JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1999, p. 3010–3012 Vol. 37, No. 9
0095-1137/99/$04.00+0
Copyright © 1999, American Society for Microbiology. All Rights Reserved.

Common Ancestry of *Borrelia burgdorferi* Sensu Lato Strains from North America and Europe

D. POSTIC,[1]* N. MARTI RAS,[1] R. S. LANE,[2] P.-F. HUMAIR,[3]†
M. M. WITTENBRINK,[4] AND G. BARANTON[1]

Unité de Bactériologie Moléculaire et Médicale, Institut Pasteur, 75724 Paris Cedex 15, France[1]*; Department of Environmental Science, Policy and Management, Division of Insect Biology, University of California, Berkeley 94720*[2]*; Département de Parasitologie, Institut de Zoologie, Neuchâtel, Switzerland*[3]*; and Institut für Veterinärbakteriologie der Universität Zürich, Zürich, Switzerland*[4]

Received 22 December 1998/Returned for modification 21 April 1999/Accepted 8 June 1999

Ten atypical European *Borrelia burgdorferi* sensu lato (*Borrelia* spp.) strains were genetically characterized, and the diversity was compared to that encountered among related *Borrelia* spp. from North America. Phylogenetic analyses of a limited region of the genome and of the whole genome extend existing knowledge about borrelial diversity reported earlier in Europe and the United States. Our results accord with the evidence that North American and European strains may have a common ancestry.

In Europe, five species of *Borrelia burgdorferi* sensu lato have been delineated so far. Three of them are pathogenic for humans, i.e., *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*, whereas the pathogenicity for humans of the two species *B. valaisiana* and *B. lusitaniae* remains uncertain (7). However, some strains isolated recently from *Ixodes ricinus* ticks in Germany (five strains) (10), Switzerland (four strains), and Russia (one strain) are atypical on the basis of their *rrf-rrl* spacer restriction patterns. Our aim was to further characterize these strains by methods involving either a limited region of the genome, specifically the *rrf-rrl* spacer and the *rrs* gene (4, 5), or the whole genome as determined by pulsed-field gel electrophoresis (PFGE) and arbitrarily primed PCR (AP-PCR) (1, 2). The observed genetic diversity was compared to that described for *Borrelia* spp. from North America (5).

When analyzing the polymorphism of the *rrf-rrl* spacer, we found that all atypical strains exhibited *Mse*I patterns close to and *Dra*I patterns quite distinct from those of B31^T (Table 1). To further evaluate the polymorphism, the spacer regions of the atypical strains and of 10 additional European strains identified earlier as *B. burgdorferi* sensu stricto were sequenced (Table 1). Next, sequences available in databases were compared with the sequences determined in this study. The phylogenetic analyses of *rrf-rrl* spacer sequences conducted by neighbor joining and unpaired group mathematical averaging distance methods yielded similar results (data not shown). They revealed that the strains NE99 and NE271 belonged to *B. burgdorferi* sensu stricto, confirming previous results (3). The remaining European strains constituted separate clusters close to the North American strains CA13 and CA28 (5). Sequences obtained from strains NE581, NE49, and Ir-3519 had 100% identity and only one nucleotide difference with the sequence of the strain Z41493. However, sequences obtained from other German strains exhibited 16 nucleotide differences from the sequence of strain Z41493. The diversity deduced from *rrf-rrl* spacer sequences of *Borrelia* spp. contrasts with the considerable homogeneity of *B. burgdorferi* sensu stricto sequences noted before (5). Since the *rrf-rrl* spacer sequence was hitherto strictly correlated with the *Borrelia* species assignment (4, 5), our results suggest that some atypical European and North American strains might represent a new genomic group.

The *rrs* sequence comparison yielded similarity values ranging from 99.5 to 99.7% for the three atypical European strains NE49, Z41293, and Z41493 and from 98.7 to 99.7% for these three strains and representatives of the three pathogenic species *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* (data not shown). The phylogenetic analysis of the *rrs* gene sequence revealed that each atypical European strain clustered separately within a large cluster comprising *B. burgdorferi* sensu stricto and *B. bissettii*. The resolution power of *rrs* sequences was low when closely related organisms were analyzed (8). At the high level of *rrs* similarity values observed between *Borrelia* spp., DNA relatedness values can either be low or approach 100%. Although our phylogenetic results suggest that European strains constitute at least one new genomic group, these data are too inconclusive to definitively assign them to a separate group at this time.

AP-PCR fingerprints were obtained from six *Borrelia* spp. strains from Europe and 14 *Borrelia* spp. strains from North America (5) using five primers (2). For each strain, 74 polymorphic characteristics yielded a data matrix that was used to draw phylogenetic trees. The results of the analysis by a parsimony method (Fig. 1) coincided with those inferred from distance analysis and confirmed the clustering deduced previously from *rrf-rrl* and *rrs* sequence analyses. Fingerprinting by AP-PCR provides taxonomic information which accords with species assignment based on DNA relatedness (6, 9). In the present study, the paucity of AP-PCR characteristics shared by strains reflects the high level of genetic divergence of *Borrelia* spp. As observed for *Leptospira* strains (6), fingerprints differed markedly between strains from different genomic groups. Except for European strains that shared many arbitrarily primed PCR markers, marked heterogeneity also was observed between strains within each genomic group. However, relationships observed with AP-PCR data are consistent with the sequencing data. Atypical European strains were closely related and constituted a single lineage, which suggests that they be-

* Corresponding author. Mailing address: Unité de Bactériologie Moléculaire et Médicale, Institut Pasteur, 28 Rue du Docteur Roux, 75724 Paris cedex 15, France. Phone: 33 1 45 68 83 37. Fax: 33 1 40 61 30 01. E-mail: dpostic@pasteur.fr.

† Present address: Molecular Parasitology Unit, Queensland Institute of Medical Research, Herston, Queensland 4029, Australia.

TABLE 1. *B. burgdorferi* sensu lato European isolates evaluated in this study

Strain	*rrf-rrl* spacer GenBank accession no.	*rrs* gene GenBank accession no.[c]	Source	Country of origin	*Mse*I patterns (bp)[b]	*Dra*I patterns (bp)[b]
B. burgdorferi sensu stricto						
NE99	AF090974[a]	NA	*I. ricinus*	Switzerland	107, 52, 40, 29, 28	146, 53, 29, 28
NE271	AF090973[a]	NA	*Sciurus vulgaris*	Switzerland	107, 52, 40, 29, 28	146, 53, 29, 28
IP1	AF090977[a]	NA	Human	France	107, 52, 38, 29, 28	144, 53, 29, 28
HII	AF090978[a]	NA	Human	Italy	107, 52, 38, 29, 28	144, 53, 29, 28
L5	AF090979[a]	NA	Human	Austria	107, 52, 38, 29, 28	144, 53, 29, 28
DK7	AF090975[a]	X85195	Human	Denmark	107, 52, 38, 29, 28	144, 53, 29, 28
NE 56	AF090981[a]	NA	*I. ricinus*	Switzerland	107, 52, 38, 29, 28	144, 53, 29, 28
20006	AF090982[a]	NA	*I. ricinus*	France	107, 52, 38, 29, 28	144, 53, 29, 28
Esp1	AF090976[a]	NA	*I. ricinus*	Spain	107, 52, 38, 29, 28	144, 53, 29, 28
35B808	AF090980[a]	NA	*I. ricinus*	Germany	107, 52, 38, 29, 28	144, 53, 29, 28
212	L30121	NA	*I. ricinus*	France	107, 52, 38, 29, 28	144, 53, 29, 28
Z51794	Z77167	NA	*I. ricinus*	Germany	107, 52, 38, 29, 28	144, 53, 29, 28
Z75892	Z77166	NA	*I. ricinus*	Germany	107, 52, 38, 29, 28	144, 53, 29, 28
MIL	AF090971[a]	NA	*I. ricinus*	Slovakia	107, 52, 40, 29, 28	146, 53, 29, 28
Z136	AF090972[a]	NA	*I. ricinus*	Germany	107, 52, 40, 29, 28	146, 53, 29, 28
Borrelia spp.						
NE49	AF090984[a]	AJ224142[a]	*I. ricinus*	Switzerland	107, 53, 38, 29, 26	144, 80, 29
NE581	AF090983[a]	NA	*I. ricinus*	Switzerland	107, 53, 38, 29, 26	144, 80, 29
Ir-3519	AF090985[a]	NA	*I. ricinus*	Russia	107, 53, 38, 29, 26	144, 80, 29
Z41493	Z77172	AF091368[a]	*I. ricinus*	Germany	107, 79, 38, 29	144, 80, 29
Z41293	Z77170	AF091367[a]	*I. ricinus*	Germany	107, 52, 38, 29, 29	144, 82, 29
Z51094	Z77171	NA	*I. ricinus*	Germany	107, 52, 38, 29, 29	144, 82, 29
Z52794	Z77169	NA	*I. ricinus*	Germany	107, 52, 38, 29, 29	144, 82, 29
Z57394	Z77168	NA	*I. ricinus*	Germany	107, 52, 38, 29, 29	144, 82, 29

[a] Sequences determined in this study.
[b] Exact sizes were deduced from *rrf-rrl* spacer sequences.
[c] NA, not available.

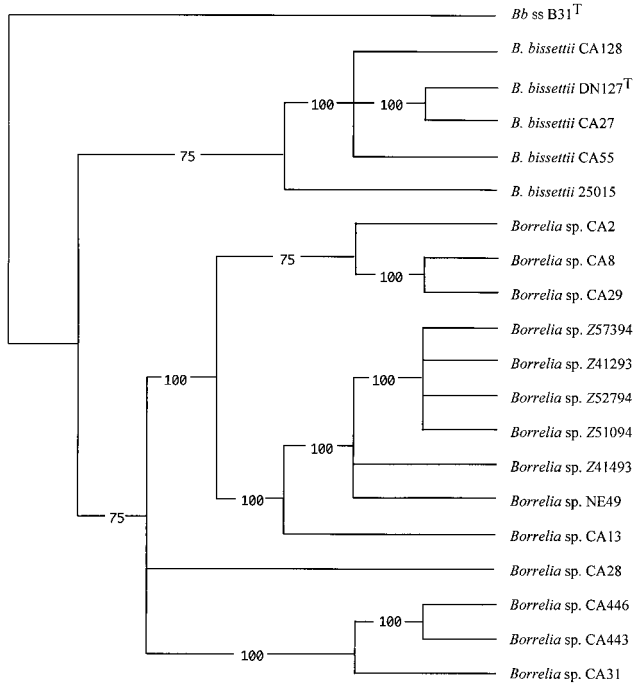

FIG. 1. The 50% majority-rule consensus of 32 trees obtained by maximum parsimony analysis. The tree was generated from the AP-PCR matrix and was solved by the heuristics method contained within the PAUP package.

long to the same genomic group, together with the North American strain CA13. *Borrelia* spp. strains isolated in North America exhibited greater diversity and were scattered on three main branches, whereas *B. bissettii* strains constituted a separate cluster.

PFGE permitted the resolution of *Mlu*I and *Bss*HII macrorestriction fragments of the strains into a large number of distinct types. *Mlu*I restriction profiles of *B. burgdorferi* sensu lato strains obtained with a pulse time ramped from 3 to 28 s for 30 h are shown in Fig. 2. Lambda concatemers (λ 48.5-kb ladder) were used as size markers. Ten *Mlu*I patterns were recorded among 15 atypical strains. Despite a high degree of polymorphism, all *B. bissettii* strains but the divergent strain 25015 shared a common *Mlu*I pattern. Strains CA446 and CA31 likewise shared a similar *Mlu*I pattern, as did the three European strains Z52794, Z41493, and NE49. Similar results were obtained after restriction by *Bss*HII (data not shown), although the polymorphism was even larger, because 12 *Bss*HII patterns were recorded among 15 strains.

AP-PCR and PFGE showed about the same discriminatory power and produced comparable results. The existence of four robust clusters was constant irrespective of the method. One cluster comprised *B. bissettii* strains, the second comprised strains CA29 and CA8, the third comprised strains CA443, CA446, and CA31, and the last one comprised the European strains. As demonstrated recently for *B. bissettii*, each of the other three groups could constitute a new genomic species. Regardless of the method used, a greater diversity was observed among *Borrelia* spp. strains from North America, which suggests a longer evolution time, as compared to European strains. Some European strains genetically resembled Californian strains, however. Taken together, these observations sug-

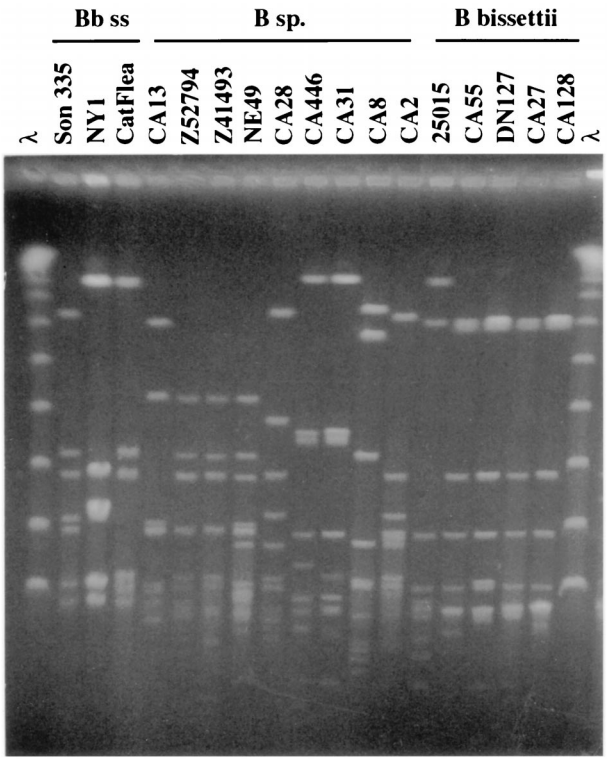

FIG. 2. *Mlu*I restriction profiles of *B. burgdorferi* sensu lato strains obtained with the BioRad apparatus with a pulse time ramped from 3 to 28 s for 30 h.

gest that *Borrelia* spp. analyzed in the present study may share a common ancestry.

We thank T. G. Schwan, R. N. Brown, and E. Korenberg for supplying some of the strains, O. Péter for his comments on strain NE49, L. Gern for critical reading of the manuscript, and E. Bellenger and N. Sertour for technical assistance.

We thank the Pasteur Institute for supporting this work. Part of this study was supported by grant AI22501 from the N.I.H. and C.D.C. Cooperative Agreement U50/CCU906594 to R.S.L. and grant 32-29964-90 from the Swiss National Science Foundation to P.F.H. N.M.R. was supported by the Commission of the European Communities (contract ERBFMBI CT96 0684).

REFERENCES

1. **Belfaiza, J., D. Postic, E. Bellenger, G. Baranton, and I. Saint Girons.** 1993. Genomic fingerprinting of *Borrelia burgdorferi* sensu lato by pulsed-field gel electrophoresis. J. Clin. Microbiol. **31:**2873–2877.
2. **Foretz, M., D. Postic, and G. Baranton.** 1997. Phylogenetic analysis of *Borrelia burgdorferi* sensu stricto by arbitrarily primed PCR and pulsed-field gel electrophoresis. Int. J. Syst. Bacteriol. **47:**11–18.
3. **Humair, P.-F., O. Péter, R. Wallich, and L. Gern.** 1995. Strain variation of Lyme disease spirochetes isolated from *I. ricinus* ticks and rodents collected in two endemic areas in Switzerland. J. Med. Entomol. **32:**433–438.
4. **Postic, D., M. V. Assous, P. A. D. Grimont, and G. Baranton.** 1994. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of *rrf* (5S) *rrl* (23S) intergenic spacer amplicons. Int. J. Syst. Bacteriol. **44:**743–752.
5. **Postic, D., N. M. Ras, R. S. Lane, M. Hendson, and G. Baranton.** 1998. Expanded diversity among Californian *Borrelia* isolates and description of *Borrelia bissettii* sp. nov. (formerly *Borrelia* group DN127). J. Clin. Microbiol. **36:**3497–3504.
6. **Ralph, D., M. McClelland, J. Welsh, G. Baranton, and P. Perolat.** 1993. *Leptospira* species categorized by arbitrarily primed polymerase chain reaction (PCR) and by mapped restriction polymorphisms in PCR-amplified rRNA genes. J. Bacteriol. **175:**973–981.
7. **Rijpkema, S. G. T., D. J. Tazelaar, M. J. C. H. Molkenboer, G. T. Noordhoek, G. Plantinga, L. M. Schouls, and J. F. P. Schellekens.** 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.
8. **Stackebrandt, E., and B. M. Goebel.** 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. **44:**846–849.
9. **Welsh, J., C. Pretzman, D. Postic, I. Saint Girons, G. Baranton, and M. McClelland.** 1992. Genomic fingerprinting by arbitrarily primed polymerase chain reaction resolves *Borrelia burgdorferi* into three distinct phyletic groups. Int. J. Syst. Bacteriol. **42:**370–377.
10. **Wittenbrink, M. M., C. Reuter, M. L. Manz, and H. Krauss.** 1996. Primary culture of *Borrelia burgdorferi* from *Ixodes ricinus* ticks. Zentbl. Bakteriol. **285:**20–28.