

Analysis of the *bmp* Gene Family in *Borrelia burgdorferi* Sensu Lato

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BmpA, BmpB, BmpC, and BmpD are homologous *Borrelia burgdorferi* lipoproteins of unknown functions, encoded by the *bmp* genes of paralogous chromosomal gene family 36. At least some of the Bmp proteins are immunogens in infected vertebrate hosts. The genetic organization of the *bmp* region has been characterized for a variety of *B. burgdorferi* sensu lato strains by Southern hybridization, PCR amplification, and DNA sequencing. All four *bmp* genes were present in the same relative order in all *B. burgdorferi* sensu lato low- and high-passage-number isolates. While there were no differences in the relative orders of the *bmp* genes in these species, variations in DNA sequence in the *bmpD*-*bmpC* and *bmpC*-*bmpA* intergenic regions were significantly more common than in the corresponding 3' *bmpD* and *bmpC* coding regions. The genetic structure of the chromosomal region containing the *bmp* genes thus appears to be well conserved across different species of *B. burgdorferi*, but variations in DNA fine structure that prevent PCR primer annealing may occur in this region and make Southern hybridization much more reliable than PCR for detection of the presence of these genes. Our results also suggest that *bmp* gene products may be used as reagents in the preparation of vaccines and diagnostic assays to protect against and diagnose Lyme disease produced by *B. burgdorferi* sensu lato.

DNA sequencing of *Borrelia burgdorferi* B31 has identified open reading frames that encode at least 105 lipoproteins belonging to several redundant gene families (6). Paralogous plasmid and chromosomal genes encoding lipoproteins are, in general, characteristic of the *B. burgdorferi* genome (6) and include *bmp* (1, 17, 19), *erp* (21), the 2.9 gene family (16), and *vls*, the *B. hermsii* *vmp* homologue (24). While DNA of any of these gene families could be a substrate for stochastic genetic rearrangement to yield variation in gene expression and/or antigenic variation (14), this phenomenon has not yet been demonstrated to occur in *B. burgdorferi* infections.

In *B. burgdorferi* B31, *bmp* genes (paralogous gene family 36) are located in tandem in the chromosome in the order *bmpD bmpC bmpA bmpB* in a region extending from nucleotides 391932 to 396563 (6). They are also present in the chromosomes of other *B. burgdorferi* strains (1, 17, 19). In *B. burgdorferi* B31, DNA sequence homologies among *bmp* genes range from 56 to 64%. DNA sequence analysis has suggested that *bmpC* is preceded by two promoters (1), that *bmpD* and *bmpA* are preceded by individual promoters (17, 19), and that *bmpB* is preceded by no promoter (19). The putative *bmpA* promoter is located within the *bmpC* coding sequence (1, 19). Although the functions of the proteins encoded by the *bmp* genes are unknown, *Borrelia* organisms in culture synthesize mRNAs of all four *bmp* genes (17; E. Dobrikova, V. Gorbacheva, and F. C. Cabello, unpublished data) and antibodies to BmpA, BmpC, and BmpD proteins are present in infected hosts (1, 2, 17, 19). These data suggest that the functions of these proteins may be necessary for in vitro and in vivo growth and that at least three members of this family may have a role in virulence (4).

Very few genes of *B. burgdorferi* that are involved in virulence have been identified as a result of obtaining the complete sequence of this organism (6). Analysis of a *B. burgdorferi*

chromosomal region whose genes code for exposed, putatively in vivo-induced and clearly immunogenic lipoproteins may therefore be relevant to *Borrelia* virulence. The presence of Bmp proteins on the surface of *B. burgdorferi*, the tandem arrangement of their genes in the chromosome of *Borrelia*, and their overlapping transcriptional signals suggest that these proteins may be virulence related and that the expression of their genes may be coregulated (1, 17, 19).

It is not known whether *bmp* genes are present in the genomes of all isolates of *B. burgdorferi* sensu lato, but at least *bmpC* and *bmpA* have been identified in *B. garinii* and *B. afzelii* (1, 17, 19). To provide a basis for understanding the role that chromosomally encoded Bmp proteins might play in the biology of *B. burgdorferi* and in the pathogenesis of Lyme disease, and to evaluate the usefulness of Bmp proteins as reagents for diagnosis of Lyme disease produced by different *Borrelia* strains (1, 18), the structures of the *bmp* regions in several *Borrelia* species were analyzed using DNA hybridization, PCR amplification, and DNA sequencing. There were no differences in the relative order of the *bmp* genes in these species, but variations in DNA sequence were significantly more common in intergenic regions than in coding regions.

Bacterial strains and culture. *B. burgdorferi* B31 (ATCC 35210) and 297 (20); 10 *B. burgdorferi* sensu stricto strains recently isolated from skin biopsies and blood samples from patients with Lyme disease and passaged only once (10); *B. garinii* G25 and N34 (from R. Marconi); *B. afzelii* Ip3 (9), ACA1 (3), VS461 (9), and VS486 (from J. Benach); *B. bissettii* 25015 (formerly *B. burgdorferi* sensu lato group DN127) (22); *B. andersonii* 21038 (from R. Marconi); *B. japonica* H014 (13); and *B. hermsii* (from R. Johnson) were grown at 32 to 34°C in BSK-H medium supplemented with 7% rabbit serum (Sigma Chemical Co., St. Louis, Mo.) (7). Strains were cloned by two rounds of limiting dilution in BSK-H medium or by subsurface agarose colony isolation (5). Cell concentration was determined by counting cells stained with acridine orange under fluorescence microscopy (23) or by counting viable cells on agarose plates (5). Comparable results were obtained by both techniques.

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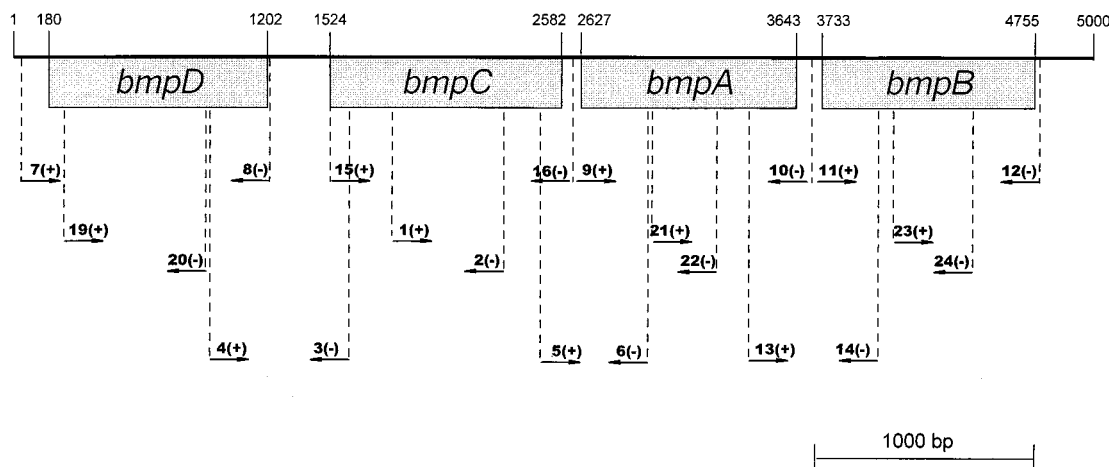


FIG. 1. Schematic representation of the PCR primer binding sites in the *bmp* chromosomal region. The sequence of this region was created on the basis of sequences of *bmpD* from *B. burgdorferi* JD1 (accession no. U35450) (17) and *bmpC*, *-A*, and *-B* genes from *B. burgdorferi* 297 (accession no. U49938) (2) using the program Primer 3 Output (Center for Genome Research). Position 1 corresponds to nucleotide 396706, and position 5000 corresponds to nucleotide 391707 on the *B. burgdorferi* B31 chromosomal map (6). The relative locations of primer binding sites are indicated by arrows. W primer pairs are for amplification of the entire indicated coding sequence, and P primer pairs are for amplification of partial regions of the indicated coding sequence. All primers with the suffix (+) are plus-strand primers, and those with the suffix (-) are minus-strand primers (i.e., reverse complement of the gene sequences). Primer sequences (5'→3') for the indicated regions were as follows. *bmpD*: 7(+), GAATGGCTGAAGCAAATAAAGC(W); 8(-), CAAATCAGCTCAATAAAAATC (W); 19(+), CTGATGATGGCAAGTCGGAG(P); 20(-), ACG CCTATACCAGAAAGCCC (P). *bmpC*: 15(+), GGCAAGGGCATATGTTTAAAGATTTATTTTATTA (W); 16(-), CGCAGATCTCCCCTTTACAAACAA AGC (W); 1(+), GATGAGGCAATGACTGAGGA (P); 2(-), GCAGCGTCATAAACTCCAAGACC (P). *bmpA*: 9(+), TGTAAGGGGAAATAGTTTATG (W); 10(-), TTCAAACAAAACCAATGTG (W); 21(+), CCAAGTTGCGGCTCTTC (P); 22(-), CTTCTACCAGCTTCAAGGTCAG (P). *bmpB*: 11(+), AACACA TTGGTTTTGTTG (W); 12(-), TCTTTCTATTCAAAAGTTTATAAC (W); 23(+), TGGTGATGATGTTTCAGATTCC (P); 24(-), TTTGCTGCCTCAATAA CACC (P). *bmpD* to *bmpC*: 3(+), AGGCCGCAAAAGAGTTGGG; 4(-), GCTACCATGAGCCAAAACACC. *bmpC* to *bmpA*: 5(+), TGATCGGGGGTTAAAG GAAGG; 6(-), TGAAGAGCCGCAACCTTGGC. *bmpA* to *bmpB*: 13(+), GGCCTTAAAGAAGGAGTTGTGGG; 14(-), CCAAATCAAGTCTGAGCC.

Southern hybridization. Total DNA from each *Borrelia* strain was purified from a mid-log-phase culture (8) and digested overnight with *Swa*I and/or *Hind*III (New England Biolabs, Inc., Beverly, Mass.) according to the manufacturer's instructions. The resulting DNA fragments were separated by agarose gel electrophoresis (1% agarose in Tris-acetate-EDTA [TAE] buffer), stained with ethidium bromide, and transferred by capillary action to a nylon membrane (Magna Graph; Micron Separation, Inc., Westboro, Mass.) (1). DNA probes were generated by PCR using partial (P) primers (Fig. 1) to amplify the central part of each *bmp* gene. Templates for these reactions were pUC19-based plasmids containing different DNA segments of the *bmp* region of *B. burgdorferi* 297 (2). A DNA probe targeting the *flaB* gene for use as a control was obtained by PCR amplification by using total DNA purified from *B. burgdorferi* 297 as a template and appropriate primers (5'-CT AGTGGGTACAGAATTAATCGAGC-3' and 5'-GCCTGC GCAATCATTGCCATTGC-3') (11). DNA probes were purified, labeled with digoxigenin-11-dUTP by the random primer method according to the instructions of the manufacturer (Boehringer Mannheim, Indianapolis, Ind.), hybridized to DNA blots at 65°C, and washed under high-stringency conditions in 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) buffer at 68°C (1). Bound probes were detected colorimetrically using nitroblue tetrazolium and BCIP (5-bromo-4-chloro-3-indolylphosphate) technology according to the manufacturer's instructions.

PCR. Primers to amplify full-length coding regions and flanking regions of *bmp* genes (whole [W] primers) or partial internal regions of each *bmp* gene (P primers) were designed on the basis of nucleotide sequences of the *bmpD* region of *B. burgdorferi* JD1 (GenBank accession no. U35450) (17) and the *bmpC*, *bmpA*, and *bmpB* regions of *B. burgdorferi* 297 (accession no. U49938) (2) by using the program Primer 3 Output (Center for Genome Research, Whitehead Institute for Bio-

chemical Research, Cambridge, Mass.) (Fig. 1) and synthesized (GenoSys Biotechnology, The Woodlands, Tex.). Primers specific for 16S rRNA genes (5'-GAATTTTACAATCTTTC GACC-3' and 5'-GGGGAATAATTATCTCTAAC-3') (10) and the *flaB* gene (see above) (17) were a gift from I. Schwartz. PCR amplifications were performed in a Rapid Cycler (Idaho Technology, Idaho Falls) according to the manufacturer's recommendations, with the final mixture in 10- μ l glass capillary tubes containing a 200 μ M concentration of each deoxynucleoside triphosphate, 2 mM MgCl₂, 50 mM Tris-HCl (pH 8.3), 0.5 mg of bovine serum albumin/ml, 0.5 to 1% Ficoll (Idaho Technology); 0.25 U of *Taq* polymerase (Gibco BRL, Gaithersburg, Md.), and 0.5 μ M concentrations of primers. Chromosomal DNA was initially denatured for 5 s at 96°C, followed by a total of 30 cycles of 94°C for 0 s, 60°C for 1 s, and 72°C for 30 s and a final cycle of 2 min at 72°C. PCR-generated products were purified and sequenced by the dideoxy chain termination method using a dye terminator-*Taq* cycle sequencing kit and a model 377 DNA sequencer (Perkin-Elmer, Foster City, Calif.). DNA sequences were aligned and analyzed with Assembly-LIGN and MacVector software (IBI, New Haven, Conn.), Clustal W 1.7, and Laling CCM Search Launcher (Human Genome Center, Baylor, Tex.). Some manual refinement of the alignments was performed.

Detection of *bmp* genes in *B. burgdorferi sensu lato*. Southern hybridization results are summarized in Table 1. DNA hybridization patterns in Southern blots with PCR-generated probes specific for each *bmp* gene indicated that a single copy of each *bmp* gene was present in the genomic DNA of *B. burgdorferi sensu lato*. Differences in hybridization patterns of *B. burgdorferi* strains consisted of variations in the lengths of the DNA restriction fragments hybridizing with the DNA probes and in band intensity. For example, total DNA from *B. burgdorferi sensu lato* strains digested with *Swa*I and hybridized with a P probe specific for *bmpC* yielded the expected single 1,271-bp

TABLE 1. Detection of *bmp* genes in different *Borrelia burgdorferi* species and isolates by PCR and Southern hybridization

| Species and isolate | Result by indicated test for: | | | | | | | | | | | | | | |
|----------------------------|-------------------------------|------------------|-------------|----------|-----|-------------|----------|-----|-------------|----------|------------------|------------------|------------------|-----|-----|
| | <i>bmpD</i> | | <i>bmpC</i> | | | <i>bmpA</i> | | | <i>bmpB</i> | | <i>bmpD-bmpC</i> | <i>bmpC-bmpA</i> | <i>bmpA-bmpB</i> | | |
| | Southern | PCR ^a | | Southern | PCR | | Southern | PCR | | Southern | PCR | | PCR | PCR | PCR |
| | | W | P | | W | P | | W | P | | W | P | | | |
| <i>B. burgdorferi</i> | | | | | | | | | | | | | | | |
| 297 | ND ^b | + | + | ND | + | + | ND | + | + | ND | + | + | + | + | + |
| B31 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| <i>B. garinii</i> | | | | | | | | | | | | | | | |
| G25 | + | - | ± | + | - | + | + | - | - | + | - | + | + | - | + |
| N34 | ND | - | + | ND | - | + | ND | - | - | ND | - | + | + | - | + |
| <i>B. afzelii</i> | | | | | | | | | | | | | | | |
| Ip3 | + | - | + | + | - | + | + | - | - | + | - | + | + | - | + |
| ACA1 | ND | - | + | ND | - | + | ND | - | - | ND | - | + | + | - | + |
| VS461 | ND | - | + | + | - | + | ND | - | - | ND | - | + | + | - | + |
| VS486 | ND | - | + | ND | - | + | ND | - | - | ND | - | + | + | - | + |
| <i>B. bissettii</i> 25015 | + | - | - | + | - | + | + | ± | - | + | - | + | ± | - | + |
| <i>B. andersonii</i> 21038 | + | - | + | + | - | + | + | - | + | + | - | + | ± | + | + |
| <i>B. japonica</i> H014 | + | - | - | + | - | + | + | - | - | + | - | + | - | - | - |
| <i>B. hermsii</i> | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

^a Detection of genes or intergenic sequences with primers to amplify entire (W) or partial (P) coding regions of the indicated *bmp* genes. See the text for details.
^b ND, not determined.

band with *B. burgdorferi* B31; a single 3,750-bp band with *B. garinii*, *B. afzelii*, *B. afzelii*, and *B. japonica*; a single 1,750-bp band with *B. andersonii*; and a major 750-bp band and a minor 550-bp band with *B. bissettii*. The additional fragment in *B. bissettii* is likely generated by an alternative restriction site for *SwaI* present inside *bmpC* in this *Borrelia* species. This possibility is supported by the facts that amplification with primers specific for the central partial coding region of *bmpC* in this strain generated only one product (Table 1) and that the sum of the molecular masses of the fragments observed during the hybridization was equal to the molecular mass of the expected fragment containing only one copy of *bmpC*. *B. hermsii* total DNA digested in the same manner used as a control failed to hybridize with any of the *bmp* DNA probes used in these experiments. A DNA probe for the highly conserved *B. burgdorferi flaB* gene (11) hybridized to all borrelial total DNAs examined (both *B. burgdorferi* sensu lato and *B. hermsii*) and gave the expected pattern and sizes of amplicons (data not shown). This result indicated that the lack of hybridization of *B. hermsii* total DNA with *bmp* DNA probes was unlikely to be due to technical problems related to hybridization itself.

PCR analysis of the *bmp* genes and their intergenic noncoding regions. PCR amplification of borrelial DNA was done using three groups of primers (Fig. 1). W primers were designed to analyze full-length coding regions of *bmp* genes and generated amplicons for *bmp* genes only from *B. burgdorferi* sensu stricto isolates as well as *B. bissettii*, from which *bmpA* was weakly amplified (Table 1). All products obtained with W primer sets were of the expected sizes (data not shown). No products were obtained from *B. hermsii* DNA with any W primers.

P primers were designed to amplify a partial internal region of each *bmp* gene. Amplicons corresponding to *bmpC* and *bmpB* were obtained from all *B. burgdorferi* sensu lato strains (Table 1), amplicons corresponding to *bmpD* were obtained

from all *B. burgdorferi* sensu lato strains but not *B. bissettii* and *B. japonica* (Table 1), and amplicons corresponding to *bmpA* were obtained only from *B. burgdorferi* sensu stricto and *B. andersonii* (Table 1). Amplicons had the same size in all tested *B. burgdorferi* strains when they were obtained with each pair of P primers. For example, all *bmpC* amplicons of *B. burgdorferi* sensu lato obtained using P primers 1 and 2 were the predicted size of 484 bp; comparable results were obtained with amplicons from *bmpB* and *bmpD* (data not shown). Similar PCR results were obtained with DNAs from cloned and uncloned *Borrelia* strains (data not shown). No products were obtained from *B. hermsii* DNA with any P primers. In these experiments, primers specific for 16S rRNA genes yielded amplicons from all borrelial DNA, including *B. hermsii* DNA (data not shown), suggesting that the failure to amplify *bmp* genes from *B. hermsii* DNA and some *B. burgdorferi* sensu lato DNAs (e.g., *bmpA*) was not due to template degradation or to the presence of PCR inhibitors and suggested the existence of DNA sequence heterogeneity in this region.

As an additional step in revealing possible DNA sequence heterogeneity in the *bmp* cluster, a third group of primers was designed to amplify *bmp* intergenic regions. These intergenic primer sets amplified regions extending from the 3' end of the upstream gene to the 5' end of the downstream gene of each *bmp*. *B. burgdorferi* sensu stricto and *B. andersonii* DNAs generated amplicons with single primer pairs designed to amplify *bmpD-bmpC*, *bmpC-bmpA*, and *bmpA-bmpB* intergenic regions (Table 1). In other *B. burgdorferi* sensu lato strains, only *bmpD-bmpC* and *bmpA-bmpB* regions were amplified (Table 1). *B. japonica* DNA did not generate a product when it was amplified with primers targeting any of the intergenic regions. Other primer sets composed of combinations of different plus- and minus-strand primers (Fig. 1) were used to provide further characterization of the *bmp* region. These additional primer sets generated amplicons from all *B. burgdorferi* sensu lato

DNAs except that of *B. japonica*. In each case, the products obtained were of the expected sizes and overlapped throughout the entire *bmp* region. This result ruled out the possibility that the observed negative PCR amplifications were caused by the presence of extensive deletions in these regions and suggested that the failure to amplify was due to sequence polymorphism. None of the primers directed at amplifying *bmp* intergenic regions generated any product from *B. hermsii* DNA.

PCR analysis of the *bmp* chromosomal region in *B. burgdorferi* low-passage-number strains. Ten *B. burgdorferi* sensu stricto strains recently isolated from skin biopsies and blood samples from patients with Lyme disease and passaged only once (10) were used to characterize the genetic structure of the *bmp* chromosomal region in low-passage-number *B. burgdorferi* strains. All primer sets directed at amplifying full-length and partial coding regions of each *bmp* gene and of the *bmp* intergenic regions yielded single DNA products identical in size to those amplified from *B. burgdorferi* 297 and B31. Although continuous in vitro cultivation can cause significant changes in the *B. burgdorferi* genome (15), low-passage-number *B. burgdorferi* sensu stricto strains recently isolated from patients with Lyme disease were identical to *B. burgdorferi* 297 and B31 in terms of both PCR pattern and amplicon size.

DNA sequence analysis of *bmp* intergenic regions in *B. burgdorferi* sensu lato. To identify the molecular basis for the polymorphism observed in the *bmp* region during PCR amplification, and to assess the genetic divergence in this region among different strains of the *B. burgdorferi* sensu lato complex, PCR products containing *bmpD-bmpC* and *bmpC-bmpA* intergenic regions were sequenced and compared (Fig. 2). In those cases when the primer sets designed to amplify the intergenic region did not yield an amplification product, combinations of different primers were used to amplify DNA sequences of an increased size that included the regions of interest. Because it was not possible to obtain amplification of *B. japonica* DNA with any available primer set, this isolate was not subjected to further analysis.

The *bmpD-bmpC* intergenic region analyzed comprised approximately 80 bases upstream from the *bmpD* stop codon to approximately 90 bases downstream from the *bmpC* start codon (Fig. 2A). The DNA sequence of this region of *B. burgdorferi* 297 was 97% identical to that of *B. burgdorferi* B31 (6). Nucleotide identities to *B. burgdorferi* 297 for this region were 82.2, 85.1, 87.0, and 88.0% for *B. afzelii*, *B. garinii*, *B. bissetii*, and *B. andersonii*, respectively. Coding sequences were relatively conserved among different strains, with few single-base replacements. Nucleotide insertions, deletions, and substitutions were concentrated in a few clusters (Fig. 2A) and were significantly greater in number per 100 nucleotides in the *bmpD-bmpC* intergenic region than in the 3' *bmpD* coding region preceding this intergenic region ($P < 0.02$, Kruskal-Wallis analysis of variance with Dunn multiple-comparison post-test). The most important characteristic of the intergenic region was the presence of two deletions: one of 12 nucleotides detected in a *B. garinii*-derived amplicon and another of 18 nucleotides found in a *B. bissetii* amplicon (Fig. 2A). Only minor differences at a few positions were found when DNA sequences were compared between two *Borrelia* strains of the same genotype; for example, *bmpD-bmpC* sequences of *B. afzelii* ACA1 and *B. afzelii* Ip3 were 98.3% identical. However, the region immediately upstream of the *bmpC* start codon was highly polymorphic between strains of different genotypes and contained various numbers of thymidines in runs of thymidines (Fig. 2A).

The *bmpC-bmpA* intergenic region analyzed comprised approximately 150 bases upstream from the *bmpC* stop codon

and approximately 310 bases downstream from the *bmpA* start codon (Fig. 2B). Here, too, nucleotide insertions, deletions, and substitutions were significantly greater in number per 100 nucleotides in the *bmpC-bmpA* intergenic region than in the preceding 3' *bmpC* coding region ($P < 0.02$, Kruskal-Wallis analysis of variance with Dunn multiple-comparison post-test). Numbers of insertions, deletions, and substitutions per 100 nucleotides were not significantly different between *bmpD-bmpC* and *bmpC-bmpA* intergenic regions. The presence of these variations in the DNA sequence was confirmed by sequencing both strands of several independently generated PCR amplicons.

This study shows that *bmp* genes are widely distributed among all strains of *B. burgdorferi* sensu lato and are not present in *B. hermsii*. These results are consistent with previous reports about the species-specific nature of the members of this family (1, 17, 19). The similarity of amplicon size obtained with each primer set used also suggests conservation of these genes among *B. burgdorferi* sensu lato strains. In all cases of positive amplification, a single product was obtained, confirming previous results demonstrating that only one copy of these genes is present in the borrelial genome (1, 17, 19). Our data also demonstrate conservation of *bmp* gene order in the borrelial chromosome (*bmpD bmpC bmpA bmpB*).

The genetic structure of the *bmp* chromosomal region appears to be similar in low- and high-passage-number *B. burgdorferi* sensu stricto strains. Whether this region undergoes any changes while the spirochetes are maintained in zoonotic cycles involving ticks and small rodents or during tick feeding and human infection is not known. The observed conservation of the size and order of the *bmp* genes is surprising, as their DNA sequence similarity could potentially generate the homology needed for recombination events to alter this order as happens with *B. burgdorferi* plasmid-borne members of paralogous gene families (15, 16). This difference in genetic behavior between chromosomally and plasmid-located paralogous genes may reflect alternative biological roles of their gene products.

Despite the constancy of the overall genetic organization of the *bmp* cluster in *B. burgdorferi* sensu lato, DNA sequence variations existed over the entire region. These variations were significantly higher in noncoding regions, where there was a tendency for them to be clustered at particular points. Our observation that primers designed to target entire *bmp* coding regions generated amplicons only in *B. burgdorferi* sensu stricto strains suggested that primer annealing (and therefore amplification) was prevented either by deletions in the central regions of these genes or by DNA sequence variations in regions flanking these genes. Our subsequent successful amplification of *bmp* genes in some *B. burgdorferi* sensu lato isolates using alternative primer combinations with different plus- and minus-strand primers generated amplicons that overlapped the entire *bmp* region with sizes corresponding to those of the *B. burgdorferi* 297 sequence. This result indicated that the previous failure to generate amplicons was not due to deletions or insertions in *bmp* coding sequences and implied that lack of amplification was due to the DNA sequence variations in flanking regions (Fig. 2). Although no amplicons were generated from *B. japonica* DNA, Southern hybridization analysis indicated that all *bmp* genes were present in the genome of this species. DNA sequence variations appear to be more pronounced in *B. japonica* than in *B. burgdorferi* sensu lato strains and may reflect evolutionary divergency (12).

In summary, the genetic structure of the chromosomal region containing the *bmp* genes appears to be well conserved across different species of *B. burgdorferi*, but variations in DNA fine structure that prevent PCR primer annealing may occur in

| | | | | | | | | | |
|-------------------|-------------------|-------------------|-------------------|------------------|------------------|------------------|---|---|-----|
| (A) | | | | | | | | | |
| Bb | gtataatgaatggta | taataaaagtcctt | atgacaaggatctt | atgataaactttg | tgcaaatggaaaat | <i>bmpD</i> stop | | | 90 |
| Bg | gtataatggaggta | taataaaagtcctt | atgacaaggatctt | atgataaactttg | tgcaaatggaaaat | A | A | A | 90 |
| Bafz | gtataatgaatggta | taataaaagtcctt | atgacaaggatctt | atgataaactttg | tgcaaatggaaaat | A | A | A | 90 |
| Bbis | gtataatgaatggta | taataaaagtcctt | atgacaaggatctt | atgataaactttg | tgcaaatggaaaat | A | A | A | 90 |
| Band | gtataatgaatggta | taataaaagtcctt | atgacaaggatctt | atgataaactttg | tgcaaatggaaaat | A | A | A | 90 |
| Bb | gagctgattttgaa | aaatctttttaattc | tttaaagatatttta | aaggg-tttttta | tgttgtaagtttga | atttaaattaatctt | | | 179 |
| Bg | gagttggctttgaa | aaatgtttttagttt | tttgaagagatctt | -----ta | tgttgtaagttt-a | atttagattaatctt | | | 166 |
| Bafz | gagttgattttgaa | aaatgatttttgctc | tttaaaaagatttta | aataagctttta | tattgcaagatt-a | atctagattaatctt | | | 178 |
| Bbis | -agttggttttgaa | aaatcttttta-ttc | tttaaagatatttta | gagag-ttttt--- | -----a | atttaaattaatctt | | | 159 |
| Band | gagttagttttgaa | aaatcttttttaattc | tttaaagatctttaa | gagaa-tttttta | tattgtaa-ttttt- | atctagattaatctt | | | 176 |
| Bb | gcaaaagagtttaa | tttgatattatg-- | gtgatgtaggaa-- | aattgatttttccta | ctactgtgtttttat | taatgctagaagtat | | | 265 |
| Bg | gcaaaagggttttaa | tttagatattatg-- | gtgatgtaggaa-- | aataattttt-ctta | ccaccatgtttttat | taatgctaaaagtat | | | 251 |
| Bafz | gcaaaagggttttaa | tttgggtattatg-- | gtgatgtaggaa-- | a-----aattttctta | ccaccatgtttttat | taatactagaagtat | | | 260 |
| Bbis | gcaaaagggttttaa | tttgatattatg-- | gtgatgtaggaa-- | aataatttttccta | ctaccgtgtttttat | taatgctagaagtat | | | 245 |
| Band | gcagaag-gtttaa | tttgatattatagt | gtgatgtaggaa | aaataatttttccta | ctaccgtgtttttat | taatgctagaagtat | | | 265 |
| Bb | tt-----ttttaa | aggattattaaaa | tttttttataaata | aagaatactgcttgt | tagtaaaataaagtt | aatatttttaattttt | | | 349 |
| Bg | ttttttttttttaa | aggtttattgagatt | tttgttttataaata | aagagtactatttgt | tagtaaaataaagtt | aatatttttaattttt | | | 341 |
| Bafz | ta-----ttttt--- | agggttattgagatt | tttttttttaaatg | aagaatactacttgt | tagtaaaataaagtt | gatgttttaattttt | | | 342 |
| Bbis | ct-----ttttaag | tatcttattgaaatt | t-tattttataaata | aagaatactacttgt | tagtaaaataaagtt | aatatttttaattttt | | | 328 |
| Band | ct-----tttagaaa | gggtttgttttaatt | ttttttttgtaaata | aagaatactacttgt | tagtaaaataaagtt | aatatttttaattttt | | | 350 |
| <i>bmpC</i> start | | | | | | | | | |
| Bb | -aaaaaatttagaat | tttaa--aaaaaat | at--aaggagagga | tttaattTTGtttaa | agattttatttttatt | actttatctttatta | | | 433 |
| Bg | -aaaaaattctaaat | ttttt--aaataaat | tttgggaagggagga | tttaattTTGtttaa | aggtttgtttttatt | gttt-a---ttat-a | | | 423 |
| Bafz | gaaaaaattctaaat | ttttt--aaataaat | tttggtaaggagagga | ttgatTTGtttaa | aggtttattttttatt | gctttatcttttttg | | | 430 |
| Bbis | -aaaaaattaaaaat | tttttttaataaat | tttggtaaggagaggg | tttgaTTGtttaa | aggtttattttttatt | actttatctttatta | | | 417 |
| Band | -agaaaatttttaaat | ttttt--aaataaat | tttggtaaggagagga | tttaattTTGtttaa | aggtttattttttatt | actttatctttatta | | | 437 |
| Bb | gtatttgcttgtttt | aaatctaataaaaa | cttattaaactctgac | aaagttgt- | | | | | 486 |
| Bg | gtatt-gcttgtttt | aaatctaataaaaa | ctctgtaagctctgac | aaagttgt- | | | | | 476 |
| Bafz | gtgttgcttgtttt | caatctaataaaaa | ctctgtaaaactctgac | aaagttgt- | | | | | 483 |
| Bbis | gtatttgcttgtttt | aaatctaataaaaa | cttattaaactctgaa | aaagttgt- | | | | | 471 |
| Band | gtatttcttgtttt | aaatctaataaaaa | cttattaaactctgac | aaagttgt- | | | | | 491 |
| (B) | | | | | | | | | |
| Bb | gttaaagggaaggagt | aatagaaattgttaa | ggatcccgatgtttt | aaacaataggttggt | tgatgaagttattga | tctagaaaaataaat | | | 90 |
| Bg | gttgaagggaaggagt | aatagaaatcgttaa | ggatccctgatgtttt | aaaataataggttagt | ttaatgaagttgttga | gctagaaaaataaat | | | 90 |
| Bafz | gtttaaagggaaggagt | aatagagggtgttaa | ggatcccgatgtttt | aaaataataggttagt | tgatgaagttattga | tctagaaaaataaat | | | 90 |
| Bbis | gttaaagggaaggagt | aatagaaattgttaa | ggatccctgatgtttt | aaaataataggttagt | tgatgaagttattga | tctagaaaaataaat | | | 90 |
| Band | gttaaagggaaggagt | aatagaaattgttaa | ggatccctgatgtttt | aaaataataggttagt | tgatgaagttattga | tctagaaaaataaat | | | 90 |
| Bb | aataagtgagaaat | tattgttcctgatag | tgaatatgcatttga | tttatttaaatcaaaa | gtaTAAactactta | aatatagctttgttt | | | 180 |
| Bg | aataagtgagaaat | tattgttcctgatag | tgaatatgcatttga | tttatttaaatcaaaa | gtaTAAactactta | ggataacttttttt | | | 180 |
| Bafz | aataagtgagaaat | tattgttcctgatag | tgaatatgcatttga | tttatttaaatcaaaa | gtaTAAactacttg | ggatagcttttgatt | | | 180 |
| Bbis | aataagtgagaaat | tattgttcctgatag | tgaatatgcatttga | tttatttaaatcaaaa | gtaTAAactactta | ggatagcttttttt | | | 180 |
| Band | aataagtgagaaat | tattgttcctgatag | tgaatatgcatttga | tttatttaaatcaaaa | gtaTAAactactta | ggatagcttttttt | | | 180 |
| <i>bmpA</i> start | | | | | | | | | |
| Bb | gtaaa--ggggaaat | agtttATGaaataaa | tattgttgttgattt | tgcttgagagtattg | tttttttatcttga | gtggtaaaggtagtc | | | 268 |
| Bg | gggaa--ggggaa-t | attttATGaaataaa | tatttattgttaatt | tgcttgaagtgattg | tttttttatcttga | gtggtaaaggatact | | | 267 |
| Bafz | taaaaagggggaaac | ggtttATGagtaaa | tattgttgttgattt | tatttgaaggtatta | tttttttatcttga | gtggcaaggtagtga | | | 270 |
| Bbis | gtaaa--ggggaa-t | agtttATGaaataaa | tatttattgttgattt | tatttgaaggtattg | tttttttatcttga | gtggtaaaggtagtc | | | 268 |
| Band | gtaaa--ggggaa-t | aggttATGaaataaa | tattgttgttgattt | tgcttgaagctatta | tttttttatcttga | gtggcaaggtagtc | | | 268 |
| Bb | ttgggagcgaattc | ctaaggtatctttaa | taattgatggaactt | ttgatgataaaactt | ttaatgagagtgctt | taaatggcgtaaaaa | | | 358 |
| Bg | ttgagaattggaattc | cdaaggtatctgttaa | tagttaatggaactt | ttgatgataaaactt | ttaatgagagtgctt | taaatggcgtaaaaa | | | 357 |
| Bafz | ttgagaattggaattc | cdaaggtatcttttaa | tagttgatggaactt | ttgatgataaaactt | ttaatgaaagtgctt | taaatggcgtaaaaa | | | 360 |
| Bbis | ttgaaagtgaactt | ctaaggtatctttaa | taattgatggaactt | ttgatgataaaactt | ttaatgagagtgctt | taaatggcgtaaaaa | | | 358 |
| Band | ttgagagtggaattc | ctaaggtatctttaa | taattgatggaactt | ttgatgataaaactt | ttaatgagagtgctt | taaatggcgtaaaaa | | | 358 |
| Bb | aagttaaagaagaat | ttaaatttgagcttg | ttttaaaagaatcct | catcaaatctttatt | tatctgatcttgaag | ggcttaaagtagcgg | | | 448 |
| Bg | aagttaaagaagaat | ttaaagattgagcttg | ttttaaaagaatcct | caaccaaatctttatt | tatctgatcttgaag | ggcttaaagtagcag | | | 447 |
| Bafz | aaattaaagaagaat | ttgaaattgaaacttg | ttttaaaagaatcct | caaccaaatctttatt | tatctgatcttgaag | ggcttaaagtagcgg | | | 450 |
| Bbis | aagttaaagaagaat | ttaaagattgaaacttg | ttttaaaagaatcct | cagcgaatctttatt | tatctgatcttgaag | ggcttaaagtagcgg | | | 448 |
| Band | aagttaaagaagagt | ttaaagattgaaacttg | ttttaaaagaatcct | catcaaatctttatt | tatctgatcttgaag | ggcttaaagtagcag | | | 448 |
| Bb | gctcagatttaattt | ggcttattgggtata | gatttagcgatgtgg | ccaaggttgcg | | | | | 504 |
| Bg | gttcaaattaattt | ggcttattggatata | gatttaattgatgtgg | ccaaggtctgtt | | | | | 503 |
| Bafz | gttcaaattaattt | ggcttattggatata | gatttagcgatgtgg | ccaaggtctgtt | | | | | 506 |
| Bbis | gttccagatttaattt | ggcttattgg-tata | gatttagcgatgtgg | ccaaggtctgtt | | | | | 503 |
| Band | gttccagatttaattt | ggcttattgggtata | gatttagcgatgtgg | ccaaggttgcg | | | | | 504 |

FIG. 2. Alignment of intergenic *BmpD*-*bmpC* (A) and *bmpC*-*bmpA* (B) sequences from selected *B. burgdorferi* sensu lato strains. Gaps introduced by alignments are indicated by hyphens, nucleotides nonidentical to those in *B. burgdorferi* 297 sequence are shaded, and translation start and stop codons are indicated by uppercase letters. *Bb*, *B. burgdorferi* 297; *Bg*, *B. garinii* G25; *Bafz*, *B. afzelii* Ip3; *Bbis*, *B. bissettii* 25015; *Band*, *B. andersoni* 21038.

this region and make Southern hybridization much more reliable than PCR for detection of the presence of these genes. Our results also suggest that *bmp* gene products may be used as reagents in the preparation of vaccines and diagnostic assays to protect against and diagnose Lyme disease produced by *B. burgdorferi* sensu lato.

Nucleotide sequence accession numbers. Nucleotide sequences of *bmpD-bmpC* and *bmpC-bmpA* intergenic regions have been deposited in GenBank under accession numbers U49934, AF222434, AF222435, AF222436, AF222437, AF222438, AF222439, AF222440, and AF222441.

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