DNA and Protein Analyses of Tick-Derived Isolates of *Borrelia burgdorferi* from California

RANCE B. LEFEBVRE,^{1*} ROBERT S. LANE,² GUEY-CHUEN PERNG,¹ JULIA A. BROWN,¹
AND RUSSELL C. JOHNSON³

Department of Veterinary Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California 95616¹; Department of Entomological Sciences, University of California, Berkeley, California 94720²; and Department of Microbiology, School of Medicine, University of Minnesota, Minneapolis, Minnesota 55455³

Received 17 August 1989/Accepted 4 December 1989

Nine isolates of *Borrelia burgdorferi* from ixodid ticks collected in northern California were characterized. Restriction endonuclease analysis, pulsed-field gel electrophoresis, and Western blot (immunoblot) analysis were used in this study. Four isolates were very similar to each other. The others shared some similarities but were classified as having unique genotypes. A strain from an *Ixodes neotomae* tick displayed the greatest genetic and antigenic diversity when compared to the isolates collected from *Ixodes pacificus* ticks. A computerized library based on DNA banding patterns of the isolates by restriction enzyme analysis is also reported. This library was created by using a scanning laser densitometer.

Borrelia burgdorferi, a cosmopolitan tick-borne spirochete, is the etiologic agent of Lyme disease (7, 16). Isolates of *B. burgdorferi* are usually classified and characterized by immunoassays, the most common of which is the reaction of monoclonal antibodies to several shared antigens on the outer surface membrane (4, 6, 34, 35). However, an analysis of the genetic diversity within *B. burgdorferi* may be a more reliable method for classifying and characterizing this spirochete. A genetic analysis may also provide important information regarding the molecular biology of the organism and the protean clinical manifestations reported in North America and Europe (31). One reported method of classifying and comparing these organisms genetically is to characterize their plasmid DNA (3). Another reported method of classifying isolates of *B. burgdorferi* is restriction endonuclease analysis (REA) (20, 21). This technique was used to examine *B. burgdorferi* isolates from North America and Europe (21). Two important findings were reported. First, genetic diversity exists between most isolates of these organisms. Second, there is a general tendency for both North American and European isolates to be more closely related to isolates from the same continent.

Here we report the results of a further investigation of the genetic characterization of *B. burgdorferi* isolates in relation to their geographical distribution. These isolates, which were cultured from ixodid ticks collected from several counties of northern California, have been characterized by immunological and immunochemical techniques (18). In this study, the isolates were characterized by REA, by pulsed-field gel electrophoresis (PFGE) (28), by Southern blot analysis (21, 22, 30), by scanning laser densitometry (SLD), and by Western blot (immunoblot) analysis (32). In addition, a computer library was created from the scanning laser densitometry data for future reference in describing other isolates.

The results suggested that several of the isolates were very similar in their genetic and protein compositions. However, there were some organisms that varied significantly from the others even though they were isolated from ticks collected in the same county.

MATERIALS AND METHODS

Source of *B. burgdorferi* isolates. All California isolates described in this study were supplied by R. S. Lane. The B31 reference strain was supplied by R. C. Johnson. See Table 1 for details. Isolates were maintained in modified Barbour-Stoenner-Kelly medium (2) at 33°C. The cells were passaged every 4 to 6 days during the logarithmic phase of growth. All but one of the isolates (CA7) were isolated from Marin, Sonoma, and Mendocino, three contiguous coastal counties north of San Francisco. Isolate CA7 was isolated from Yuba County, which is located in the north central part of the Sacramento Valley (Fig. 1).

DNA preparation for REA. Whole-cell DNA was isolated by the method of LeFebvre et al. (19). The following modification was made: 200 µl of cold (4°C) 3 M potassium acetate was added to precipitate proteins instead of 20 µl of 3 M potassium-5 M acetate solution. REA was performed as previously described (21, 22). Enzymes used in this study consisted of *Bam*HI, *Eco*RI, *Sma*I, *Hind*III, and *Hha*I (Bethesda Research Laboratories, Gaithersburg, Md.) and were used according to the manufacturer's specifications. The restriction fragments were separated by agarose gel electrophoresis in a 0.7% agarose gel (20 by 25 cm) at 60 V for 15.5 h. After electrophoresis, the gel was stained with ethidium bromide, illuminated by UV irradiation, and photographed.

Scanning laser densitometry. Photographs and autoradiographs of digested DNA were scanned by using a laser densitometer (Biomed Instruments, Fullerton, Calif.). Biomed one-dimensional and utilities superimposition computer programs were used to analyze the data. These are programs that permit the manipulation of scanned lanes for comparison purposes. The sensitivity was set at 1, and the scan speed was set at 100.

PFGE and hybridization. Samples for PFGE were prepared by the encapsulation method of Overhauser and Radic (25). Molecular weight markers consisted of lambda phage DNA (33). Approximately 3 µg of DNA was added to each well of a 1% agarose gel. The electrophoresis chamber was a Pharmacia LKB pulsaphor system (Pharmacia, Uppsala, Sweden). The electrodes were set for south and east at 30°, 120°, and 210°. The electrodes for north and west were set at

* Corresponding author.

TABLE 1. Geographical distribution and classification of strains

Isolate	Source	Location	Passage no.
B31 reference strain ATCC 35210	<i>Ixodes dammini</i>	New York	
CA2	<i>I. neotomae</i>	Mendocino	7
CA3	<i>I. pacificus</i>	Marin	7
CA4	<i>I. pacificus</i>	Sonoma	5
CA5	<i>I. pacificus</i>	Sonoma	5
CA6	<i>I. pacificus</i>	Sonoma	7
CA7	<i>I. pacificus</i>	Yuba	8
CA8	<i>I. pacificus</i>	Sonoma	8
CA9	<i>I. pacificus</i>	Marin	3

60°. The gels were run for 24 h at a constant voltage of 330 V. The gels were stained, illuminated, and photographed as described above. The gel was exposed to UV irradiation for 4 min to ensure breakage of the large-molecular-weight DNA for efficient transfer to nylon membranes. The DNA was transferred to a nylon membrane by the method of Southern (30). Whole-cell DNA of *B. burgdorferi* B31 was radiolabeled (9) and used as the probe for the blot. The hybridization and subsequent washes were carried out under stringent conditions (24). The hybridization was carried at 37°C with 50% formamide in the hybridization solution. The membrane was washed in 2× SSC (diluted from a 20× stock containing 175.3 g of NaCl and 88.2 g of sodium citrate in 800 ml of water [pH 7.0]; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.5% sodium dodecyl sulfate (SDS). This solution was incubated at room temperature for 5 to 10 min. The membrane was then placed in a solution of 2× SSC and 0.1% SDS and incubated for 15 min at room temperature. The final wash consisted of 0.1× SSC and 0.5% SDS at 68°C for 2 h. The buffer was changed, and the wash was continued for another 30 min.

Polyclonal antiserum production. Reference strain B31 was used for the production of antibodies directed against antigens of *B. burgdorferi*. Approximately 10⁸ B31 cells were washed in phosphate-buffered saline (pH 8.0)–5 mM MgCl₂.

FIG. 1. California counties in which organisms were isolated.

The cells were suspended in a 0.3% Formalin solution containing 0.85% NaCl. The cells were inoculated intradermally into an adult male New Zealand White rabbit. The rabbit was boosted weekly for 5 weeks with the same number of Formalin-treated cells administered intravenously.

SDS-polyacrylamide gel electrophoresis and Western blot analysis. Proteins from whole cell lysates were characterized by separation in 10 to 15% polyacrylamide gradient gels by discontinuous SDS-polyacrylamide gel electrophoresis as described by Laemmli (17). Approximately 20 µg of whole-cell lysates of each isolate was added to the gel. The gels were run at 60 mA (constant current) for 6.5 h to 7 h. Antigens reacting with the rabbit anti-B31 polyclonal antiserum were identified by transfer of electrophoresed proteins to nitrocellulose membranes with the Pharmacia Transphor system according to the manufacturer's specifications. The membranes were blocked and probed by standard procedures (8). Rabbit antiserum raised against *B. burgdorferi* (titer of 1:10,000, determined by enzyme-linked immunosorbent assay) was used to probe the blots at a dilution of 1:5,000. The rest of the assay was performed as previously described (32).

RESULTS

REA. The genetic characterization of the nine California *B. burgdorferi* isolates was performed using the five restriction enzymes mentioned above. Figure 2A demonstrates the banding patterns of these isolates digested with *EcoRI* and electrophoresed. The banding patterns of CA4, CA9, CA6, and CA5 (lanes 2 through 5, respectively) were very similar at all molecular weight ranges. Three of these (CA4, CA5, CA6) originated in Sonoma County, and one (CA9) originated in nearby Marin County. Isolates CA3, CA8, and CA7 (lanes 7 through 9, respectively) exhibited similarities in their banding patterns. More pronounced differences were observed when the DNA was digested with other restriction enzymes (Fig. 2B through D). Lanes 6 represent the banding patterns of an isolate from Mendocino County (CA2). This organism demonstrated the greatest divergence from the other isolates in banding patterns, regardless of the restriction endonuclease used. This isolate also was the only one isolated from *Ixodes neotomae* in this study. All other isolates were isolated from *Ixodes pacificus*, which is thought to be the primary vector of *B. burgdorferi* in the far western states, particularly in California and Oregon.

Figure 2B represents a *HindIII* digest of DNA isolated from these organisms and loaded in the same sequence as in Fig. 2A. Figures 2C and D represent *SmaI* and *BamHI* digests of the DNA from these isolates, respectively. In the *BamHI* digest, CA3 was loaded in the last lane (lane 9). The other isolates were loaded in the same order as above. Isolates CA4, CA5, CA6, and CA9 were judged to be genotypically similar as determined by each of the restriction enzyme digests. Isolates CA7 and CA3 appeared to share similar banding patterns; however, noticeable differences were consistently detected in the higher-molecular-size ranges (5 to 20 kilobase pairs [kbp]) in the *BamHI*, *SmaI*, and *HindIII* digests of their DNA. Isolates CA2 and CA8 appeared to be distinct from the other isolates.

Scanning laser densitometry. The results of the laser densitometry tracings of photographs of the REA gels substantiated the above observations. A scan of a *HindIII* digest of CA4, CA5, CA6, and CA9 shows the similarity of their banding patterns (Fig. 3A). Scans of the same isolates

FIG. 2. Photographs of ethidium bromide-stained gels representing *Eco*RI digests (A), *Hind*III digests (B), *Sma*I digests (C), and *Bam*HI digests (D) of the *B. burgdorferi* isolates.

FIG. 3. Scanning laser densitometry of *Hind*III digests of isolates C4, C5, C6, and C9 (A) and of isolate C5 superimposed onto C6 (C5 + C6) and C6 superimposed onto C2 (C6 + C2) (B).

digested with enzymes other than *Hind*III also yielded nearly identical patterns (data not shown).

Figure 3B depicts a scan of an *Hind*III digest of CA5 superimposed onto a scan of CA6; as stated above, these isolates were very similar in their overall patterns (bottom scan). Figure 3B also illustrates a scan of *Hind*III-digested CA6 superimposed onto a scan of CA2 (top scan). This clearly demonstrated the differences in the banding patterns of the CA6 and CA2 genomes. The densitometry tracings of these gels corroborated the visual results of Fig. 2. The scans

of the REA patterns were classified into sublibraries as future references in comparing other *Borrelia* isolates.

PFGE. Figure 4A is a photograph of whole-cell DNA from the isolates fractionated by PFGE. Some of the lanes were underloaded, but it is still apparent that the isolates differ in their linear plasmid content. The smallest lambda DNA band represents approximately 50 kbp and increases by 50-kbp increments. The three DNA bands of B31 reference strain between the approximate sizes of 16 and 70 kbp were linear plasmid molecules, as determined by running several pulsed-

FIG. 4. (A) PFGE of whole-cell DNA from the isolates: c.c., circular chromosome; l.c., linearized chromosome; l.p., linear plasmid. (B) Autoradiograph of the pulsed-field gel in panel A containing whole-cell DNA from the *B. burgdorferi* isolates and reference strain B31. Whole-cell DNA from B31 was radiolabeled and used as the probe. The arrows indicate the locations of the linear plasmids of the B31 genome: 1, approximately 16 kbp; 2, 48 kbp; 3, 65 to 75 kbp; 4, 140 kbp.

field gels with different switching and voltage parameters (5, 13, 23; unpublished results). The approximate sizes of these linear DNA molecules were 16, 48, and 65 to 75 kbp.

Isolates C4, C5, and C7 contained three visible linear plasmids of the same molecular weight. C6 was underloaded but revealed the same plasmid pattern as the other three by hybridization with B31 DNA and autoradiography (Fig. 4B). The DNA in the gel was transferred to a nylon membrane and probed as described below.

Figure 4B demonstrates an autoradiograph of a pulsed-field gel probed with radiolabeled genomic DNA from reference strain B31. The lambda molecular weight marker DNA was radiolabeled before the gel was run. The band at approximately 140 kbp also appeared to be a linear molecule or a relaxed circular plasmid molecule (13, 14, 29), based on the same criteria. The band at approximately 250 kbp appeared to be degraded, broken, or linearized DNA from the circular chromosomal DNA (11). The 250 kbp is probably an underestimate of the actual size due to the fact that the gels were run to provide the best resolution of the linear plasmids. Other gels run with higher voltages and longer switching times revealed this band to be much larger (unpublished results). The DNA at the top of the figure represents intact chromosomal DNA and possibly some larger circular plasmid DNA that did not enter the gel. It has been reported that *B. duttonii* and *B. burgdorferi* contain linear chromosomes rather than classical circular chromosomes (10, 12). We refer to the high-molecular-size DNA (>250 kbp) as linearized chromosomal DNA and the DNA remaining in the loading well as intact circular chromosomal DNA. This is based on work describing a circular chromosome in these *Borrelia* species rather than linear DNA molecules (G. C. Perng, R. B. LeFebvre, and R. C. Johnson, submitted for publication).

Isolates CA6, CA4, CA5, CA7, CA9, and CA3 demonstrated hybridization patterns that were similar to those of each other and to that of the reference strain. Isolate CA8 did not hybridize to the smallest linear plasmid of B31 (approximately 16 to 20 kbp). Isolate CA2 DNA did not hybridize to the 48-kbp plasmid of B31.

Other differences noted in the ethidium bromide-stained gel (Fig. 4B) before blotting included the apparent absence of the 16- to 20-kbp plasmid in CA8 and the presence of three other bands between 48 and 60 kbp that were not hybridized by B31 DNA. Additionally, CA7 appeared to contain a plasmid banding at approximately 47 kbp that did not hybridize to the B31 reference strain (unpublished results).

Protein and antigen analyses. Figure 5A represents a stained acrylamide gel of the California isolates and the B31 reference strain. Overall, the protein compositions of these organisms were similar, but some distinctive differences were noted consistently. For example, CA8 and CA3 were missing a major band at approximately 34K. Isolate CA2, isolated from the *I. neotomae* tick, had major proteins of slightly higher molecular masses in the 30- to 36-kilodalton range. The same isolate demonstrated compression of a doublet band at approximately 50 kilodaltons and had only a single band, whereas all of the other isolates showed two bands at approximately 55 kilodaltons. There also were subtle differences exhibited by CA8, CA7, CA2, and CA9 at the lower-molecular-mass ranges (15 to 25 kilodaltons). CA5 demonstrated two strongly staining proteins of high molecular weight that were not observed in the other isolates.

Figure 5B is an immunoblot of antigens from the California isolates reacted with polyclonal rabbit antiserum raised against whole cells of the B31 reference strain. There appeared to be a high degree of similarity in antigen reaction among isolates; the only minor variations observed were

FIG. 5. (A) SDS-polyacrylamide gel electrophoresis of whole-cell proteins from the *B. burgdorferi* isolates and the B31 reference strain. The gel was stained with Coomassie blue. (B) Western blot of the *B. burgdorferi* isolates and the B31 reference strain probed with rabbit polyclonal antibody raised against whole cells of reference strain B31.

primarily associated with CA2. CA8 contained a distinct antigen of approximately 90 to 95 kilodaltons that reacted with the antiserum.

DISCUSSION

The *B. burgdorferi* isolates characterized genetically and, in part, antigenically in this report were isolated from *Ixodes* ticks collected in several counties of northern California. Previously, these isolates were identified and characterized antigenically as *B. burgdorferi* (18).

The restriction enzyme analysis revealed close similarities between three isolates from Sonoma County (CA4, CA5, CA6) and one isolate from adjacent Marin County (CA9). The remaining isolate from Marin County (CA3), the isolate from Yuba County (CA7), and the fourth isolate from Sonoma County (CA8) exhibited some similarities in their restriction fragment distributions, although they were classified as distinct genotypes on the basis of their collective banding patterns. The isolate from Mendocino County (CA2) was readily distinguishable from the other isolates. It was of interest to note that CA2 was isolated from *I. neotomae*, whereas the other isolates were isolated from *I. pacificus*. *B. burgdorferi* may have adaptive mechanisms for survival in various vectors which may include such modifications as

methylation of different base pairs. It also is possible that the variation observed in CA2 represents yet another significantly divergent genetic variant of *B. burgdorferi*, as has been reported previously for other isolates (1, 21).

A consideration that cannot be overlooked when characterizing *Borrelia* isolates either by DNA or antigen analysis is the possibility that the cultures are composed of more than one genotype or antigen type. However, previous work describing the genetic characterization of a related spirochete, *Leptospira interrogans*, demonstrated highly conserved and reproducible genotypes of individual cultures regardless of passage number (19, 22). Preliminary work involving deliberately mixed cultures of *B. burgdorferi* demonstrated that over a period of time a single genotype identical to that of one of the starting cultures ultimately predominated (unpublished results). Thus, although it is possible that more than one genotype of *B. burgdorferi* could be cultured, it is unlikely that the proportionate numbers of the different genotypes would remain constant over extended periods. Therefore, it would be expected that if more than one genotype were represented in a culture that fluctuations or variations in REA would be observed over time. The cultures studied in this report were assayed several times by REA, and no appreciable changes were detected in their genotype. Using PFGE, we detected the loss of one or more linear plasmids over prolonged growth in vitro. The loss of this plasmid did not change the REA pattern when compared with that from an earlier passage of the same strain still containing the plasmid (manuscript in preparation). Finally, the fact that isolates CA4-6 and CA9 were virtually identical genetically, both chromosomally and in linear plasmid content, indicated that it is possible to culture single genotypes from infected ticks. As a final analytical step, we cloned several *B. burgdorferi* isolates, and we are in the process of determining whether the cultured populations are homogeneous at the genetic level.

The restriction fragment banding patterns discussed herein demonstrated the usefulness of this technique in accurately classifying *B. burgdorferi* isolates. The results of the scanning laser densitometry provided a means for accurately analyzing genotypes of *B. burgdorferi* isolates. Figure 3 demonstrated the ease with which a large number of restriction fragments representing the genome from a single isolate was condensed into graphs. These graphs, in turn, were readily compared and contrasted with the banding patterns of genomic DNA from the other *B. burgdorferi* isolates after digestion with the same enzyme. The high resolution of the laser scanner and the capacity of the computer to sort and store libraries of scanned genomes permits isolates of *B. burgdorferi* from North America and elsewhere to be classified and catalogued as to genotype. Such a library of *B. burgdorferi* will provide important information regarding the degree of genetic variation within the species. This information may corroborate antigen expression and facilitate attempts to develop a reliable vaccine and diagnostic reagents. Such studies also may reveal whether there is an association between the geographical distribution of *B. burgdorferi* genotypes and the clinical manifestations of Lyme borreliosis.

The PFGE of the California *B. burgdorferi* isolates and the B31 reference isolate permitted the analysis of undigested DNA in its native state. The results of the PFGE and hybridization with B31 DNA demonstrated homologous sequences in both the chromosome and the plasmids of the nine isolates and the B31 reference strain from New York. Some differences were noted in the linear plasmid composi-

tion in the range of 16 to 65 kbp. These distinctions may be significant since it has been reported that a 49-kbp linear plasmid codes for major outer surface antigens in *B. burgdorferi* (3, 15, 27). Thus, the variations in the linear plasmid composition within these isolates may reflect the capacity of these isolates to synthesize different surface antigens.

The whole-cell protein analysis of the California isolates corroborated the results of the monoclonal antibody analysis performed by Lane and Pascocello (18). Polyacrylamide gels stained with Coomassie blue demonstrated that all nine isolates were similar in their overall protein composition. However, only CA4, CA5, CA6, and CA9 appeared to be identical in their protein profiles. As stated above, these were the four isolates that appeared to have identical genomes. The other isolates displayed minor but distinct protein differences that corroborated their distinct genotypes. However, the protein distinctions may be temporary, since *B. burgdorferi* isolates have been shown to vary their expressed antigens after prolonged passage in BSKII medium (6, 26).

The immunoblot analyses of the *B. burgdorferi* isolates using rabbit polyclonal antibodies likewise revealed that considerable cross-reacting antigens were present. Thus, it appears that these California isolates, like those characterized previously (6), are relatively homogeneous in their antigen profile. These results further substantiate the relative homogeneity of antigen presentation in North American isolates in comparison to European isolates (4, 34, 35).

B. burgdorferi isolates collected from California appear to be similar antigenically and genetically, based on the results of this and previous studies (6, 18). However, sufficient diversity has been detected within this species from this state alone to suggest that the protean clinical manifestations of Lyme borreliosis may be dependent, in part, on the genotype of the infecting organism. Should this be the case, further analysis of *B. burgdorferi* from different geographical regions may reveal whether particular genotypes are more commonly associated with certain clinical symptoms (e.g., neurological) than others.

ACKNOWLEDGMENTS

We thank Jane Shapiro and Judith A. Pascocello for their expert technical assistance in this study.

This research was supported in part by Public Health Service grant AI22501 from the National Institutes of Health to Robert S. Lane, by Public Health Service grant AR 34744 from the National Institutes of Health to Russell C. Johnson, and by a grant from the School of Veterinary Medicine, U.C. Davis Companion Animal Laboratory Funds, to Rance B. LeFebvre.

LITERATURE CITED

- Anderson, J. F., L. Magnarelli, R. B. LeFebvre, T. G. Andreadis, J. B. McAninch, G. C. Perng, and R. C. Johnson. 1989. Antigenically variable *Borrelia burgdorferi* isolated from cotton-tail rabbits and *Ixodes dentatus* in rural and urban areas. *J. Clin. Microbiol.* 27:13-20.
- Barbour, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.* 57:521-525.
- Barbour, A. G. 1988. Plasmid analysis of *Borrelia burgdorferi*, the Lyme disease agent. 1988. *J. Clin. Microbiol.* 26:475-478.
- Barbour, A. G., R. H. Hieland, and T. R. Howe. 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. *J. Infect. Dis.* 152:478-484.
- Beverly, S. M. 1988. Characterization of the 'unusual' mobility of large circular DNAs in pulsed field-gradient electrophoresis. *Nucleic Acids Res.* 16:925-939.
- Bissett, M. L., and W. Hill. 1987. Characterization of *Borrelia burgdorferi* strains isolated from *Ixodes pacificus* ticks in California. *J. Clin. Microbiol.* 25:2296-2301.
- Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis. 1983. Lyme disease: a tickborne spirochetosis? *Science* 216:1317-1319.
- Burnette, W. N. 1981. Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulfate polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195-203.
- Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
- Ferdows, M. S., and A. G. Barbour. 1989. Megabase-sized linear DNA in the bacterium *Borrelia burgdorferi*, the Lyme disease agent. *Proc. Natl. Acad. Sci. USA* 86:5969-5973.
- Frutos, R., M. Pages, M. Bellis, G. Roizes, and M. Bergoin. 1989. Pulsed-field gel electrophoresis determination of the genome size of obligate intracellular bacteria belonging to the genera *Chlamydia*, *Rickettsiella*, and *Porochlamydia*. *J. Bacteriol.* 171:4511-4513.
- Hayes, L. J., D. J. M. Wright, and L. C. Archard. 1988. Segmented arrangement of *Borrelia duttonii* DNA and location of variant surface antigens. *J. Gen. Microbiol.* 134:1785-1793.
- Hightower, R. C., D. W. Metge, and D. V. Santi. 1987. Plasmid migration using orthogonal-field-alternation gel electrophoresis. *Nucleic Acids Res.* 15:8387-8398.
- Hightower, R. C., and D. V. Santi. 1989. Migration properties of circular DNA using orthogonal-field-alternation gel electrophoresis. *Electrophoresis* 10:283-290.
- Howe, T. R., L. W. Mayer, and A. G. Barbour. 1985. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. *Science* 227:645-646.
- Johnson, R. C., F. W. Hyde, G. P. Schmid, and D. J. Brenner. 1984. *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease—a tickborne spirochetosis? *Int. J. Syst. Bacteriol.* 34:496-497.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- Lane, R. S., and J. A. Pascocello. 1989. Antigenic characteristics of *Borrelia burgdorferi* isolates from ixodid ticks in California. *J. Clin. Microbiol.* 27:2344-2349.
- LeFebvre, R. B., J. W. Foley, and A. B. Thiermann. 1985. Rapid and simplified protocol for the isolation and characterization of leptospiral chromosomal DNA for taxonomy and diagnosis. *J. Clin. Microbiol.* 22:606-608.
- LeFebvre, R. B., and G. C. Perng. 1989. Genetic and antigenic characterization of *Borrelia coriaceae*, putative agent of epizootic bovine abortion. *J. Clin. Microbiol.* 27:389-393.
- LeFebvre, R. B., G. C. Perng, and R. C. Johnson. 1989. Characterization of *Borrelia burgdorferi* isolates by restriction endonuclease analysis and DNA hybridization. *J. Clin. Microbiol.* 27:636-639.
- LeFebvre, R. B., and A. B. Thiermann. 1986. DNA homology studies of serogroups Sejroe and Pomona from cattle and swine. *Am. J. Vet. Res.* 47:959-963.
- Levene, S. D., and B. H. Zimm. 1987. Separation of open-circular DNA using pulsed-field electrophoresis. *Proc. Natl. Acad. Sci. USA* 84:4054-4057.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Overhauser, J., and M. Z. Radic. 1987. Encapsulation of cells in agarose beads for use with pulsed field gel electrophoresis. *BRL Focus* 9:8-9.
- Schwan, T. G., and W. Burgdorfer. 1987. Antigenic changes of *Borrelia burgdorferi* as a result of *in vitro* cultivation. *J. Infect. Dis.* 156:852-853.
- Schwan, T. G., W. Burgdorfer, and C. F. Garon. 1988. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of *in vitro* cultivation. *Infect.*

- Immun. 56:1831-1836.
28. Schwartz, D. C., and C. R. Cantor. 1984. Separation of yeast chromosome-sized DNA by pulsed field gradient gel electrophoresis. *J. Mol. Biol.* 98:503-518.
29. Simske, J. S., and S. Scherer. 1989. Pulsed-field gel electrophoresis of circular DNA. *Nucleic Acids Res.* 17:4359-4365.
30. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 47:61-66.
31. Steere, A. C., S. E. Malawista, N. H. Bartenhagen, P. N. Spieler, J. H. Newman, D. W. Rahn, G. T. J. Hutchinson, J. Green, D. R. Snyderman, and E. Taylor. 1984. The clinical spectrum and treatment of Lyme disease. *Yale J. Biol. Med.* 57:453-461.
32. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76:4350-4354.
33. Waterbury, P. G., and M. J. Lane. 1987. Generation of lambda phage concatemers for use as pulsed field electrophoresis size markers. *Nucleic Acids Res.* 15:3930.
34. Wilske, B., V. Preac-Mursic, S. F. Hayes, J. L. Benach, and K. V. Busch. 1986. Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. *Zentralbl. Bakteriologie. Hyg. A* 263:92-102.
35. Wilske, B., V. Preac-Mursic, G. Schierz, R. Kuhbeck, A. G. Barbour, and M. Kramer. 1988. Antigenic variability of *Borrelia burgdorferi*. Lyme disease and related disorders. *Ann. N.Y. Acad. Sci.* 539:126-142.