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Analysis and Comparison of Plasmid Profiles of *Borrelia burgdorferi* Sensu Lato Strains

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The relationship between plasmid profiles and genospecies of the Lyme disease borreliae was investigated by using 40 strains from diverse biological and geographical sources. The genospecies of the strains were determined by examination of rRNA gene restriction patterns with cDNA probes complementary to the 16S and 23S rRNAs of *Escherichia coli*. Plasmid profiles were obtained by pulsed-field gel electrophoresis. The number of plasmids per strain and the size of these plasmids ranged from 4 to 10 and from 13.3 to 57.7 kb, respectively. The strains all contained a single large plasmid of 50 to 57.7 kb, with the exception of two *Borrelia garinii* strains that contained two or three of the large plasmids. The large plasmids of *Borrelia burgdorferi* sensu stricto strains ranged in size from 51.4 to 52.7 kb and were consistently smaller than the 54.0- to 57.7-kb plasmids present in *B. garinii* and *Borrelia afzelii*. The exceptions to this observation were the two *B. garinii* strains with multiple large plasmids; in this case the large plasmids were 50.6 to 53 kb. Although a large degree of heterogeneity in the sizes and frequencies of occurrence of smaller plasmids was observed, there were some differences among the three genospecies. The differences in plasmids were further studied by using two *Bam*HI DNA fragments from a 28.7-kb plasmid of *B. burgdorferi* sensu stricto 297 as probes. Both probes hybridized with the 27- to 29-kb plasmids of *B. burgdorferi* sensu stricto strains. In contrast, two patterns of hybridization were observed with *B. garinii* and *B. afzelii*. One pattern was the hybridization of both probes, with each probe hybridizing with a different plasmid. The other pattern was the hybridization of a plasmid of a strain with only one of the two probes. Some strains of the three genospecies did not hybridize with either probe. Our results suggest that the plasmid profiles of *B. burgdorferi* sensu lato have genospecies characteristics and that the hybridization patterns of similar-sized plasmids are different for the three genospecies.

The etiologic agent of Lyme disease in the United States was discovered in 1982 (10) and was isolated from patients in 1983 (6, 30). Initially, one species, *Borrelia burgdorferi* sensu lato (18) was believed to be responsible for this illness. However, differences in disease manifestation suggested that more than one species was responsible for this disease in Eurasia. Subsequently, it was shown that *B. burgdorferi* sensu lato responsible for Lyme disease is a complex of three genospecies, *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* (1, 11). *B. burgdorferi* sensu stricto strains have been identified in both North America and Europe, whereas *B. garinii* and *B. afzelii* have been found only in Eurasia (31).

The genome of *B. burgdorferi* sensu lato is composed of one linear chromosome of 950 kb (5, 13) and linear plasmids as well as supercoiled circular plasmids (4, 16, 23). The copy number of linear and circular plasmids is approximately the same as that of the chromosome (8, 14). Both linear and circular plasmids may be lost as a result of in vitro cultivation, and the loss of plasmids has been associated with reduced infectivity in laboratory animals (27). The size and number of plasmids vary among strains of *B. burgdorferi* sensu lato (3, 15, 29). To investigate the correlation between plasmid profiles and genospecies, we studied 40 *B. burgdorferi* sensu lato strains obtained from diverse biological sources and geographic locations. The genospecies of these strains were identified by rRNA gene restriction patterns, and the plasmid profiles were analyzed by pulsed-field gel electrophoresis and Southern hybridization.

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MATERIALS AND METHODS

Strain sources and cultivation. The *B. burgdorferi* sensu lato strains used in this study are shown in Table 1. The infectivity of *B. burgdorferi* sensu lato was determined in hamsters as previously described (17). If hamsters could be infected by intraperitoneal injection of 10^6 cells of a strain, the strain was considered to be virulent. These strains were subcultured fewer than 10 times after isolation from hamsters before plasmid analysis. Strains noninfectious for hamsters were considered to be avirulent. The infectivities of three strains were not determined. The strains were cultivated in Barbour-Stoenner-Kelly (BSK) medium (2) with minor modifications (7). Cultures were incubated at 34°C and harvested when the cell concentration reached 10^8 cells per ml.

PFGE. Plasmid preparations were carried out as described by Barbour (3). Contour-clamped homogeneous electric field pulsed-field gel electrophoresis (CHEF-PFGE) was performed with a Pharmacia LKB apparatus (Pharmacia, Uppsala, Sweden) with a hexagonal electrode array (Pulsaphor 2105). Agarose gels (0.9 to 1.1%) in 0.5× TBE (0.5× TBE is 45 mM Tris-borate plus 1 mM EDTA, pH 8.0) were used. The electrophoresis was performed at a constant voltage (200 V) at 9°C with different pulsed-field parameters. Different parameters were used to obtain better resolution of plasmids with some strains. Lambda DNA monocut mixture (New England Biolabs, Beverly, Mass.) and lambda DNA digested with *Hin*dIII (Boehringer Mannheim, Indianapolis, Ind.) were used as molecular size markers. The gels were stained with ethidium bromide, illuminated with UV light, and photographed. The photographic negatives were analyzed by using Image public domain software (21) and GelReader (National Center for Supercomputering Application) computer programs. The relative sizes of plasmid bands were calculated by the GelReader program.

rRNA gene restriction pattern. DNA from whole cells was prepared as described by Brenner et al. (9). DNA was digested with *Hin*dIII or *Eco*RV (Boehringer Mannheim) according to the manufacturer's instructions, separated in a 0.9% agarose gel, and transferred onto positively charged nylon membranes. cDNAs complementary to *Escherichia coli* 16S and 23S rRNAs were used to probe the target DNA on nylon membranes. cDNA probes to rRNA were synthesized by reverse transcription, and the products were labeled with digoxigenin-11-dUTP by using the random-primed labeling method according to the manufacturer's instructions (Boehringer Mannheim).

Preparation of DNA probes. Plasmid DNA was digested with *Bam*HI and separated in 0.7% agarose gels in 0.5× TBE. DNA fragments of interest were excised from the gels and purified with GeneClean II (Bio 101, La Jolla, Calif.) according to the manufacturer's instructions. The purified DNA fragments were labeled by the random-primed DNA-labeling method with the Genius System

TABLE 1. *B. burgdorferi* sensu lato strains studied

Genospecies and strain	Geographic location	Origin	Virulence[a]
B. burgdorferi sensu stricto			
B31	United States	*Ixodes scapularis*	VIR
MMT1/59	United States	*I. scapularis*	VIR
MM1	United States	*Peromyscus leucopus*	VIR
NCH-1	United States	Human, skin	VIR
LAX#7	United States	*Peromyscus leucopus*	VIR
297	United States	Human, CSF[b]	VIR
10293	United States	Veery, liver	VIR
CT-1	United States	*I. scapularis*	VIR
IPS	United States	*Ixodes pacificus*	VIR
PKa 1	Germany	Human, CSF	AVIR
ZS7	Germany	*Ixodes ricinus*	VIR
IP1	France	Human, CSF	AVIR
20001	France	*I. ricinus*	VIR
20004	France	*I. ricinus*	VIR
IP2	France	Human, CSF	AVIR
IRS	Switzerland	*I. ricinus*	VIR
H11	Italy	Human, blood	AVIR
B. garinii			
2226	China	*Ixodes persulcatus*	AVIR
2223	China	*I. persulcatus*	AVIR
Fuji P1	Japan	*I. persulcatus*	VIR
Fuji P2	Japan	*I. persulcatus*	VIR
GERTICK#3	Germany	*I. ricinus*	ND
20047	France	*I. ricinus*	VIR
G25	Sweden	*I. ricinus*	AVIR
SWTICK#1	Sweden	*I. ricinus*	ND
VSBP	Switzerland	Human, CSF	AVIR
BITS	Italy	*I. ricinus*	AVIR
IP89	Russia	*I. persulcatus*	VIR
IP553	Russia	*I. persulcatus*	AVIR
Ir210	Russia	*I. ricinus*	AVIR
PD89	China	Human, blood	VIR
PBi	Germany	Human, CSF	VIR
B. afzelii			
M7	China	*I. persulcatus*	AVIR
2246	China	*I. persulcatus*	AVIR
IPF	Japan	*I. persulcatus*	AVIR
PGau	Germany	Human, skin	VIR
PKo	Germany	Human, skin	ND
BO23	Germany	Human, skin	VIR
ECM-1	Sweden	Human, skin	AVIR
VS461	Switzerland	*I. ricinus*	AVIR

[a] VIR, infectious in hamsters at a dose of $\leq 10^8$ cells per ml; AVIR, noninfectious in hamsters at a dose of $\geq 10^8$ cells per ml; ND, infectivity not investigated.
[b] CSF, cerebrospinal fluid.

(Boehringer Mannheim). Probe concentrations were quantified by comparison with control labeled DNA provided in the labeling kit.

Southern blotting. Nucleotide hybridization was carried out at 62°C for 8 to 12 h according to the manufacturer's instructions for the Genius System. Membranes to be reprobed were stripped as follows: the membranes were rinsed thoroughly in water, incubated in probe-stripping solution (60% formamide, 50 mM Tris-HCl [pH 8.0], 1% sodium dodecyl sulfate) at 75°C for 60 min, and rinsed thoroughly in water.

RESULTS

Identification of genospecies. The genospecies of the 40 strains of *B. burgdorferi* sensu lato were identified by comparison of the rRNA gene restriction patterns with those of reference strains of each genospecies. The reference strains used were *B. burgdorferi* sensu stricto B31 (ATCC 35210) and 297, *B. garinii* 20047 and G25, and *B. afzelii* PGau and VS461 (1). Seventeen of the 40 strains were *B. burgdorferi* sensu stricto; these included 9 North American and 8 European strains. The 15 *B. garinii* and 8 *B. afzelii* strains were from Europe and Asia (Table 1).

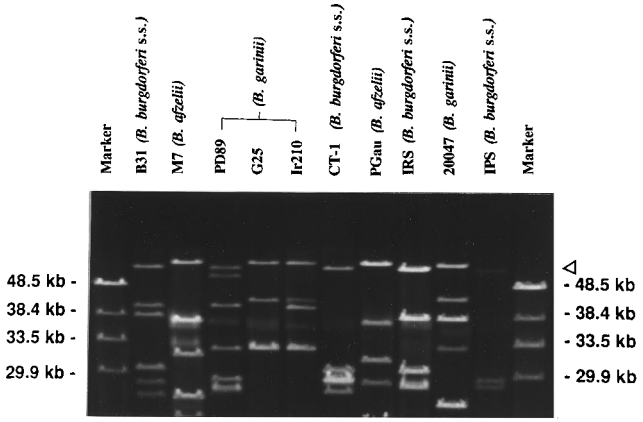

FIG. 1. Sizes and number of plasmids in the 50- to 59-kb range in *B. burgdorferi* sensu lato. The arrowhead indicates the large plasmids in the 50- to 59-kb range. The marker is lambda DNA monocut mixture. s.s., sensu stricto.

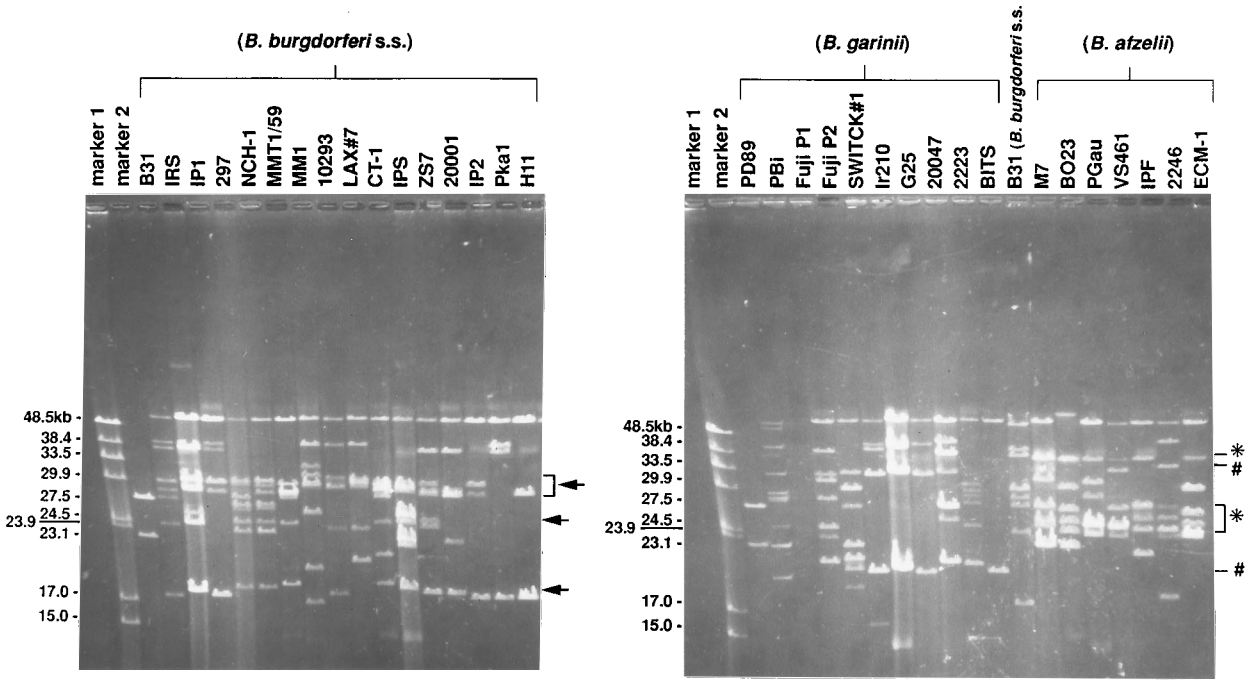

FIG. 2. Plasmid profiles of *B. burgdorferi* sensu lato determined by PFGE. (Left) Plasmid profiles of *B. burgdorferi* sensu stricto (s.s.) strains. ←, plasmids (30, 29, 28, 24, and 18 kb) occurring at a high frequency. (Right) Plasmid profiles of genospecies *B. garinii* and *B. afzelii*. #, plasmids (32 and 21 kb) occurring with a high frequency in *B. garinii*; *, plasmids (36, 27, 26, 25, and 24 kb) occurring with a high frequency in *B. afzelii*. *B. burgdorferi* sensu stricto B31 was used for comparison. Marker 1 is lambda DNA monocut mixture, and marker 2 is *Hin*dIII-digested lambda DNA.

Plasmid profiles. The plasmid profiles of the 40 strains of *B. burgdorferi* sensu lato were determined by CHEF-PFGE, and the results for the majority of the strains are shown in Fig. 1 and 2. The sizes and number of plasmids present in each strain were calculated and are listed in Tables 2, 3, and 4. The number of plasmids in each strain of the three genospecies varied from 4 to 10, and the plasmid size ranged from 13.3 to 57.7 kb. The majority of the plasmids of the three genospecies were in

TABLE 2. Plasmid profiles of *B. burgdorferi* sensu stricto

Strain	Size(s) (kb) of plasmid(s) in the following size range:					No. of plasmids
	50–59 kb	40–49 kb	30–39 kb	20–29 kb	<20 kb	
B31	52.1	40.0	38.3, 30.3	28.9, 27.5, 24.3	18.1	8
MMT1/59	51.6			29.9, 28.5, 27.9, 24.4	18.9, 14.9	7
MM1	52.0	40.0	32.7, 31.0	29.6, 29.2, 25.7, 20.2	17.1, 14.3	10
NCH-1	51.4		30.2	29.4, 28.4, 26.7, 26.2, 24.5, 23.5	18.7	9
LAX#7	51.8	40.0	30.1	29.7, 28.9, 23.7, 20.7		7
IRS	52.0		38.3, 30.4	28.7, 25.5, 24.6	18.6	7
297	51.5		30.1	29.6, 28.7, 28.0, 26.8, 26.3, 24.6, 23.4	18.5	10
10293	52.2	40.3	31.0	29.8, 23.9	18.2	6
CT-1	52.1		30.2	29.4, 28.0, 24.9, 22.3	19.0, 13.8	8
IPS	52.1			29.5, 28.9, 25.7, 24.9, 23.3, 22.6	18.4	8
ZS7	51.9		38.5, 30.4	29.0, 28.6, 25.2, 24.5	18.1	8
20001	52.1		38.6	29.1, 23.2	18.2	5
20004	52.1		38.4, 30.5	28.5, 24.4, 22.1	18.2, 17.0	8
IP2	52.1		30.7	28.7	17.8	4
PKa1	52.1	40.2	38.3		17.9	4
H11	52.1		38.6	29.4	18.2	4
IP1	52.7	40.3	38.6, 30.1	28.2	17.9	6
Total no. of plasmids[a]	17	6	21	55	20	119
Avg no. of plasmids in each strain[b]	1.00	0.3	1.3	3.3	1.2	7.0 (1.9)[c]

[a] Total number of plasmids observed in all 17 strains investigated.
[b] Ratio of the total number of plasmids observed to the total number of strains (17 strains) investigated.
[c] The number in parentheses is the standard deviation.

TABLE 3. Plasmid profiles of *B. garinii*

Strain	Sizes (kb) of plasmid(s) in the following size range					No. of plasmids
	50–59 kb	40–49 kb	30–39 kb	20–29 kb	<20 kb	
20047	54.0	43.4	38.0, 32.8	27.2, 25.5, 21.9		7
Ir210	54.5	42.3, 40.6	32.6	22.3, 21.4	13.7	7
G25	54.3	42.3	32.7	21.3		4
VSBP	54.9	44.2, 42.7	32.9	29.5, 21.5	15.9	7
GERTICK#3	54.7	42.5	32.6	20.8	19.0	5
SWTICK#1	54.2	42.3, 40.6	32.4	20.7	15.6	6
Fuji P1	54.1	40.0	32.7, 31.9	28.9, 25.1, 24.3, 22.1		8
Fuji P2	54.1		33.1, 30.6	27.7, 23.5, 22.2, 21.7, 21.2	19.5	9
BITS	54.2		35.7, 32.0	20.7		4
IP89	54.8		32.5, 30.9, 30.1	26.6, 25.1, 22.0, 21.2, 21.0	19.3	10
IP553	54.3		33.2, 32.0, 30.1	27.6, 26.2	19.9, 19.6, 15.4	9
2226	54.9		33.8, 30.1, 31.4	26.4, 20.2	19.5	7
2223	56.1		31.6, 30.3	29.5, 28.1, 25.2, 21.8		7
PD89	52.9, 50.7	42.3	32.4	29.0, 28.2, 24.9, 23.1, 20.6		9
PBi	53.0, 51.6, 50.6			28.4, 24.2, 23.7, 22.8, 21.1		8
Total no. of plasmids[a]	18	11	25	43	10	107
Avg no. of plasmids in each strain[b]	1.2	0.7	1.7	2.9	0.7	7.1 (1.8)[c]

[a] Total number of plasmids observed in all 15 strains investigated.
[b] Ratio of the total number of plasmids observed to the total number of strains (15 strains) investigated.
[c] The number in parentheses is the standard deviation.

the 20- to 29-kb size range, and the smallest number were in the 40- to 49-kb size range. The average number of plasmids per strain was essentially the same for the three genospecies, ranging from 7.0 for *B. burgdorferi* sensu stricto to 7.1 for *B. garinii* and *B. afzelii*.

Thirty-eight of the 40 strains examined contained a single large plasmid in the 50.6- to 57.7-kb size range; the exceptions were two *B. garinii* strains, PD89 (a human blood isolate from China) and PBi (a human spinal fluid isolate from Germany), which contained two and three plasmids of this size, respectively. The largest plasmid, 57.7 kb, was present in *B. afzelii* BO23, a human skin isolate from Germany. The smallest plasmid in this size range, 50.6 kb, was one of the three large plasmids present in PBi. The single large plasmid present in strains of *B. burgdorferi* sensu stricto ranged in size from 51.8 to 52.7 kb. These plasmids were consistently smaller than those present in *B. garinii* and *B. afzelii* strains, which ranged in size from 54 to 57.7 kb. Again, the exceptions to this observation were the two *B. garinii* strains that contained multiple large plasmids; in this case the plasmids ranged in size from 50.6 to 53 kb (Tables 2, 3, and 4; Fig. 1 and 2).

A large degree of heterogeneity in the sizes and frequencies of occurrence of plasmids smaller than 50 kb was observed. However, some differences between the three genospecies were observed for the smaller plasmids that occurred in more than 50% of the strains. The plasmids present at this frequency were 30, 29, 28, 24, and 18 kb for *B. burgdorferi* sensu stricto, 32 and 21 kb for *B. garinii*, and 36, 27, 26, 25, and 24 kb for *B. afzelii* (Tables 2, 3, and 4; Fig. 2 and 3).

Hybridization patterns. Two *Bam*HI DNA fragments from

TABLE 4. Plasmid profiles of *B. afzelii*

Strain	Size(s) (kb) of plasmid(s) in the following size range:					No. of plasmids
	50–59 kb	40–49 kb	30–39 kb	20–29 kb	<20 kb	
M7	54.4		37.1, 33.0, 31.79	27.5, 25.2, 23.5		7
PGau	54.5		37.2, 31.3	29.1, 25.3, 24.5	13.3	7
VS461	54.4		36.6, 33.3	27.7, 26.1, 24.3		6
IPF	54.9		37.4	27.8, 26.2, 25.1, 22.9		6
2246	54.7	44.9	35.1	27.6, 26.5, 24.8, 24.2	18.6	8
ECM-1	54.2		36.4, 30.0	27.7, 26.1, 24.1		6
PKo	54.3		36.8, 33.5	28.5, 27.1, 25.2, 24.6, 24.2	16.9	9
BO23	57.7		36.8, 30.5	27.3, 25.9, 24.9, 23.2	14.5	8
Total no. of plasmids[a]	8	1	15	29	4	57
Avg no. of plasmids in each strain[b]	1.0	0.1	1.9	3.6	0.5	7.1 (1.1)[c]

[a] Total number of plasmids observed in all eight strains investigated.
[b] Ratio of the total number of plasmids observed to the total number of strains (eight strains) investigated.
[c] The number in parentheses is the standard deviation.

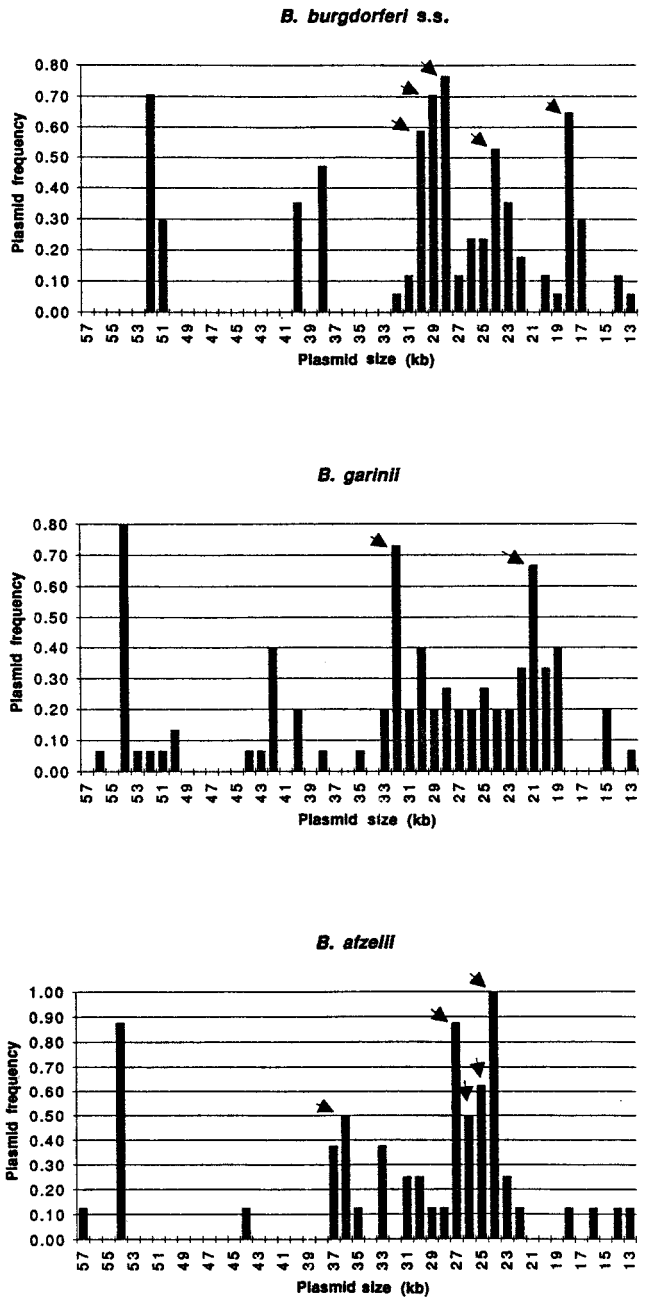

FIG. 3. Histogram showing the frequencies of plasmids in each genospecies of *B. burgdorferi* sensu lato. The frequency of plasmids in each kilobase range was calculated by the ratio of the total plasmid number observed in each kilobase range to the total number of strains in a genospecies. The arrows indicate the plasmids (<50 kb) occurring with a frequency of 50% or greater in each genospecies. s.s., sensu stricto.

the 28.7-kb plasmid of *B. burgdorferi* sensu stricto 297 were purified and used as probes to detect the presence of homologous nucleotide sequences among the plasmids of the three genospecies of Lyme disease borreliae. *Bam*HI probe 1 was 5.2 kb, and *Bam*HI probe 2 was 6.4 kb. The hybridization of the two probes with plasmids of representative members of the three genospecies is shown in Fig. 4, and the results with all of the test strains are tabulated in Table 5. If a strain of *B. burgdorferi* sensu stricto contained a plasmid that hybridized with one *Bam*HI probe, then that same plasmid hybridized with the second *Bam*HI probe. In contrast, most strains of *B. garinii* did not contain plasmids that hybridized with these probes. Two strains hybridized with both probes but did so with different plasmids, and one strain hybridized with only a single probe. The same pattern of hybridization was observed among the *B. afzelii* strains. However, all of the strains hybridized with the *Bam*HI probes. The sizes of *B. burgdorferi* sensu stricto plasmids that hybridized with the probes were all approximately the same, 27.5 to 29.8 kb. With the exception of one 44.8-kb plasmid, the plasmids of *B. afzelii* that hybridized with the probes were 24.0 to 27.7 kb, a size similar to those of the plasmids of *B. burgdorferi* sensu stricto. In contrast, the plasmids of *B. garinii* that hybridized with the *Bam*HI probes were dissimilar in size, ranging from 23.7 to 51.5 kb.

DISCUSSION

We investigated the relationship between plasmid profile and genospecies for 40 strains of *B. burgdorferi* sensu lato. Fifty-three percent of the strains were infectious for hamsters and probably lost few, if any, of their original complement of plasmids as a result of in vitro cultivation. Although the distribution of virulent and avirulent strains studied was uneven for *B. burgdorferi* sensu stricto and *B. afzelii*, virulent strains usually had a larger number of plasmids than avirulent strains. This pattern was most apparent with strains of *B. burgdorferi* sensu stricto. Virulent strains contained an average of 7.8 plasmids per cell, whereas the avirulent strains had an average of only 4.5 plasmids per cell. This observation is in agreement with reports of plasmid loss during continued in vitro cultivation and the associated loss of infectivity and virulence (3, 14, 22, 27, 28). Heterogeneity in plasmid profiles among strains of *B. burgdorferi* sensu lato has been reported by a number of investigators (3, 5, 15, 20, 27–29). Since different methods for plasmid analysis were used, it is difficult to directly compare the results of the various studies. The majority, if not all, of the plasmids we observed by using CHEF-PFGE are probably linear (12). For example, we observed eight plasmids in *B. burgdorferi* B31 (Fig. 2) by using CHEF-PFGE. When this strain was examined by two-dimensional agarose gel electrophoresis, three circular plasmids in addition to the eight linear plasmids were observed (unpublished data). Accordingly, the number of plasmids that we reported to be present in the strains may be less than the total number of plasmids actually occurring. We found that the plasmid sizes in the three genospecies ranged from 13.3 to 57.7 kb. These results are similar to those of Baril et al. (5). They reported that the plasmids of European and North American strains of *B. burgdorferi* sensu lato ranged from 15 to 60 kb.

We found that differences in the plasmid profiles of the three genospecies were most apparent for the large 50.6- to 57.7-kb plasmids. Heterogeneity in the size of the large *ospA*-encoding plasmid has been reported by a number investigators (3, 19, 25, 29). Samuels et al. (26) reported that the *ospA*-containing plasmid of *B. burgdorferi* sensu stricto was statistically significantly smaller (50 kb) than the equivalent plasmids (55 to 56 kb) of *B. garinii* and *B. afzelii*. We also found that large plasmids of *B. burgdorferi* sensu stricto were consistently smaller (51.8 to 52.7 kb) than the corresponding plasmids of *B. garinii* and *B. afzelii*. The latter two genospecies contained large plasmids of approximately the same size (54.0 to 54.9 kb). The exceptions to this finding were 3 of 15 *B. garinii* strains and 1 of 8 *B. afzelii* strains. The role of the multiple large plasmids of *B. garinii* PD89 and PBi is an area deserving further investigation. Although the profiles of the smaller plasmids (<50

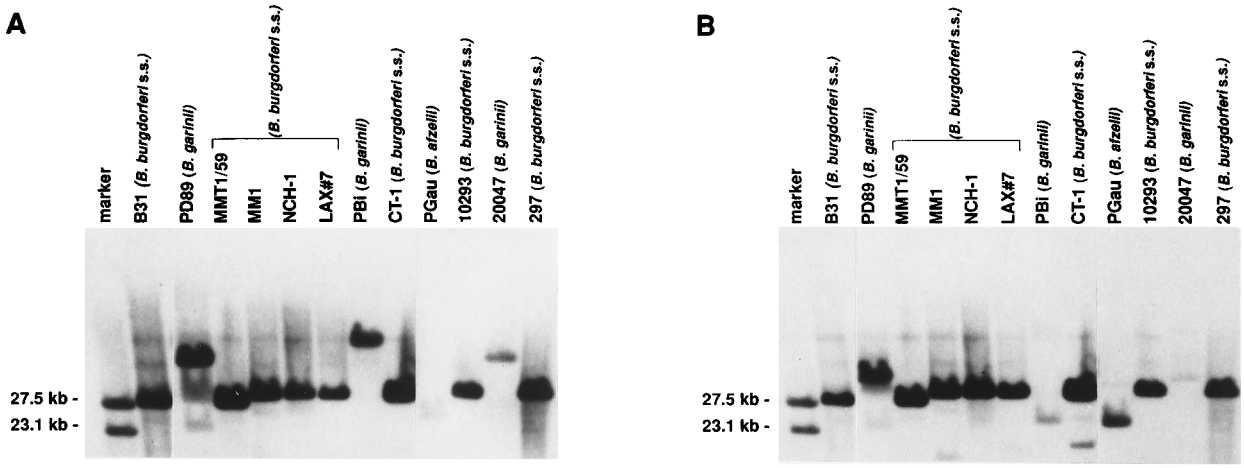

FIG. 4. Plasmid hybridization patterns of *B. burgdorferi* sensu lato with probes (*Bam*HI bands 1 and 2). After hybridization with *Bam*HI band 1 (A) the probe was stripped off and the membrane was used for hybridization with *Bam*HI band 2 (B). The marker is lambda DNA digested with *Hin*dIII and labeled with digoxigenin (Boehringer Mannheim).

TABLE 5. Plasmid hybridization patterns with *Bam*HI probes

Genospecies and strains	Size (kb) of plasmid hybridizing with *Bam*HI probe:	
	1	2
B. burgdorferi sensu stricto		
B31	27.5	27.5
MMT1/59	27.9	27.9
MM1	29.2	29.2
NCH-1	28.4	28.4
LAX#7	28.9	28.9
297	28.7	28.7
10293	29.8	29.8
CT-1	27.9	27.9
IPS	28.9	28.9
PKa 1	—[a]	—
ZS7	29.0	29.0
IP1	—	—
20001	29.1	29.1
20004	28.5	28.5
IP2	—	—
IRS	28.7	28.7
H11	—	—
B. garinii		
2226	—	—
2223	—	—
PD89	40.6	32.3
Fuji P1	—	—
Fuji P2	—	—
PBi	51.5	23.7
20047	43.4	—
G25	—	—
VSBP	—	—
BITS	—	—
IP89	—	—
IP553	—	—
Ir210	—	—
B. afzelii		
M7	27.5	25.2
2246	44.8	26.4
IPF	27.7	—
PGau	—	24.5
BO23	24.0	25.0
ECM-1	24.0	26.1
VS461	24.3	—

[a] —, no plasmid hybridized.

kb) were quite heterogeneous, it was possible to detect differences between the three genospecies when comparing those plasmids that were present in more than 50% of the strains. A plasmid occurring with a high frequency in a genospecies tended to occur at a very low frequency in the other genospecies, with the exception of the 24-kb plasmids, which occurred with high frequencies in both *B. burgdorferi* sensu stricto and *B. afzelii.*

Differences in the plasmid hybridization patterns of the three genospecies were demonstrated by using two *Bam*HI DNA fragments from a 28.7-kb plasmid of *B. burgdorferi* sensu stricto 297 as probes. A high degree of plasmid gene homogeneity was present in strains of *B. burgdorferi* sensu stricto whether they were of North American or European origin. Thirteen of 17 strains of this genospecies contained a plasmid of 27.5 to 29.8 kb that hybridized with both probes and were infectious for hamsters. The four strains that did not hybridize with the probes were noninfectious. These results suggested that these plasmids may play a role in the virulence of *B. burgdorferi* sensu stricto. However, an ongoing study has demonstrated that this plasmid is not necessary for the infectivity of *B. burgdorferi* sensu stricto (data not shown). All seven strains of *B. afzelii* contained plasmids that hybridized with the two probes. However, hybridization of the two probes did not occur on a single plasmid, as in the *B. burgdorferi* sensu stricto strains, but on different plasmids. Only 3 of the 13 strains of *B. garinii* contained plasmids that hybridized with the two probes, following the same pattern of hybridization as strains of *B. afzelii*. These results suggest that *B. garinii* and *B. afzelii* are more closely related to each other than they are to *B. burgdorferi* sensu stricto. These observations are in agreement with the interspecies DNA-DNA hybridization data of Postic et al. (24).

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