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Genetic Diversity among *Borrelia* Strains Determined by Single-Strand Conformation Polymorphism Analysis of the *ospC* Gene and Its Association with Invasiveness

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Lyme borreliosis (LB) is a tick-borne spirochetal infection caused by three *Borrelia* species: *Borrelia afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto. LB evolves in two stages: a skin lesion called erythema migrans and later, different disseminated forms (articular, neurological, cardiac. . .). Previous research based on analysis of *ospC* sequences allowed the definition of 58 groups (divergence of <2% within a group and >8% between groups). Only 10 of these groups include all of the strains isolated from disseminated forms that are considered invasive. The aim of this study was to determine whether or not invasive strains belong to restricted *ospC* groups by testing human clinical strains isolated from disseminated forms. To screen for *ospC* genetic diversity, we used single-strand conformation polymorphism (SSCP) analysis. Previously known *ospC* sequences from 44 different strains were first tested, revealing that each *ospC* group had a characteristic SSCP pattern. Therefore, we studied 80 disseminated-form isolates whose *ospC* sequences were unknown. Of these, 28 (35%) belonged to previously known invasive groups. Moreover, new invasive groups were identified: six of *B. afzelii*, seven of *B. garinii*, and one of *B. burgdorferi* sensu stricto. This study confirmed that invasive strains are not distributed among all 69 *ospC* groups but belong to only 24 groups. This suggests that OspC may be involved in the invasiveness of *B. burgdorferi*.

Lyme borreliosis (LB) is the most commonly reported tick-borne infection in Europe and North America. The etiological agent belongs to the complex *Borrelia burgdorferi* sensu lato, which comprises at least 11 species, of which only 3 are pathogenic for humans: *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* (2, 4). LB evolves in two stages that may occur independently. The first is a primary skin lesion that spreads from the site of the tick bite and is called erythema migrans (EM). Later, bacteria may disseminate to different organs and induce different clinical manifestations, i.e., neurological (neuroborreliosis [NB]), articular (arthritis), cardiac, cutaneous (multiple EM [MEM]), acrodermatitis chronica atrophicans (ACA), and lymphocytoma benigna cutis (LBC). Each of these manifestations is associated with a distinct pathogenic species, and more frequently a given patient experiences only one of these delayed manifestations (1, 5, 22, 23). The expression patterns of outer surface protein A (OspA) and OspC play an important role in the infection cycle. In unfed ticks, spirochetes express OspA but not OspC. During tick feeding, OspA synthesis is repressed whereas OspC synthesis is induced. The switch is associated with increased temperature and with exposure to tick hemolymph factor (10, 16, 19). Thus, OspC is the major outer surface protein expressed in early infection. Moreover, Masuzawa et al. (14) have shown that OspC expression is associated with infectivity. The *ospC* gene is highly variable (9, 12, 21, 25, 26). On the basis of *ospC* sequence analysis, Wang et al. (24) defined major *ospC* groups (divergence of <2% within a group and >8% between groups). Furthermore, Seinost et al. (17) and Baranton et al. (3) found that, among 149 sequences from data banks, 58 *ospC* groups can be defined within the three pathogenic species. Only 10 of these groups contain all of the strains (76 sequences) isolated from different clinical samples in disseminated forms (referred to secondary sites). These strains are hereafter called invasive. Two groups are individualized within *B. afzelii*, four within *B. garinii*, and four within *B. burgdorferi* sensu stricto. These groups are defined as invasive. Other *ospC* groups comprise strains isolated either from primary skin lesions or from ticks and are defined as noninvasive or of unknown invasiveness. In this study, we extended the investigation to 80 clinical strains isolated from secondary sites and of which the *ospC* sequence was unknown. We used single-strand conformation polymorphism (SSCP) analysis to study the genetic variability of the *ospC* gene. We determined the distribution among defined invasive groups. Our results confirm that invasive strains belong to given *ospC* groups. This suggests that the *ospC* gene could be one of the determinants involved in the invasiveness of strains leading to disseminated forms of the disease.

MATERIALS AND METHODS

***Borrelia* strains.** One hundred twenty-four *B. burgdorferi* strains were tested in this study. Forty-four strains whose *ospC* sequences are known were chosen, as indicated in Table 1. Eighty human-invasive strains were selected, including 61 Slovenian isolates. Fifty strains were isolated from different clinical samples (skin, blood, and cerebrospinal fluid [CSF]) in disseminated forms (ACA, MEM, LBC, and NB). Thirty strains isolated in the early stage were also included in this study. As they were isolated from the blood of patients with EM, they were considered to be invasive. Strains were cultured at 33°C in BSK-H medium (Sigma). The cultures were routinely monitored by dark-field microscopy for growth and contamination.

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TABLE 1. Strains previously identified as invasive[a] (A1 to G4) or noninvasive on the basis of their sequences

Strain(s)	Vector(s)	Source (condition)	Origin(s)	*ospC* group	Seinost *ospC* group designation (17)
B. afzelii					
ACA1		Skin (ACA)	Sweden	A1	
DK3, DK8		Skin (ACA)	Denmark	A2	
PKo, DK26		Skin (EM)	Germany, Denmark	A2	
E61		Skin (EM)	Austria	NI[b]	
Simon		Skin (EM)	Austria	NI	
H9		Skin (EM)	Austria	NI	
J1	*Ixodes persulcatus*		Japan	NI	
VS461	*I. ricinus*		Switzerland	NI	
B. burgdorferi sensu stricto					
28354	*I. scapularis*		United States	B1	K
297		CSF (NB)	United States	B1	K
DK7		Skin (ACA)	Denmark	B2	B
61BV3		Skin (EM)	Germany	B2	B
ZS7	*I. ricinus*		Switzerland	B2	B
HB19		Blood (arthritis)	United States	B3	I
Pka	*I. ricinus*		Germany	B4	A
IP1, IP2, IP3		CSF (NB)	France	B4	A
HII		Blood (arthritis)	Italy	B4	A
P1F		Synovia (arthritis)	Switzerland	B4	A
B31, 26816	*I. scapularis*		United States	B4	A
Mil	*I. ricinus*		Slovakia	NI	J
20006	*I. ricinus*		France	NI	P
212	*I. ricinus*		France	NI	Q
ESP1, Ne-56	*I. ricinus*		Spain, Switzerland	NI	R
Z136	*I. ricinus*		Germany	NI	S
Son188	*I. pacificus*		United States	NI	F
B. garinii					
N34, Far03	*I. ricinus; I. uriae*		Germany, Sweden	G1	
W		CSF (NB)	Austria	G2	
VSDA		CSF (NB)	Switzerland	G3	
PBi		CSF (NB)	Germany	G4	
DK6		CSF (NB)	Denmark	G4	
KL11	*I. ricinus*		Czech Republic	G4	
NBS16	*I. ricinus*		Sweden	NI	
NBS23	*I. ricinus*		Sweden	NI	
153	*I. ricinus*		France	NI	
20047	*I. ricinus*		France	NI	
T25	*I. ricinus*		Germany	NI	
BITS	*I. ricinus*		Italy	NI	

[a] A strain was considered invasive if it was isolated from secondary sites or when it belonged to a group comprising strains isolated from secondary sites.
[b] NI, noninvasive group.

DNA isolation. Bacterial cultures were harvested by centrifugation (10,000 × *g*; 10 min). The bacterial pellet was washed in phosphate-buffered saline, resuspended in water, heated at 100°C for 10 min, and then stored at −20°C.

PCR. A 277-bp fragment of the variable central part of *ospC*, suitable in size for SSCP analysis, was amplified by using forward primer SC3 (5′-AAAGCTA TTGGTAAAGTAAT-3′; bp 226 to 245; Genset) and reverse primer OspC92 (5′-GTTTTTAAAATAGCTTTTTTTG-3′; bp 491 to 470; Eurogentec), which are based on consensus sequences for the three pathogenic species.

Amplification was processed in 25 μl of a solution containing 0.2 μM each primer, 0.2 mM each deoxynucleoside triphosphate, 0.625 U of *Taq* polymerase (Q. Bio gene), and 1× *Taq* buffer (1.5 mM $MgCl_2$). The amplification reaction was carried out in a DNA thermal cycler (Touch Down Hybaid) under the following conditions: initial denaturation at 93°C for 1 min, followed by 35 cycles of denaturation at 93°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 30 s. Negative controls were included to check for contamination. Amplification was checked by agarose gel electrophoresis. A 5-μl volume of each sample was loaded onto a 1% TBE 1X (Tris-borate-EDTA) agarose gel and revealed by ethidium bromide staining.

SSCP. A 3-μl volume of the PCR product was added to 3 μl of denaturation solution (94% formamide, 0.05% xylene cyanol) and heated at 95°C for 5 min. Samples were loaded on a nondenaturing polyacrylamide gel (GeneGel Excel 12,5/24; Amersham Pharmacia Biotech). Electrophoresis was performed in a temperature-controlled electrophoresis system (GenePhor; Amersham Pharmacia Biotech) at 6°C with a first run at 600 V, 25 mA, and 15 W for 10 min and then at 600 V, 37 mA, and 21 W for 2 h 30 min.

Gels were revealed by silver staining (Plus One DNA Silver Staining Kit; Amersham Pharmacia Biotech) in accordance with the manufacturer's instructions.

DNA sequencing. The partial *ospC* gene (sizes ranged from 534 to 601 bp) was sequenced ($n = 25$) as previously described (13), by Genome Express, Montreuil, France.

Phylogenetic analysis. *ospC* gene sequences recorded from the GenBank database and new sequences (sizes ranged from 445 to 460 bp) were aligned manually by using VSM software and analyzed by the unweighted pair group method with mathematic averages (UPGMA) (18). Phylogenic trees were drawn with Mega software (11).

RESULTS AND DISCUSSION

SSCP is a screening method based on the secondary structure of a single-stranded DNA fragment. Different single-

TABLE 2. Clinical strains isolated from secondary sites with unknown *ospC* sequences

Strain(s)	Clinical data	Origin(s)	*ospC* group	Accession no. of *ospC* sequences determined in this study
B. afzelii				
Spet1793/01, Sbri	Skin (ACA)	Slovania	A1	
Svod, Srej	Blood (EM)	Slovenia	A1	
ACA2[a]	Skin (ACA)	Sweden	New invasive group A3	AY150206
P/sto[a]	Skin (ACA)	Germany	New invasive group A4	AY150205
Skoz	Blood (EM)	Slovenia	A4	
5 isolates (Skol,[a] Ssim[a])	Blood (MEM)	Slovenia	New invasive group A5	AY150203, AY150202
Shri	Blood (meningitis)	Slovenia	A5	
22 isolates	Blood (EM)	Slovenia	A5	
Spri[a]	Blood (MEM)	Slovenia	New invasive group A6	AY150204
Sspe, Sdol	Blood (EM)	Slovenia	A6	
Sobl[a]	Skin (ACA)	Slovenia	New invasive group A7	AY150201
Shrv[a]	Skin (ACA)	Slovenia	New invasive group A8	AY150200
Srav, Svir[b]	CSF (NB)	Slovania	A1	
GR4135[b]	CSF (NB)	Austria	A1	
Sjam, Spec[b]	CSF (NB)	Slovania	A5	
Sabu[b]	CSF (NB)	Slovania	A6	
B. garinii				
60, Schenk, PATO3	CSF (NB)	Austria	G1	
387[a]	CSF (NB)	Germany	G1	AY150188
Skos,[a] Sspa[a]	CSF (NB)	Slovenia	G1	AY150194, AY150197
57, GR4229	CSF (NB)	Austria	G2	
Sdom	CSF (NB)	Slovenia	G2	
Silc	Blood (EM)	Slovenia	G2	
239, VSBP	CSF (NB)	Austria, Switzerland	G4	
Scar, Stam[a]	CSF (NB)	Slovenia	G4	AY150187
Skle, Ssko	Blood (EM)	Slovenia	G4	
VSBM[a]	CSF (NB)	Switzerland	New invasive group G5	AY150185
PBr[a]	CSF (NB)	Germany	New invasive group G6	AY150186
GrLil	CSF (NB)	Austria	New invasive group G7	
Sbos[a]	CSF (NB)	Slovenia	New invasive group G8	AY150193
IBS-8,[a] Smrz[a]	CSF (NB)	France, Slovenia	New invasive group G10	AY150189, AY150196
Smar[a]	CSF (NB)	Slovenia	New invasive group G11	AY150195
IBS-9[a,b]	Skin (LBC)	France	G1	AY150190
Skot[b]	Skin (ACA)	Slovenia	G4	
Spet 114/95[a,b]	Skin (ACA)	Slovenia	G5	AY150192
Sles,[a,b] Ssob[a,b]	Skin (ACA)	Slovenia	G7	AY150199, AY150198
Spet 1058/01[a,b]	Skin (ACA)	Slovenia	New invasive group G9	AY150191
B. burgdorferi sensu stricto				
Lenz, Holzer	Cardiac muscle, blood	Austria	B4 (A)[c]	
Shaj[a,b]	Skin (ACA)	Slovenia	B2 (B)[c]	AY150208
Szid,[a,b] BRE-13[a,b]	Skin (ACA), CSF (NB)	Slovenia, France	New invasive group B5 (Q)[c]	AY150209, AY150207

[a] Strain whose *ospC* sequence was sequenced in this study.
[b] Strain isolated in a unusual clinical manifestation.
[c] Seinost *ospC* group designation (17).

stranded DNA sequences result in different conformational foldings. These conformational polymorphisms can be discriminated by their electrophoretic mobilities on polyacrylamide gels (8, 15). This method is widely used for mutation analysis (20). Theoretically, three bands are detectable on the gel (two single DNA strands and one double DNA strand), but heteroduplex formation causes several conformations that coexist in the gel. Under our conditions, different electrophoretic mobilities were indicative of sequence heterogeneities, irrespective of the genetic distance between sequences and of their mutation rates. This method was previously used (17, 24) to provide evidence for different groups of *ospC* mobility classes within *B. burgdorferi* sensu stricto. The authors also demonstrated that each mobility class had a unique sequence. Our first objective was to confirm that such data are also applicable to *B. garinii* and *B. afzelii*. The analysis was initiated by selecting strains from our collection ($n = 44$) that belong to the three pathogenic species and whose *ospC* sequence is available in data banks. Therefore, these strains could be classified into either invasive ($n = 26$) or noninvasive ($n = 18$) groups on the basis of their sequences (Table 1). Within each *ospC* group, the sequences were very similar, with less than 2% nucleotide differences. SSCP patterns were determined for all of the strains. Moreover, SSCP reproducibility was checked, revealing that the PCR product obtained in repeated experiments from a given DNA always yielded the same SSCP pattern and one PCR product always yielded the same SSCP pattern in different migrations.

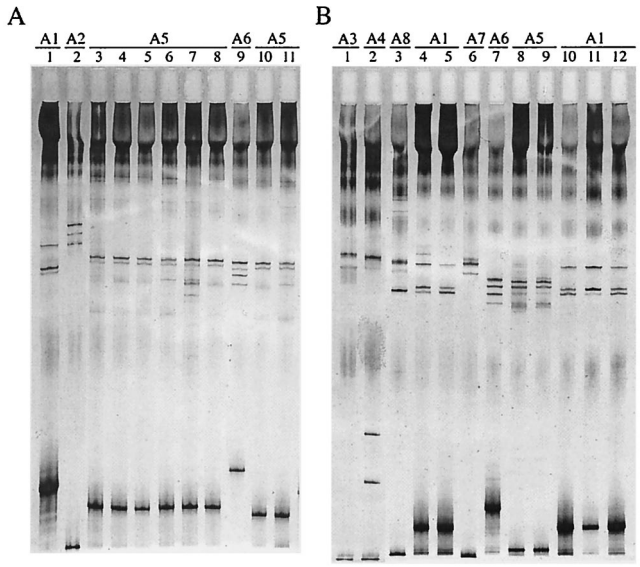

FIG. 1. *ospC* SSCP patterns of 21 invasive *B. afzelii* isolates. A 277-bp fragment of the central variable part of the *ospC* gene was amplified by PCR, denatured by heating and formamide, and electrophoresed in a nondenaturing polyacrylamide gel. (A) Lanes: 1, *B. afzelii* ACA1; 2, *B. afzelii* DK8; 3, *B. afzelii* Shri; 4, *B. afzelii* Skol; 5: *B. afzelii* Szaj (a strain of the five Slovenian isolates from blood [MEM]); 6: *B. afzelii* Savb (a strain of the five Slovenian isolates from blood [MEM]); 7, *B. afzelii* Ssim; 8, *B. afzelii* Svre (a strain of the five Slovenian isolates from blood [MEM]); 9, *B. afzelii* Spri; 10, *B. afzelii* Sper (a strain of the 22 Slovenian isolates from blood [EM] [Table 2]), 11, *B. afzelii* Slet (a strain of the 22 Slovenian isolates from blood [EM] [Table 2]). (B) Lanes: 1, *B. afzelii* ACA2; 2, *B. afzelii* P/sto; 3, *B. afzelii* Shrv; 4, *B. afzelii* Spet 1793/01; 5, *B. afzelii* Sbri; 6, *B. afzelii* Sobl; 7, *B. afzelii* Sabu; 8, *B. afzelii* Sjam; 9, *B. afzelii* Spec; 10, *B. afzelii* Srav; 11 *B. afzelii* Svir; 12, *B. afzelii* GR4135.

Within the 26 invasive strains, we identified 11 distinct patterns, each corresponding to 1 of the 10 invasive groups previously described (A1 and -2, B1 to -4, and G1 to -4; Table 1) (3, 17). However, within invasive group G4, two different SSCP patterns were observed. From the 18 noninvasive strains, 17 different SSCP patterns were recorded, in complete accordance with the sequence analysis (data not shown). These results confirmed that each *ospC* group should correspond to a specific SSCP pattern, in agreement with previous studies (17, 24). Therefore, SSCP patterns could be used as references for the assignment of clinical strains to invasive or noninvasive groups.

Further, since SSCP methodology has been validated as a powerful and reproducible screening tool, we tested 80 clinical invasive European isolates from different secondary sites (Table 2). Additionally, some *ospC* sequences were determined in order to confirm the assignment of strains to a given invasive group.

Forty-six invasive *B. afzelii* strains were analyzed by SSCP. Seven distinct SSCP patterns were recorded (Fig. 1), corresponding to seven different *ospC* groups in accordance with the phylogenetic analysis of sequences (Fig. 2). Seven strains exhibited the typical pattern of invasive group A1. No pattern corresponding to previously described group A2 was observed. The 39 remaining strains belonged to six new distinct invasive groups designated A3 to A8 (Table 2 and Fig. 2). Strains E61 and Simon, which have been previously designated noninvasive (3), had the same SSCP pattern as invasive strains ACA2 and P/sto, respectively (Table 1 and data not shown). *ospC* sequencing and phylogenetic analysis confirmed that these strains should now be included in new invasive groups A3 and A4 (Table 2 and Fig. 2). Invasive group A5 comprised isolate Orth, which was not previously considered to be invasive on the basis of the sequence data (Fig. 2). Although invasive strains Spri, Sspe, and Sdol exhibited identical SSCP patterns that were different from that of noninvasive strain H9, sequencing data allowed them to be assigned to new invasive group A6 (data not shown; Table 2 and Fig. 2). Therefore, group A6 exhibited two different patterns.

Twenty-nine invasive *B. garinii* strains were also tested by SSCP. Eighteen strains exhibited a pattern previously assigned to an invasive group; seven patterns referred to invasive group G1, four patterns referred to G2, and seven patterns referred to G4. Seven distinct SSCP patterns were identified among the 11 remaining strains (data not shown). *ospC* sequencing confirmed that these *B. garinii* strains belonged to seven new distinct invasive groups designated G5 to G11 (Table 2 and Fig. 2). The SSCP patterns of groups G5 (strains VSBM, Spet 114/95, and BITS), G9 (strains Spet 1058/01 and 20047), and G10 (strains IBS-8, Smrz, and T25), which have been previously designated distinct noninvasive groups (3), should be now assigned to invasive groups (data not shown; Table 1 and Fig. 2). A sequence homologous to that of group G8 was also found in data banks (Fig. 2). The three remaining invasive groups (G6, G7, and G11) constituted new *ospC* groups (Table 2 and Fig 2).

Finally, we tested five *B. burgdorferi* sensu stricto isolates. Two SSCP patterns referred to invasive group B4, and one pattern referred to B2. The two remaining strains, which had the same SSCP pattern as strain 212 and were previously identified as noninvasive, should now be included in new invasive group B5 (data not shown; Table 1 and Fig. 2). Wang et al. (24) and Seinost et al. (17) have screened a large number of *ospC* genes from North American human and tick *B. burgdorferi* sensu stricto isolates. This study allowed us to list all of the *ospC* groups found in North America. As additional invasive group B5 found in this study comprised only strains from Europe, it had not been identified in those previous studies. Genetic diversity studies based on the whole genome (7) or on the *ospC* gene (13) have shown that North American *B. burgdorferi* sensu stricto isolates are more heterogeneous than European ones and that some groups are restricted to either North America or Europe. Analysis of all of the *B. burgdorferi* sensu stricto *ospC* sequences found in data banks revealed that invasive groups B1 to -4 are all found in North America, whereas only groups B2, B4, and B5 are found in Europe, suggesting that groups B1 and B3 are restricted to North America and B5 is restricted to Europe.

Previous studies based on *ospC* sequences (3, 17) allowed the definition of 10 invasive groups within 58 groups. Within the 80 strains tested, 28 (35%) belonged to previously defined invasive groups and the 52 remaining strains (65%) belonged to the 14 new invasive *ospC* groups identified in this study. Nine of these 14 groups comprise isolates that, on the basis of their *ospC* sequences, were previously classified as noninvasive,

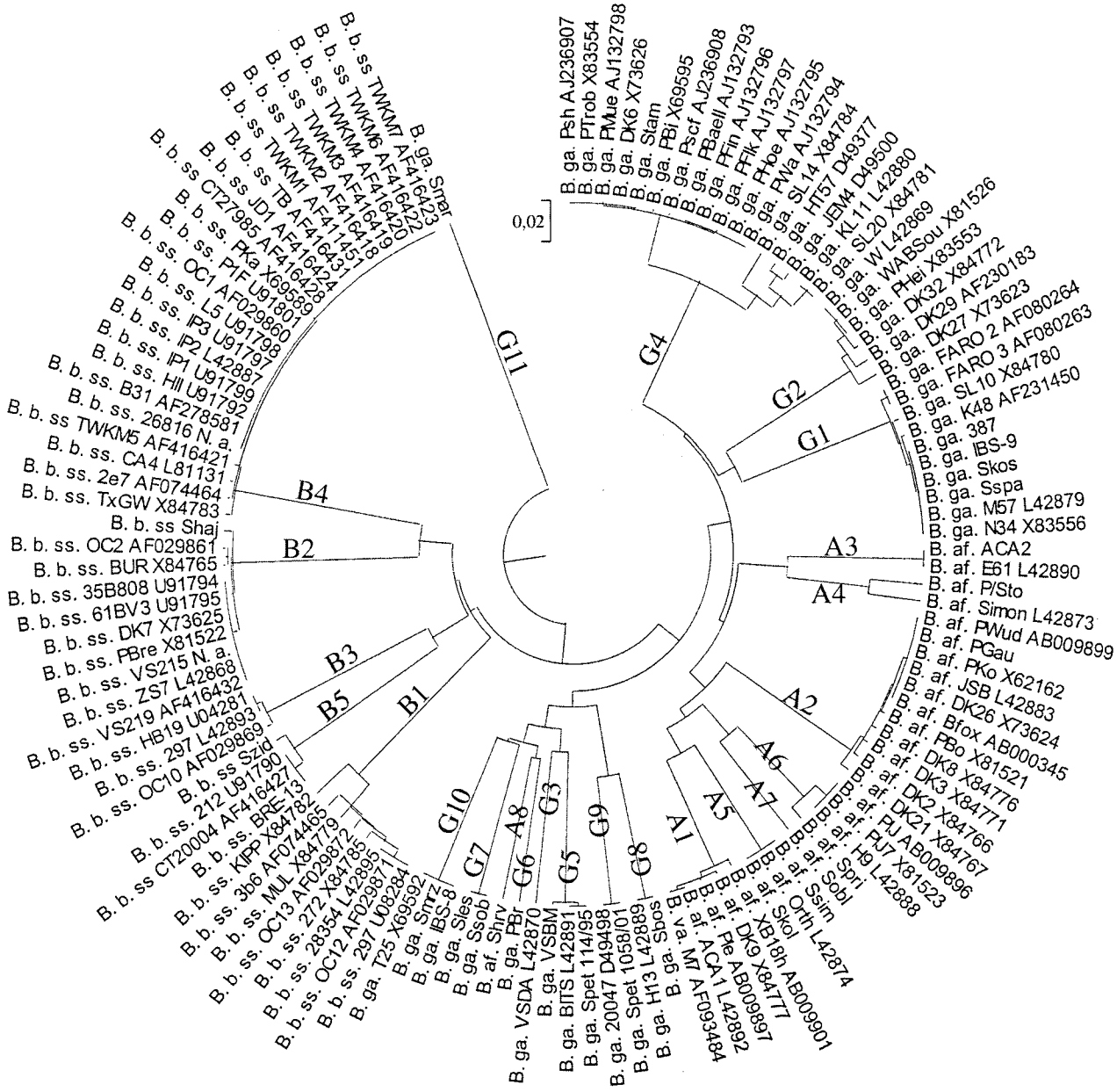

FIG. 2. UPGMA-based tree of invasive *ospC* sequences from 100 isolates available in data banks (indicated by their accession numbers) and the 25 *ospC* sequences determined in this study. B. b. ss, *B. burgdorferi* sensu stricto; B. ga., *B. garinii*; B. af., *B. afzelii*; B. va., *B. valaisiana*.

increasing the proportion of invasive groups. However, invasive *ospC* groups A7, A8, G6, G7, and G11 constituted previously unobserved *ospC* groups. At the same time, new *ospC* groups were also identified and added to data banks, increasing the global *ospC* diversity to 69 *ospC* groups. Thus, *ospC* sequence analysis allowed us to define 69 groups among 204 sequences, including 24 invasive groups (125 sequences) (Fig. 3 [a table listing nucleotide identities for the newly described *ospC* groups is available]). All of these results confirmed that invasive strains were not distributed within all *ospC* groups but belonged to restricted *ospC* groups (24 [35%] of 69) (Fig. 3). Moreover, the strains additionally studied in this work were selected for their invasiveness, thus increasing the probability of finding only new invasive groups.

Fingerprinting studies (12, 25, 26) indicate that *B. garinii* is the most heterogeneous species, whereas *B. afzelii* is the most homogeneous one. We found 11 invasive groups for *B. garinii*. Within group G4, which was very heterogeneous, as indicated by the phylogenetic tree in Fig. 2, two different patterns were observed. These data confirmed that *B. garinii* is heterogeneous. However, surprisingly, we found eight invasive groups of *B. afzelii* and two different patterns within the A6 group. Thus, *B. afzelii* was as heterogeneous as *B. garinii* regarding the *ospC* gene.

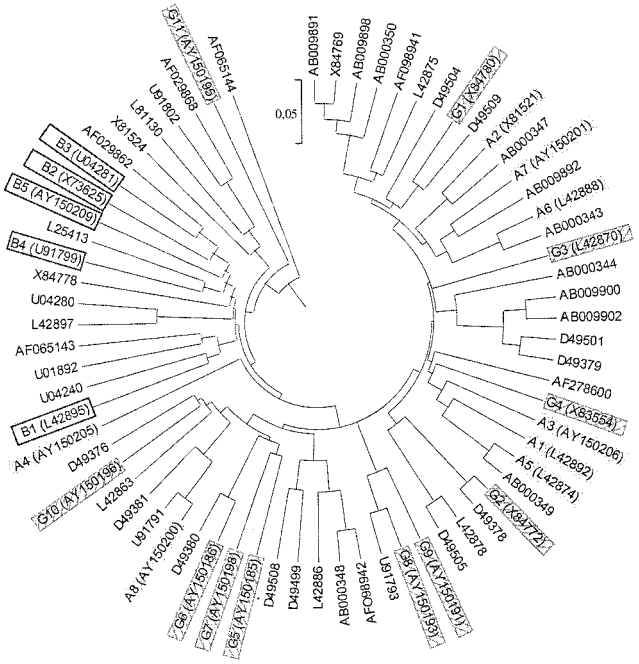

FIG. 3. *ospC* genetic diversity tree (UPGMA method) drawn from one representative sequence, indicated by accession numbers, from each of 69 *ospC* groups of the three pathogenic species (204 sequences). Invasive groups are identified by their coding abbreviations (A1 to G11). *B. afzelii* invasive group, plain gray shading; *B. burgdorferi* sensu stricto invasive group, no hatching or shading; *B. garinii* invasive group, cross-hatched and shaded gray.

Wang et al. (24) have shown that the genetic diversity of *B. burgdorferi* sensu stricto within a local population of ticks is almost equal to the worldwide genetic diversity. Our study revealed that *ospC* groups identified from human disseminated forms were found in different areas, suggesting that these groups are widely distributed. Moreover, the analysis of 61 Slovenian strains showed that most invasive groups were found in a local area (Table 2). These results were in agreement with those of Wang et al. (24). However, a heterogeneous distribution of the sequences of human origin was found. For instance, in data banks, 11 out of 14 sequences from humans in group G4 were from strains isolated in Germany. Regarding the Slovenian strains tested in this study, 30 (70%) out of the 43 invasive *B. afzelii* strains fell into group A5. These data suggested that all of the *ospC* groups had a wide range but some groups could be selected, in humans, in a restricted geographical area.

Each pathogenic *Borrelia* species is predominantly associated with a given late clinical manifestation: *B. afzelii* with cutaneous manifestations, *B. garinii* with neurologic manifestations, and *B. burgdorferi* sensu stricto with articular manifestations (1, 22). However, sequences from strains isolated from each clinical manifestation are scattered along the tree (data not shown). Fifteen strains included in our study were responsible for a clinical manifestation different from that expected (six *B. afzelii* strains isolated from CSF, five *B. garinii* strains from ACA, two *B. burgdorferi* sensu stricto strains from ACA, one *B. garinii* strain from LBC, and one *B. burgdorferi* sensu stricto strain from CSF; Table 2). Indeed, lateral transfer, which is common in the *ospC* gene (6, 9, 12, 13), could have been responsible for such interspecific organotropism. However, our results showed that *ospC* sequences from these particular isolates clustered together with strains isolated from expected clinical manifestations. For example, *B. afzelii* strains Srav and Svir, isolated from the CSF of a patient with NB, belonged to group A1, as did Spet 1793/01 and Sbri, which were isolated from a patient with ACA (Table 2). Moreover, sequence analysis revealed that *B. burgdorferi* sensu stricto strains P1F (arthritis) and IP1-2-3 (NB) in group B4 have exactly the same *ospC* sequence (Table 1 and Fig. 2). Furthermore, it was found that *B. afzelii* strain Shrv, which was involved in a genetic transfer from *B. garinii*, was responsible for a case of ACA in a Slovenian patient. Our data indicate that clinical presentation is not associated with a given *ospC* sequence.

Our results and those of others (17, 24) have demonstrated that SSCP can be used for epidemiological studies of tick isolates or first-stage EM isolates in order to evaluate the proportion of invasive strains and to predict evolution toward disseminated forms. Extensive geographical studies could determine the distribution and frequency of *ospC* groups according to the area.

OspC expression is induced during tick feeding (16), and it is the major outer surface protein expressed in early infection, but its role is still unknown. Our results show that invasive strains belong to restricted *ospC* groups, suggesting that OspC is one of the factors involved in *Borrelia* invasiveness. Further studies to determine the role of OspC in *Borrelia* invasiveness are in progress in our laboratory.

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