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Whole-Genome Sequences of *Borrelia bissettii*, *Borrelia valaisiana*, and *Borrelia spielmanii*

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It has been known for decades that human Lyme disease is caused by the three spirochete species *Borrelia burgdorferi*, *Borrelia afzelii*, and *Borrelia garinii*. Recently, *Borrelia valaisiana*, *Borrelia spielmanii*, and *Borrelia bissettii* have been associated with Lyme disease. We report the complete genome sequences of *B. valaisiana* VS116, *B. spielmanii* A14S, and *B. bissettii* DN127.

The bacteria that cause human Lyme disease belong to a group of at least 15 species, referred to as *Borrelia burgdorferi* sensu lato, or the Lyme disease agent bacterial group (20). Among these, *B. burgdorferi* sensu stricto causes Lyme disease in North America, while in Europe and eastern Asia *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia bavariensis* sp. nov. are the best-known causes (reference 15 and references therein); however, more recently, *Borrelia*

bissettii, *Borrelia lusitaniae*, *Borrelia spielmanii*, and *Borrelia valaisiana* have been isolated from Lyme disease patients (5–8, 16, 17). Other species in this bacterial group, such as *Borrelia japonica* and *Borrelia sinica* in Asia, have not been associated with human disease. To date, genome sequences have been reported for 14 *B. burgdorferi* isolates (1, 9, 19), 2 *B. afzelii* isolates (4, 11), 2 *B. garinii* isolates (4, 11), 1 *B. bavariensis* sp. nov. isolate (10), and 1 isolate of unassigned species (2).

We report here the complete genome sequences for three additional *Borrelia* species: *B. valaisiana* isolate VS116 (from an *Ixodes ricinus* tick [Switzerland]) (14, 18), *B. bissettii* isolate DN127 clone 9 (*Ixodes pacificus* tick [northern California]) (12), and *B. spielmanii* isolate A14S (human skin [The Netherlands]) (21). DNA samples from low-passage isolates were sequenced to minimize plasmid loss, and genomes were sequenced to about 8-fold coverage as previously described (13). Genome annotation was performed using the JCVI Prokaryotic Annotation Pipeline (www.jcvi.org/cms/research/projects/annotation-service/overview/). The DN127 chromosome and 35 of 39 plasmid sequence contigs were closed, but in order to maximize the use of available funds, the sequences of a few replicons were not closed and some gaps remained in these sequences (two chromosomes and one cp9 and three cp32 plasmids, because they are much less variable than the other plasmids).

These three genome sequences include 3,914,891 bp in total (1,258,865, 1,403,466, and 1,252,560 bp for strains VS116, DN127, and A14S, respectively), with an average of 1,304,497 bp/genome. Like the sequences of other *Borrelia* species, they include numerous linear plasmids (6, 7, and 7, respectively) and circular plasmids (2, 2, and 2, respectively). Plasmid numbers in these three strains range from 11 in VS116 and 12 in A14S to 16 in DN127. Plasmids that are very similar to *B. burgdorferi* sensu stricto cp26, cp32 (7 in DN127, versus 3 in the other two strains analyzed), and lp54 plasmids are present in each of these isolates,

TABLE 1 *B. valaisiana*, *B. bissettii*, and *B. spielmanii* sequence accession numbers

| Replicon name ^a | Accession no. for strain (GPID) ^b | | |
|---------------------------------|--|-----------------------------------|--|
| | <i>B. valaisiana</i> VS116 (19843) | <i>B. bissettii</i> DN127 (29363) | <i>B. spielmanii</i> A14S (28635) |
| Chromosome | ABCY02000001 ^c | CP002746 | ABKB02000001 to -13 ^c |
| lp17 | CP001439 | CP002756 | CP001468 |
| lp25 | CP001437 | CP002757 | |
| lp28-2 | | | |
| lp28-3 | CP001440 | CP002758 | CP001471 |
| lp28-4 | | CP002759 | CP001470 |
| lp28-7 | | CP002760 | |
| lp28-8 | CP001442 | | CP001465 |
| lp36 | CP001436 | | CP001466 |
| lp38 | | | CP001464 |
| lp54 | CP001433 | CP002761 | CP001469 |
| lp56 | | CP002762 | |
| cp9 | CP001438 ^e | CP002755 | ABKB02000016, ^c ABKB02000020 ^{c,e} |
| cp26 | CP001432 | CP002747 | CP001467 |
| cp32-3 | | CP002749 | ABKB02000026 ^c |
| cp32-4 | | CP002750 | |
| cp32-5 | CP001441 | CP002751 | ABKB02000031 ^c |
| cp32-6 | | CP002752 | |
| cp32-7 | CP001434 | CP002753 | |
| cp32-quad (cp32-9) ^f | | CP002754 | |
| cp32-10 | CP001435 | | |
| cp32-11 | | CP002748 | |
| cp32-12 | | Fused to cp32-quad | ABKB02000021 ^c |
| cp32-13 | | Fused to cp32-quad | |
| Unidentified plasmid contigs | | | ABKB02000014, -15, -17 to -19, -22 to -25, -27 to -30, -32 to -41 ^{c,d} |

^a Plasmids are named according to their PFam32 partition/replication protein (see Casjens et al. [3]).

^b Genome project ID number.

^c Draft sequence; contigs not joined.

^d All but ABKC02000036 are cp32-like.

^e Unlike other cp9 plasmids in *B. burgdorferi*, VS116 cp9 (and probably a very similar sequence in A14S) carries a *parA*-type gene in the partition gene cluster.

^f The DN127 plasmid that carries cp32-9, cp32-12, and cp32-13 type partition genes (i.e., is a fusion between these three plasmids and a fourth whose partition genes are deleted) was named cp32-quad.

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and DN127 also contains an unusual fusion of four partial cp32 plasmids. Plasmids with predicted lp17 compatibility (3) are also present in all three genomes, making it the only other plasmid type, in addition to cp26 and lp54, to be found in all 23 *B. burgdorferi* sensu lato sequenced genomes. However, the gene contents of the lp17s are much more variable than the other universally present plasmids.

The detailed analyses of these genome sequences will be a major step forward in attaining a complete understanding of *B. burgdorferi* sensu lato diversity. They will contribute to the development of species- and group-specific vaccines and diagnostic tools, as well as inform us whether these species are in genetic contact with the more-common Lyme disease-associated agents. These foundational sequencing efforts can now be further developed with the use of evolving deep sequencing methods.

Nucleotide sequence accession numbers. The nucleotide sequences for these three strains and relevant replicons have been deposited in the GenBank database, and their accession numbers are listed in Table 1.

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