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# Sequence and phylogenetic analysis of the *Borrelia burgdorferi secA* gene [1]

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**Abstract**

A *Borrelia burgdorferi secA* homologue was cloned and the complete DNA sequence was determined. The deduced protein sequence consists of 899 amino acids and shows a high degree of homology to SecA homologues from other Bacteria and photosynthetic plastids. The presence of the *secA* gene in Spirochetes suggests that this gene is present in most if not all major lineages within Bacteria. The ease of isolation of *secA* by conservation of its ATP-binding motifs combined with its extreme conservation in protein secretion pathways and the presence of a phylogenetic sequence marker in one of its ATP-binding domains makes this gene useful for phylogenetic analysis of Bacteria and photosynthetic plastids. © 1998 Elsevier Science B.V.

*Keywords:* SecA ATPase; Protein secretion; (*Borrelia burgdorferi*)

Outer membrane proteins (OMPs) of *Borrelia burgdorferi* have important roles for the maintenance of this spirochete in different host environments and in virulence. Differential expression of OMPs enables *B. burgdorferi* to disseminate through host tissues [1], to evade the host's immune response [2–4], and to acquire nutrients essential for the growth of the bacterium [5]. Most of these proteins contain fatty acids covalently attached to an N-terminal cysteine which is produced after processing by signal peptidase II [6]. Precursors of *B. burgdorferi* OMPs contain typical signal sequences and are transported by a *sec*-dependent pathway across the cytoplasmic membrane when expressed in *Escherichia coli* [7]. The SecA protein plays a central role in protein export in *E. coli* due to its ability to interact with most other components of the protein translocation system [8], and it is conserved among various Bacteria and certain photosynthetic plastids [9–19]. SecA recognizes conventional signal peptides and unknown elements of the mature domain of precursor proteins [20,21], and it couples the hydrolysis of ATP to the process of translocation of preprotein across the plasma membrane [22]. SecA also autoregulates itself in *E. coli* by binding its mRNA and competing with and dislodging initiating ribosomes [23].

Spirochetes are a kingdom of Bacteria remote from purple and Gram-positive bacteria where the Sec-dependent protein secretion pathway has been discovered and characterized. Furthermore, the absence of a *secA* homologue in the recent sequence of the Archaea, *Methanococcus jannaschii* [24], and the pres-

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[1] GenBank accession number for *B. burgdorferi secA* gene is AF003354.


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ence of signal recognition particle and signal recognition particle receptor homologues in Archaea and Bacteria [25,26] render uncertain the origin and retention of a SecA-dependent protein secretion pathway in various members of the domain Bacteria. In order to resolve this query and initiate studies of the protein translocation mechanism and its regulation in *B. burgdorferi*, we have attempted to clone a *secA* homologue from this organism.

Using similarity data for different *secA* homologues, degenerate 17-mer oligonucleotide primers homologous to the highly conserved ATP-binding sites of SecA [27] were designed according to the reported codon usage for *B. burgdorferi* [28]. These primers were employed in polymerase chain reactions (PCR) to amplify corresponding regions of a putative *secA* homologue from *B. burgdorferi* genomic DNA. DNA fragments of sizes comparable to those ampli-

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TATTACATGATATAATTGCAAAAAGCAGGCTTAATATGAGAGCTTTATAGTGTTTTTTCATATTTTTTATATTATTATTTAAAATGACTT   90
TAAGTCAATTATTTAGGAGAATTAATACCTACCATGTTAAAAGCAGTACTTGAGACAACTATTGGCTCAAAAAGTAAAAAGAGATTTAAAA  180
                                  M  L  K  A  V  L  E  T  T  I  G  S  K  S  K  R  D  L  K
GATTATCTTCCGACTTTAAGAAATATTAATAAGCTTGAGCGTTGGGCATTATTGTTGGCAGATGAAGATTTTTCAAAGGAGACAGAAAAG  270
 D  Y  L  P  T  L  R  N  I  N  K  L  E  R  W  A  L  L  L  A  D  E  D  F  S  K  E  T  E  K
CTTAAAGATGAATTAAAATCGGGTAATTCTTTAGAGAATATTTTAGAGCGAGCTTTTACTCTGTCTAGAGAAGCTGCTAGAAGGCGTCTT  360
 L  K  D  E  L  K  S  G  N  S  L  E  N  I  L  E  R  A  F  T  L  S  R  E  A  A  R  R  R  L
AAAGAAAGGCCTTATGATGTGCAAATCATTGCGGGGCTTGCTCTTCACAAAGGCAAAATAATAGAAATGAAAACGGGAGAAGGAAAAACT  450
 K  E  R  P  Y  D  V  Q  I  I  A  G  L  A  L  H  K  G  K  I  I  E  M  K  T  G  E  G  K  T
CTCTCCTCAGTTCAAGCGGCGTATTTAAATAGTTTAACAGGAGATGGGGTTATTATTGTTACTGTTAATGACTATCTTGCAGAACGTGAT  540
 L  S  S  V  Q  A  A  Y  L  N  S  L  T  G  D  G  V  I  I  V  T  V  N  D  Y  L  A  E  R  D
TCCAATTGGATGAAGCCGGTTTTTGATCTTTGGGTGTTAGCGTGGGGGTTGTTCTATCTAATATGGATTATGAGCTAAGAAAGGCTCAA  630
 S  N  W  M  K  P  V  F  D  L  L  G  V  S  V  G  V  V  L  S  N  M  D  Y  E  L  R  K  A  Q
TATGCTAAAGATATTACTTATGTTACAAATAATGAACTTGGATTTGATTATCTAAGAGATAATATGCGTTATGACTTGAATGAAAAATCC  720
 Y  A  K  D  I  T  Y  V  T  N  N  E  L  G  F  D  Y  L  R  D  N  M  R  Y  D  L  N  E  K  S
TTGAGAAAGTTTAATTATTGTATTATTGATGAAATTGATTCTATTTTGATCGATGAGGCAAGAACCCCATTGATTATTTCAGGGCCTACT  810
 L  R  K  F  N  Y  C  I  I  D  E  I  D  S  I  L  I  D  E  A  R  T  P  L  I  I  S  G  P  T
GAAGGCAACACTAATGCTTATCTTGAAGTTAATTCTCTTGTATCTTTTTTAAAAGAATGTTCCAAGGATCCCAAAACAGGTGATTATCCT  900
 E  G  N  T  N  A  Y  L  E  V  N  S  L  V  S  F  L  K  E  C  S  K  D  P  K  T  G  D  Y  P
TTAGAAATAGACGACTTTGATGGTGATTATACTGTTGATGAAAAAGCCAAAAGAATTTCTTTTACTGCTAAAGGGCTTAATAATCTTGAA  990
 L  E  I  D  D  F  D  G  D  Y  T  V  D  E  K  A  K  R  I  S  F  T  A  K  G  L  N  N  L  E
CAGCTTTTAGTTTCTAAAGGCATTATTAGTGGGTCTATGTATACTGATTCAAATTTTAATTATGTTCATTATATGACTCAAGCCTTAAAA 1080
 Q  L  L  V  S  K  G  I  I  S  G  S  M  Y  T  D  S  N  F  N  Y  V  H  Y  M  T  Q  A  L  K
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GGGCGAAGATATTCAGATGGACTTCACCAAGCTATTGAGGCTAAAGAAGGAGTTAGAGTTGCTAATGAAAATAAGACTATGGCAACTATT 1260
 G  R  R  Y  S  D  G  L  H  Q  A  I  E  A  K  E  G  V  R  V  A  N  E  N  K  T  M  A  T  I
ACTTTTCAAAATTTATTTAGAATGTTTGATAAAATTTCTGGCATGACAGGTACAGCTGATACAGAAGCTAAGGAATTTGATAAAATATAT 1350
 T  F  Q  N  L  F  R  M  F  D  K  I  S  G  M  T  G  T  A  D  T  E  A  K  E  F  D  K  I  Y
AATCTTGATGTAGTTGTAGTTCCAACAAATAGATTGTTAGCGCGAATAGATGAGGATGATACTATTTATTATACGGAAGAATTTAAATTT 1440
 N  L  D  V  V  V  V  P  T  N  R  L  L  A  R  I  D  E  D  D  T  I  Y  Y  T  E  E  F  K  F
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 N  A  I  T  D  E  V  Y  K  T  Y  K  K  G  Q  P  V  L  V  G  T  V  S  I  E  K  S  E  I  L
TCAGCTATGTTTAAAAGCAGAGGGATTAAGCATGAAGTTCTTAATGCAAAAAATCATTCTCGAGAAGCATTTATTATTGCTGAAGCTGGA 1620
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GCAAAACATGCGGTTACAATAGCAACAAATATGGCTGGTCGTGGTACGGATATTAAGCTTGGGGGAAATATTGAGCACAGAGTTAGAAAA 1710
 A  K  H  A  V  T  I  A  T  N  M  A  G  R  G  T  D  I  K  L  G  G  N  I  E  H  R  V  R  K
AAAATCGGAACTAATGTAAGCCTTGAAGAATTTCAAGAGGCTGTTAAAAATGAGAGAGAAAATTACCTGAAAGACTACAATGAGGTTAAA 1800
 K  I  G  T  N  V  S  L  E  E  F  Q  E  A  V  K  N  E  R  E  N  Y  L  K  D  Y  N  E  V  K
AGTCTTGGTGGGCTTTATGTTATTGGTAGTGAACGTCACGAATCAAGGCGAATAGACAATCAGCTTCGTGGGCGTAGTGGAAGACAAGGT 1890
 S  L  G  G  L  Y  V  I  G  S  E  R  H  E  S  R  R  I  D  N  Q  L  R  G  R  S  G  R  Q  G
GACCCTGGGCGCTCAAGATTTTATGTGTCCCTTGAGGACGATTTAATGCGCCTTTTTGCTGGGGACAACCTAAGATCATTAATGGGTAAG 1980
 D  P  G  R  S  R  F  Y  V  S  L  E  D  D  L  M  R  L  F  A  G  D  N  L  R  S  L  M  G  K
CTTGGAATGGCAACAGGAGAGCCTATTACACATTCTCTTTTAACTAAATCTTTGATTAATGCTCAAAAACGAGTAGAAGACAGAAATTTT 2070
 L  G  M  A  T  G  E  P  I  T  H  S  L  L  T  K  S  L  I  N  A  Q  K  R  V  E  D  R  N  F
GAAATTAGAAAGCATTTGTTAGAGTATGACGATGTTATTACAAAACAAAGAGATTTTATTTATGCTCAGAGAAACTCCATTCTTGAAGAT 2160
 E  I  R  K  H  L  L  E  Y  D  D  V  I  T  K  Q  R  D  F  I  Y  A  Q  R  N  S  I  L  E  D
ACAGCTATTAAGGATCGTATTCTTGTTGCTTTAGAAGAATATCTTAGTTTTTTGCTTGAAGGGACAAAAAGTAGCACGGTTTCAAATGTT 2250
 T  A  I  K  D  R  I  L  V  A  L  E  E  Y  L  S  F  L  L  E  G  T  K  S  S  T  V  S  N  V
TTTTTAAATGAAGTAAATTCAATTTTTGCTTATATGCTTGAGAGTCTTGGTTCTATTGAAAATATTAGTTCTCTTGATTTAAAGGCTAAG 2340
 F  L  N  E  V  N  S  I  F  A  Y  M  L  E  S  L  G  S  I  E  N  I  S  S  L  D  L  K  A  K
CTAATGCAAATAGCAAAAGCAAATTTAGATGAAAAGGAAAATTTGATTGGTAGAGATCTTTTTAATGGATTTTTAAGATATGAATATTTG 2430
 L  M  Q  I  A  K  A  N  L  D  E  K  E  N  L  I  G  R  D  L  F  N  G  F  L  R  Y  E  Y  L
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 P  I  T  E  Y  K  E  E  G  F  S  I  F  S  E  L  I  K  D  I  K  V  S  T  I  R  R  V  L  Q
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 L  K  L  D  S  N  S  S  D  F  K  S  T  K  K  S  R  N  V  K  P  I  H  K  E  L  S  G  I  V
ATTAATGAGAATAAAAGCGCTTCTAATGTTCAAGTGGTTAGAAGTTCTCCTAAAATAGGCAGAAATGAGCCTTGTTATTGTGGAAGTGGG 2790
 I  N  E  N  K  S  A  S  N  V  Q  V  V  R  S  S  P  K  I  G  R  N  E  P  C  Y  C  G  S  G
AAGAAATATAAAAATTGTCATGGCAAAAGTTAAAACAGGAGGTTTTTATGTTTAAGCTGCCAGAACTTGGTTATGATTATGATGCTGTTG 2880
 K  K  Y  K  N  C  H  G  K  S  *
AGCCTTATATTGATGCTAAAACTATGGAAATTCATCATAGCAAGCATCATAATGGTTTTGTAATGAATTTGAATTCCTATATTATTAGTA 2970
GTATTATTAATACACTCCCATTTAACACTACTATTTATTTGATTAAATTTTTTTATTCTT                                 3027
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Fig. 1. Nucleotide and deduced amino acid sequence of *B. burgdorferi* N40 *secA* gene (GenBank accession number AF003354). A predicted Shine–Dalgarno sequence for *secA* is underlined.

fied from *E. coli secA* were obtained. Additional degenerate primers containing restriction enzyme sites *Eco*RI (Eco) and *Bam*HI (Bam) were then designed as follows: Eco-1F (GGAATTCAC[A/T]GG[A/T]GAAGG[A/T]AAAAC, where the underlined sequence represents nucleotides 310 to 326 in the Walker A motif [29] of the high-affinity ATP-binding site of the noncoding strand of *E. coli secA*; see Fig. 2) and Bam-2R (CGGGATCCGG[A/T]GTTCT[A/T]GCTTCATC, where the underlined sequence represents nucleotides 665 to 649 in the Walker B motif of the high-affinity ATP-binding site of the coding strand of *E. coli secA*), and employed in comparable PCR reactions. Amplified DNA fragments were digested with *Eco*RI or *Bam*HI, resolved on an agarose gel, purified and ligated into the pBluescriptII SK-plasmid (Stratagene) which had been digested similarly. The ligation mixture was transformed into *E. coli* strain XL1-Blue (New England Biolabs), and recombinants were selected by plating on Luria broth (LB) plates supplemented with 100 $\mu$g ml$^{-1}$ of ampicillin.

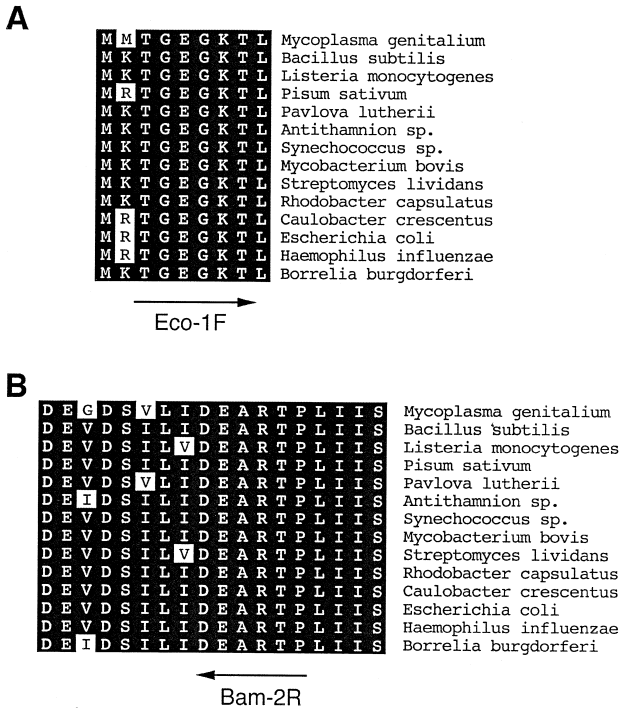



Fig. 2. Sequence alignment around the Walker A (A) and B (B) motifs of the high-affinity ATP-binding site of SecA protein. Blackened areas depict positions of identical amino acid residues. The location of the primers used to amplify a portion of the *B. burgdorferi secA* gene is shown.

Plasmid DNA was isolated from the resulting clones, and the DNA sequence of the *B. burgdorferi* DNA fragment was determined using a Sequenase kit™ (United States Biochemical). This fragment contained a 358 bp open reading frame exhibiting very high identity to the corresponding regions of *E. coli* SecA (58%) and *B. subtilis* SecA (57%). Oligonucleotide primers specific for the cloned DNA fragment were end-labeled with [$\gamma$-$^{32}$P]-ATP using T4 polynucleotide kinase and used to screen a *B. burgdorferi* N40 genomic library (gift of Erol Fikrig). This library was created using random-sheared *B. burgdorferi* genomic DNA and a Lambda Zap II vector (Stratagene). Only two ($\lambda$13 and $\lambda$20) of 20,000 phage plaques screened gave positive hybridization signals. These phage were purified and pBluescript SK-plasmids were excised from the phage genome and cloned into the 'excision strain' (SOLR™, Stratagene) according to the manufacturer's protocol. The resulting plasmid DNA (pC-13 and pC-20) was isolated and the approximate size of the cloned DNA fragments was determined to be 4.0 kb and 2.2 kb, respectively. The complete nucleotide sequence of the *B. burgdorferi* DNA fragments was determined for both strands. The insert in pC-13 contained a large open reading frame of 2697 bp encoding a protein of 899-amino acids ($M_r$ 102,084; Fig. 1), which was of similar size to *E. coli* SecA (901 amino acids; $M_r$ 101,900). Comparison of this sequence to the GenBank database using TBLASTN showed that this open reading frame exhibited a very high similarity with *E. coli* and *Bacillus subtilis* SecA proteins (Table 1). However, the polyclonal antisera raised against either of these two proteins failed to recognize any protein of the predicted molecular weight on a Western blot containing total cellular protein of *B. burgdorferi* B31. *Borrelia* SecA protein could be immunologically distinct from these two homologues, or the SecA protein may be expressed at a lower level in *B. burgdorferi* under these conditions where the amount of protein export would be substantially lower than *E. coli* or *B. subtilis* given the slow growth rate of *B. burgdorferi* in culture. It was shown previously that polyclonal antisera raised against either *E. coli* or *B. subtilis* SecA protein was only weakly cross-reactive to the other protein [30].

SecA homologues from different bacterial species were aligned by the Clustal method using DNAstar-

Table 1
The similarity between *B. burgdorferi* SecA and other bacterial and photosynthetic plastid homologues

| Organism | % Similarity |
|---|---|
| *Bacillus subtilis* | 42.3 |
| *Caulobacter crescentum* | 35.9 |
| *Escherichia coli* | 43.2 |
| *Haemophilus influenzae* | 41.9 |
| *Listeria monocytogenes* | 42.0 |
| *Mycobacterium bovis* | 37.3 |
| *Mycoplasma genitalium* | 35.5 |
| *Rhodobacter capsulatus* | 40.9 |
| *Streptomyces lividans* | 39.7 |
| *Synechococcus* sp. | 35.7 |
| *Antithamnion* sp. | 36.8 |
| *Pavlova lutherii* | 35.0 |
| *Pisum sativum* | 35.8 |

The GenBank accession numbers are as follows: *Antithamnion* sp. (red alga), X64705; *B. subtilis*, D10279 and D90218; *C. crescentus*, U06928; *E. coli*, M20791; *H. influenzae*, U32772 and L42023; *L. monocytogenes*, L32090; *M. bovis*, U66080; *M. genitalium*, U39687 and L43967; *P. lutherii* (chromophytic alga), X65961; *P. sativum* (pea), X82404; *R. capsulatus*, X89411; *S. lividans*, U21192; *Synechococcus* sp., X74592.

MegAlign software, version 1.02 (DNAStar). *B. burgdorferi* SecA exhibited a high similarity at the amino acid level to its other bacterial and photosynthetic plastid homologues (Table 1), especially at the highly conserved high-affinity ATP-binding site (Fig. 2). However, *B. burgdorferi* SecA is more divergent from the other bacterial SecA homologues then they are from each other. *B. burgdorferi* SecA shows only 35.0% to 43.2% similarity to the other bacterial and plastid homologues, and its evolutionary divergence is exceeded only by *Mycoplasma genitalium* (data not shown). A phylogenetic analysis of the different SecA homologues (Fig. 3) revealed that SecA appears to be a useful marker for evolutionary analysis of bacteria and photosynthetic plastids, since it represents an intermediate case between highly conserved sequences such as 16S ribosomal RNA and proteins serving a variety of less essential metabolic functions. The utility of SecA for phylogenetic analysis is underscored by the ability to isolate this gene from any bacterial source of interest utilizing sequences at the highly conserved ATP-binding sites (Figs. 2 and 5). In performing our analysis of the different SecA proteins we noticed the presence of a highly variable sequence immediately following the Walker A motif of the low-affinity ATP-binding site that appears to serve as a useful phylogenetic sequence marker (Fig. 4). The cyanobacterium, *Synechococcus* PCC7942, as well as the photosynthetic plastids, which share a common ancestor according to the endosymbiotic theory, possess a longer version of this sequence compared to *B. burgdorferi* and Gram-negative bacteria, while the Gram-positive bacteria and *M. genitalium* lack this sequence. Barring parallel evolutionary pathways, this finding suggests that this sequence was present in the common ancestor of Spirochetes and Eubacteria, and that it was subsequently deleted in the Gram-positive line. We have recently shown that this sequence is essential in *E. coli* and that it corresponds to a periplasmically-accessible loop in the integral-membrane form of SecA protein [31]. In order to show the utility of this sequence for phylogenetic analysis we utilized primers flanking this sequence, (GA[C/T]GA[A/G]GCN[A/C]GNACNCCN[C/T]TNAT[A/C/T]AT, where the underlined sequence represents nucleotides 649 to 674 in the Walker B motif of the high-affinity ATP-binding site of the noncoding strand of *E. coli secA*, and NC[G/T]NCCNC[G/T]NA[A/G][C/T]TG

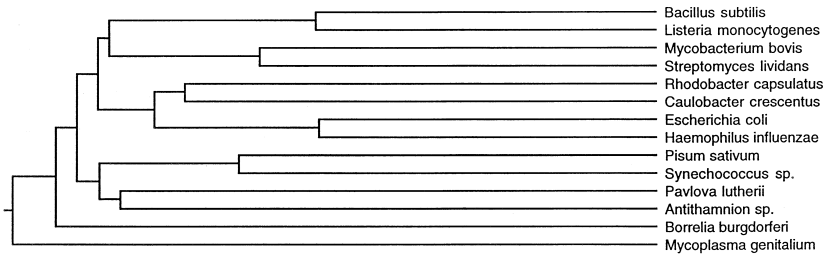
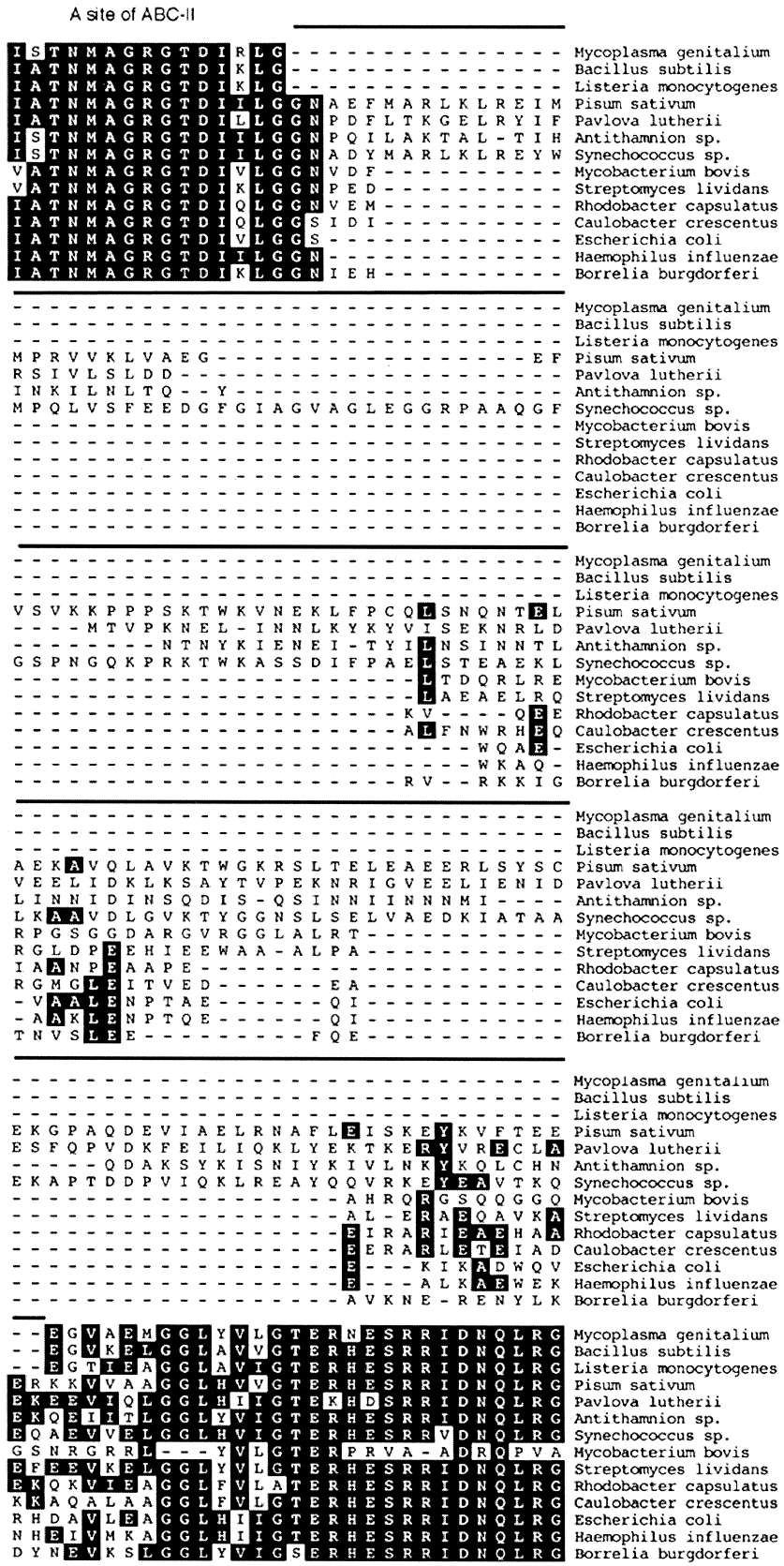



Fig. 3. Evolutionary relationship of the SecA homologues using the Clustal method with a PAM250 residue weight table (MegAlign, DNAStar).


A site of ABC-II
Mycoplasma genitalium
Bacillus subtilis
Listeria monocytogenes
Pisum sativum
Pavlova lutherii
Antithamnion sp.
Synechococcus sp.
Mycobacterium bovis
Streptomyces lividans
Rhodobacter capsulatus
Caulobacter crescentus
Escherichia coli
Haemophilus influenzae
Borrelia burgdorferi

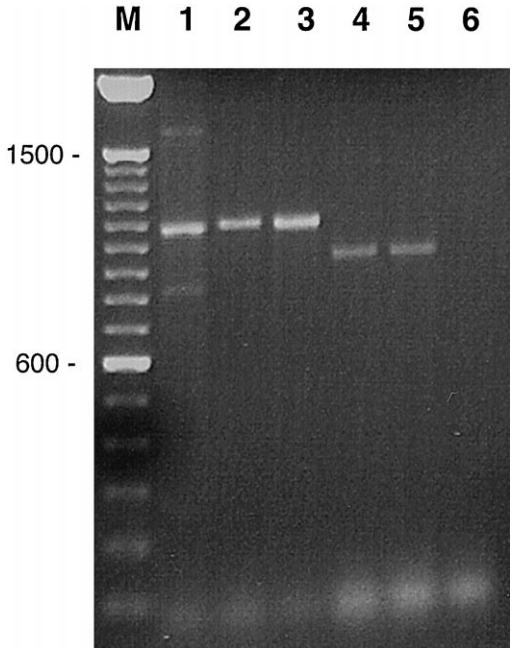



Fig. 5. PCR analysis of the phylogenetic sequence market with *secA*. Chromosomal DNA (50–100 $\mu$g ml$^{-1}$) of each species and 1 $\mu$M final concentration of each primer were used for PCR amplification according to the manufacturer's protocol (Perkin-Elmer). Thirty cycles (94°C, 55°C, and 72°C, each for 1 min) were performed, and 15 $\mu$l of each reaction product was analyzed on a 1.2% agarose gel, which was photographed after ethidium bromide staining. M, 100 bp ladder from Gibco-BRL. Lanes 1–6, PCR products from reactions containing chromosomal DNA from *E. coli* MC4100, *Borrelia burgdorferi* B31, *Borrelia burgdorferi* N40, *Bacillus subtilis* 841, *Bacillus stearothermophilus* ATCC7953, no added DNA.

[A/G]TT[A/G]TC[A/G/T]AT, where the underlined sequence represents nucleotides 1722 to 1699 immediately downstream of the Walker A motif of the low-affinity ATP-binding site of the coding strand of *E. coli secA*) to amplify this region by PCR (Fig. 5). The difference between Gram-negative bacteria and Spirochetes, which contain a shorter version of this sequence marker, and Gram-positive bacteria that entirely lack this sequence, can readily be detected by this method. Finally we note that there is a probable sequencing error in the *secA* gene of *Mycobacterium bovis* (accession number U66080), since a single nucleotide frameshift within the region immediately downstream of the Walker A motif of the low affinity ATP-binding site results in a perfect match of the predicted amino acid sequence with all other very highly conserved sequences of this region of SecA (see Fig. 4), including *Mycobacterium tuberculosis* (accession number Z95121).

The presence of the *secA* gene in Spirochetes suggests that this gene is present in most if not all major lineages within Bacteria. Since *B. burgdorferi* is evolutionarily remote from many well characterized eubacteria [32], possesses a unique membrane lipid composition [33], and must exist in both vertebrate and arthropod hosts, it is likely that at least some of the components and mechanisms of protein secretion and its regulation will be novel for this organism. Cloning of the *B. burgdorferi secA* gene is a first step in the analysis of protein secretion mechanisms in this spirochete.

## References

[1] A. Sadziene, A.G. Barbour, P. Rosa, D.D. Thomas, Infect. Immun. 61 (1993) 3590–3596.
[2] A. Sadziene, P. Rosa, P. Thompson, D. Hogan, A.G. Barbour, J. Exp. Med. 176 (1992) 799–809.
[3] J.-R. Zhang, J.M. Hardham, A.G. Barbour, S.J. Norris, Cell 89 (1997) 275–285.
[4] T.G. Schwann, J. Piesman, W.T. Golde, M.C. Dolan, P.A. Rosa, Proc. Natl. Acad. Sci. USA 92 (1995) 2909–2913.
[5] M. Theisen, J. Bacteriol. 178 (1996) 6435–6442.
[6] M.E. Brandt, B.S. Riley, J.D. Radolf, M.V. Norgard, Infect. Immun. 58 (1990) 983–991.
[7] M. Giladi, C.I. Champion, D.A. Haake, D.R. Blanco, J.F. Miller, J.N. Miller, M.A. Lovett, J. Bacteriol. 175 (1993) 4129–4136.
[8] D.B. Oliver, Mol. Microbiol. 7 (1993) 159–165.
[9] M. Schmidt, E. Rollo, J. Grodberg, D. Oliver, J. Bacteriol. 170 (1988) 3404–3414.
[10] Y. Sadaie, H. Takamatsu, K. Nakamura, K. Yamane, Gene 98 (1991) 101–105.
[11] C. Scaramuzzi, R. Hiller, H. Stokes, Curr. Genet. 22 (1992) 421–427.

Fig. 4. Phylogenetic sequence marker within SecA protein. Amino acid sequences within and immediately following the Walker A motif of the low-affinity ATP-binding site of the different SecA homologues (A site of ABC-II) were aligned. Blackened areas depict positions of identical amino acid residues. The position of the phylogenetic sequence marker is indicated by the bar. The *secA* gene of *Mycobacterium bovis* contains a probable sequencing error (frameshift) based on this alignment (see text).

[12] K. Valentin, Mol. Gen. Genet. 236 (1993) 245–250.
[13] P.J. Kang, L. Shapiro, J. Bacteriol. 176 (1994) 4958–4965.
[14] M. Nakai, T. Nohara, D. Sugita, T. Endo, Biochem. Biophys. Res. Commun. 200 (1994) 844–851.
[15] R. Fleischmann, M. Adams, O. White, R. Clayton et al., Science 269 (1995) 496–512.
[16] C. Fraser, J. Gocayne, O. White, M. Adams et al., Science 270 (1995) 397–403.
[17] M. Klein, J. Meens, R. Freudl, FEMS Microbiol. Lett. 131 (1995) 271–277.
[18] T. Nohara, M. Nakai, A. Goto, T. Endo, FEBS. Lett. 364 (1995) 305–308.
[19] M. Gilbert, S. Ostiguy, D. Kluepfel, R. Morosoli, Biochim. Biophys. Acta 1296 (1996) 9–12.
[20] M. Akita, S. Sakaki, S. Matsuyama, S. Mizushima, J. Biol. Chem. 265 (1990) 8164–8169.
[21] R. Lill, W. Dowhan, W. Wickner, Cell 60 (1990) 271–280.
[22] W. Wickner, A.J.M. Driessen, F.-U. Hartl, Annu. Rev. Biochem. 60 (1991) 101–124.
[23] R. Salavati, D. Oliver, RNA 1 (1995) 745–753.
[24] C. Bult, O. White, G. Olsen, L. Zhou et al., Science 273 (1996) 1058–1073.
[25] C. Ramirez, A. Matheson, Mol. Microbiol. 5 (1991) 1687–1693.
[26] J. Luirink, B. Dobberstein, Mol. Microbiol. 11 (1994) 9.
[27] C. Mitchell, D.B. Oliver, Mol. Microbiol. 10 (1993) 483–497.
[28] N. Burman, S. Bregstrom, B.I. Restrepo, A.G. Barbour, Mol. Microbiol. 4 (1990) 1715–1726.
[29] J.E. Walker, M. Saraste, M.J. Runswick, N.J. Gay, EMBO J. 1 (1982) 945–951.
[30] H. Takamatsu, S.-I. Fuma, K. Nakamura, Y. Sadaie, A. Shinkai, S.-I. Matsuyama, S. Mizushima, K. Yamane, J. Bacteriol. 174 (1992) 4308–4316.
[31] V. Ramamurthy, D. Oliver, J. Biol. Chem. 272 (1997) 23239–23246.
[32] R.S. Gupta, K. Bustard, M. Falah, D. Singh, J. Bacteriol. 179 (1997) 345–357.
[33] J. Belisle, M. Brandt, J.D. Radolf, M.V. Norgard, J. Bacteriol. 176 (1994) 2151–2157.