin Microbiol 16:192–198. https://doi.org/10.1016/j.mib.2013.01.011.
9. Schwan TG, Battisti JM, Porcella SF, Raffel SJ, Schrumpf ME, Fischer ER, Carroll JA, Stewart PE, Rosa P, Somerville GA. 2003. Glycerol-3-phosphate acquisition in spirochetes: distribution and biological activity of glycerophosphodiester phosphodiesterase (GlpQ) among *Borrelia* species. J Bacteriol 185:1346–1356. https://doi.org/10.1128/JB.185.4.1346-1356.2003.
10. Miller SC, Porcella SF, Raffel SJ, Schwan TG, Barbour AG. 2013. Large linear plasmids of *Borrelia* species that cause relapsing fever. J Bacteriol 195:3629–3639. https://doi.org/10.1128/JB.00347-13.