

Whole Genome Sequence of an Unusual *Borrelia burgdorferi* Sensu Lato Isolate[∇]

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Human Lyme disease is caused by a number of related *Borrelia burgdorferi* sensu lato species. We report here the complete genome sequence of *Borrelia* sp. isolate SV1 from Finland. This isolate is to date the closest known relative of *B. burgdorferi* sensu stricto, but it is sufficiently genetically distinct from that species that it and its close relatives warrant its candidacy for new-species status. We suggest that this isolate should be named “*Borrelia finlandensis*.”

The bacteria that cause human Lyme disease belong to a clade of 16 named species called *Borrelia burgdorferi* sensu lato, the Lyme agent group, or the Lyme borreliosis group (14). Among these species, *B. burgdorferi*, *B. afzelii*, *B. garinii*, and “*B. bavariensis*” sp. nov. are well-known causes of Lyme disease in North America and western Europe (reference 11 and references therein). Recently, *B. lusitaniae*, *B. spielmanii*, and *B. valaisiana* have also been isolated from Lyme disease patients in Europe (2–5, 13). Other species in this clade, such as *B. californiensis* sp. nov. and *B. andersonii* in North America and *B. japonica*, *B. turdi*, and *B. sinicia* in Asia, have not been associated with Lyme disease. To date, genome sequences have been completed for 14 *B. burgdorferi* sensu stricto isolates (1, 6, 15) as well as for two *B. afzelii* isolates, for two *B. garinii* isolates, for single *B. bissettii*, *B. valaisiana*, and *B. spielmanii* isolates, and for one *B. bavariensis* sp. nov. isolate (7, 8; our unpublished work).

We announce here the whole genome sequence of Lyme agent group strain SV1, which was isolated from an *Ixodes ricinus* tick in Finland (12). DNA from a low-passage culture was sequenced to minimize plasmid loss, and sequencing proceeded to about 8-fold coverage as previously described (10). Genome annotation was performed using the JCVI prokaryotic annotation pipeline (www.jcvi.org/cms/research/projects/prokaryotic-annotation-pipeline/overview/). The SV1 genome sequence contains 1,281,782 bp and, like the genomes of other borrelias, includes numerous linear and circular plasmids. Nine

of its 10 plasmid sequence contigs were closed, but to maximize the use of available funds, the sequences of the plasmid lp32-6 and the ~900-kbp chromosome were not closed (currently in two and five contigs, respectively).

Traditionally, new Lyme agent group species have been defined by genetic distances between discrete clusters of isolates (reference 14 and references therein). Qiu et al. (12) previously showed that isolate SV1 and its only known close relative strain, Ri5 (also from Finland), fall convincingly outside the *B. burgdorferi* sensu stricto clade by multilocus sequence typing (MLST) analysis using six chromosomal housekeeping genes as well as by comparison of the sequences of the *rrs-rrlA* intergenic spacer and three plasmid genes, *ospC*, *dbpA*, and *bdb14*. The SV1 genome sequence supports this notion. Single-nucleotide polymorphism trees for the chromosome and plasmids cp26 and lp54 all place SV1 on a robust branch outside the 14 sequenced *B. burgdorferi* genomes (unpublished results). Margos et al. (9) have recently argued that a new species, *B. bavariensis*, should be split from *B. garinii*. The chromosomal sequence of *B. bavariensis* sp. nov. isolate PBi (7) is 2.23% different from those of our *B. garinii* isolates PBr and Far04 (our unpublished results), and the chromosome of SV1 is 1.75% different from those of *B. burgdorferi* sensu stricto isolates. Thus, to retain uniformity in *Borrelia* taxonomy, the SV1/Ri5 group should also probably have species status. We suggest “*Borrelia finlandensis*” sp. nov. as a potential name, because both known isolates were found in Finland.

Nucleotide sequence accession numbers. Sequences have been deposited in the GenBank database as follows. For the chromosome, the accession number is ABJZ02000001-5. For the plasmids, the accession numbers are as follows: for lp17, CP001519; for lp28-2, CP001518; for lp28-4, CP001523; for lp54, CP001524; for cp26, CP001522; for cp32-3, CP001517; for cp32-4, CP001520; for lp32-6, ABJZ02000006-7; for cp32-7, CP001521; and for cp32-12, CP001516.

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REFERENCES

1. Casjens, S., et al. 2000. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. *Mol. Microbiol.* **35**:490–516.
2. Collares-Pereira, M., et al. 2004. First isolation of *Borrelia lusitaniae* from a human patient. *J. Clin. Microbiol.* **42**:1316–1318.
3. de Carvalho, I. L., et al. 2008. Vasculitis-like syndrome associated with *Borrelia lusitaniae* infection. *Clin. Rheumatol.* **27**:1587–1591.
4. Diza, E., et al. 2004. *Borrelia valaisiana* in cerebrospinal fluid. *Emerg. Infect. Dis.* **10**:1692–1693.
5. Fingerle, V., et al. 2007. Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* s.l. from southern Germany with special respect to the new species *Borrelia spielmanii* sp. nov. *Int. J. Med. Microbiol.* **298**:279–290.
6. Fraser, C. M., et al. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* **390**:580–586.
7. Glockner, G., et al. 2004. Comparative analysis of the *Borrelia garinii* genome. *Nucleic Acids Res.* **32**:6038–6046.
8. Glockner, G., et al. 2006. Comparative genome analysis: selection pressure on the *Borrelia vls* cassettes is essential for infectivity. *BMC Genomics* **7**:211.
9. Margos, G., et al. 2009. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. *Appl. Environ. Microbiol.* **75**:5410–5416.
10. Nelson, K. E., et al. 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic Acids Res.* **32**:2386–2395.
11. Piesman, J., and L. Gern. 2004. Lyme borreliosis in Europe and North America. *Parasitology* **129**(Suppl.):S191–S220.
12. Qiu, W. G., et al. 2008. Wide distribution of a high-virulence *Borrelia burgdorferi* clone in Europe and North America. *Emerg. Infect. Dis.* **14**:1097–1104.
13. Rijpkema, S. G., et al. 1997. Detection of *Borrelia afzelii*, *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. *Clin. Microbiol. Infect.* **3**:109–116.
14. Rudenko, N., et al. 2009. Delineation of a new species of the *Borrelia burgdorferi sensu lato* complex, *Borrelia americana* sp. nov. *J. Clin. Microbiol.* **47**:3875–3880.
15. Schutzer, S. E., et al. 2011. Whole genome sequences of thirteen isolates of *Borrelia burgdorferi*. *J. Bacteriol.* **193**:1018–1020.