Molecular analysis of a *Leptospira borgpetersenii* gene encoding an endoflagellar subunit protein

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A flagellin gene, *flaB*, from *Leptospira borgpetersenii* (formerly *L. interrogans*) serovar *hardjo* was cloned and expressed in *Escherichia coli*. Expression of the 32 kDa FlaB protein was dependent upon the *lacZ* promoter from pUC18. Nucleotide sequence data showed an open reading frame encoding 283 amino acid residues, corresponding to a protein of molecular mass 31.3 kDa. The G + C content of the *flaB* gene was 54.7 mol%. Comparison of the deduced FlaB amino acid sequence with flagellins from other bacteria revealed a high level of identity with the *Treponema pallidum* FlaB proteins.

Introduction

Motility in leptospires is dependent on the presence of endoflagella located between the protoplasmic cylinder and the outer membrane sheath (Smibert, 1973). In these organisms there are two flagella inserted subterminally into the protoplasmic cylinder at each pole of the cell (Chang & Faine, 1970). Like many other flagella studied, leptospiral flagella consist of a basal body, hook and filament (Smibert, 1973). Using Western blotting techniques, flagellar proteins have been identified in both pathogenic and saprophytic strains of leptospires. In particular, a major protein doublet of 33–34 kDa has been shown to be conserved among the different genera of the family *Leptospiraceae* (Kelson *et al.*, 1988).

The genes encoding the flagellar proteins from another member of the order *Spirochaetales*, *Treponema pallidum*, have been cloned and sequenced (Pallesen & Hindersson, 1989; Isaacs *et al.*, 1989; Champion *et al.*, 1990). Three of these proteins, a 31 and 31.3 kDa doublet and a 34.5 kDa species, have been identified as endoflagellar subunit proteins (Pallesen & Hindersson, 1989; Champion *et al.*, 1990) designated FlaB1 to FlaB3 respectively. An additional 37 kDa protein has been identified as the endoflagellar sheath protein, FlaA (Isaacs *et al.*, 1989). In this paper we report the cloning, expression and sequencing of a gene encoding a 32 kDa endoflagellar subunit gene from *L. borgpetersenii* (formerly *L. interrogans*) serovar *hardjo* (Yasuda *et al.*, 1987).

Methods

Bacterial strains and plasmids. *L. borgpetersenii* serovar *hardjo* strain L171 (Farrelly *et al.*, 1987) was cultured in Tween 80-bovine albumin (EMJH) medium with added pyruvate (Johnson *et al.*, 1973). All *Escherichia coli* recombinants were derivatives of strain DH5 α (Bethesda Research Laboratories) and were cultured in 2 $\times$ YT medium (Vieira & Messing, 1982), supplemented with ampicillin (100 μ g ml⁻¹) as required. All recombinant plasmids were derivatives of the cloning vector pUC18 (Yanisch-Perron *et al.*, 1985).

Preparation and analysis of DNA. Whole-cell leptospiral DNA was prepared from serovar *hardjo* strain L171 as described previously (Marshall *et al.*, 1984; Doherty *et al.*, 1989). *E. coli* plasmid DNA was isolated by the alkaline lysis method (Birnboim & Doly, 1979). Ligation, transformation and agarose gel electrophoresis methods were as described by Maniatis *et al.* (1982). Restriction endonucleases, T4 ligase and calf intestinal alkaline phosphatase (molecular biology grade) were from either Promega or Boehringer-Mannheim.

Preparation of *L. borgpetersenii* gene bank. *L. borgpetersenii* DNA (20 μ g) was partially digested with *Sau*3A, excised from a 1% low-melting-point agarose gel and ligated overnight at 15 °C, within the gel matrix, with 1 μ g of *Bam*HI-digested, dephosphorylated pUC18 (Yanisch-Perron *et al.*, 1985). The ligation mixture was used to transform competent *E. coli* DH5 α cells to ampicillin resistance. Transformants were selected on 2 $\times$ YT-ampicillin (100 μ g ml⁻¹) plates containing X-Gal (5-bromo-4-chloro-3-indolyl β -D-galactopyranoside). Colourless recombinant clones were screened by colony immunoassay as described previously (Doherty *et al.*, 1989).

Antiserum production. All antisera were produced in New Zealand White rabbits. Antiserum used in the colony immunoassay, SB288, was kindly provided by S. Ballard (Department of Microbiology, Monash University) and contained antibodies against a large range of leptospiral serovar *hardjo* proteins as assessed by Western blotting against leptospiral whole-cell lysate. It was absorbed with lysed *E. coli* DH5 α cells prior to use. The R390 antiserum used to detect the flagellar subunits in Western blotting was prepared against isolated, SDS-PAGE-denatured flagellum proteins consisting primarily of the genus

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cross-reactive 33–34 kDa doublet (Kelson *et al.*, 1988), from *L. interrogans* serovar *pomona* (Boulard & Lecroisey, 1982).

Protein electrophoresis and immunoblotting. Leptospiral and *E. coli* components were separated by electrophoresis on discontinuous 10–15% (w/v) SDS-polyacrylamide gels (Laemmli, 1970). Leptospiral sonicates were prepared as described previously (Adler *et al.*, 1980). *E. coli* preparations were prepared in sample buffer containing SDS (1.3%, w/v), bromophenol blue (0.002%, w/v) and glycerol (4%, v/v) immersed in boiling water for 10 min and then centrifuged at 11 600 *g* for 5 min prior to loading. Flagellar protein was prepared by boiling in sample buffer for 5 min. Electrophoresis conditions, Western blotting and immunostaining procedures have been described previously (Chapman *et al.*, 1987), as has the method for eluting antibodies from nitrocellulose (Beall & Mitchell, 1986).

Isolation of flagella. Flagella isolated from approximately 5×10^{11} cells of strain L171 were prepared as described by Chang & Faine (1970) and were further purified by caesium chloride density-gradient centrifugation (Kelson *et al.*, 1988).

DNA hybridization. Southern blots were performed as described by Southern (1975). Randomly primed DNA was labelled with [32 P]dATP (Amersham) using an oligolabelling kit (Bresatec) following the supplied protocol. Prehybridization and hybridization solutions contained 50% (v/v) formamide, 1% (w/v) SDS, 0.5% (w/v) skim milk powder, 4 mM-EDTA, 32 mM-NaOH, 40 mM-NaH₂PO₄, 0.72 M-NaCl and 0.3 mg salmon sperm DNA ml⁻¹. Following hybridization overnight, blots were washed under high-stringency conditions prior to autoradiography at -70 °C using intensifying screens.

DNA sequencing and computer analysis. DNA sequencing was performed using [α - 35 S]dATP (Amersham) and a T7 sequencing kit (Pharmacia) by following the manufacturer's protocol for double-stranded sequencing. DNA to be sequenced was purified on a caesium chloride gradient or by using GeneClean (Bio 101). Unequivocal sequence was obtained on both DNA strands. Nucleotide and amino acid sequence data were analysed using MELBDBSYS, a suite of programs developed by the Walter and Eliza Hall Institute, the Ludwig Institute for Cancer Research and the Howard Florey Institute, Melbourne, Australia. The Treealign program, which performs phylogenetic alignment of homologous sequences (Hein, 1989*a, b*), was used to compare the FlaB amino acid sequence with other flagellin proteins.

Results

Cloning and definition of the *flaB* coding region

Recombinant clones containing chromosomal DNA isolated from serovar *hardjo* strain L171 were initially screened by colony immunoassay using SB288 anti-*hardjo* antiserum. Positive clones were further analysed by Western blotting. One clone, carrying the recombinant plasmid pLBA21, reacted weakly in colony immunoassays and two weak bands, 32 and 28 kDa in size were detected by Western blotting. To determine whether the cloned products were flagellar antigens they were further analysed by Western blotting using R390 antiserum raised against denatured flagellar components. The antiserum reacted strongly with the 32 and 28 kDa bands (Fig. 1*a*). Antiserum raised against

undenatured whole flagella did not react with the cloned products by Western blotting (data not shown).

The plasmid pLBA21 (Fig. 2) contained a 3.9 kb *Sau*3A leptospiral DNA fragment inserted into the *Bam*HI site of pUC18. By subcloning and subsequent testing for antigen expression using Western blotting with R390 antiserum, the gene encoding the 32 kDa protein was localized to a central 1.2 kb *Nru*I fragment (Fig. 2), which also contained an internal *Nru*I site. In accordance with the terminology used for the cloned *T. pallidum* genes (Pallesen & Hindersson, 1989; Champion *et al.*, 1990), the gene encoding the 32 kDa protein was designated *flaB*. There was no expression of the 32 or 28 kDa proteins when the entire 3.9 kb region encoded by pLBA21, or the 1.2 kb *Nru*I fragment from pLBA38, was inserted in the opposite orientation relative to the *lacZ* promoter (Fig. 2). Expression of the cloned products encoded by pLBA21 therefore appeared to be under the control of the *lacZ* promoter in pUC18, indicating a direction of transcription from left to right in Fig. 2. Interestingly, the strain harbouring the *Pst*I subclone pLBA31 produced an 18 kDa protein identified by R390 antiserum. To confirm the origin of the insert, chromosomal DNA isolated from strain L171 was digested with *Eco*RV and *Cla*I and probed by Southern blotting using the 1.2 kb *Nru*I fragment from pLBA38. The probe hybridized to a single 3.4 kb chromosomal band which was of identical size to the fragment obtained after *Eco*RV/*Cla*I digestion of pLBA21 (data not shown). To determine whether any other regions of pLBA21 had *flaB*-related sequences, a 0.5 kb *Pst*I/*Sal*I fragment contained wholly within the *flaB* gene was used to probe pLBA21 digested with *Nru*I. The probe hybridized to three bands, of 1.2, 0.7 and 0.5 kb, which corresponded to the partial *Nru*I digestion of the central 1.2 kb *Nru*I region encoded by pLBA38 (Fig. 2).

Serological relatedness of the cloned antigens

A cell lysate of *E. coli* containing pLBA21 was separated by SDS-PAGE, and following Western transfer and overnight incubation with anti-flagellar serum (R390), antibodies were eluted from the 32 and 28 kDa protein bands. These antibodies were reacted by Western blotting with serovar *hardjo* L171 sonicate, isolated flagella and a cell preparation of the FlaB clone, harbouring pLBA21 (Fig. 1*b, c*). Antibodies eluted from the 32 kDa band reacted with the 28 kDa band and also with the 33 kDa band present in both the leptospiral sonicate and the purified flagella preparation and corresponding to the lower doublet band described by Kelson *et al.* (1988). Antibodies eluted from the 28 kDa band showed a similar reactivity pattern, and reacted with the 32 kDa band.

Fig. 1. Western blot analysis of the cloned flagellin proteins immunostained with anti-flagellar serum R390 (a), antibodies eluted from the 32 kDa FlaB band (b), or antibodies eluted from the 28 kDa band (c). Lanes: A, E and H, L171 whole-cell sonicate; B, F and I, purified L171 flagella; D, G and J, cell extracts prepared from *E. coli* DH5 α cells containing pLBA21; C, control strain of *E. coli* DH5 α containing pUC18. The 32 kDa FlaB protein and the 28 kDa protein (p28) are indicated by arrows on the right. Minor bands in lanes G and J are due to co-eluted *E. coli* antibodies and are also seen in the *E. coli* control (lane C). The numbers on the left indicate the positions of the standard molecular mass markers.

Nucleotide sequence of the cloned *flaB* gene and the deduced amino acid sequence

DNA sequence analysis of a series of overlapping subclones was used to obtain the unequivocal nucleotide sequence of the 1.2 kb *Nru*I fragment from pLBA21, on both DNA strands (Fig. 3). An open reading frame of 849 nucleotides, corresponding to 283 amino acids, was identified. The calculated molecular mass of the encoded protein was 31.3 kDa. A consensus *E. coli* Shine-Dalgarno sequence was present 7 base pairs upstream from the initiation codon. An inverted repeat sequence [$\Delta G = -22.8 \text{ kcal mol}^{-1}$ ($-95.4 \text{ kJ mol}^{-1}$)] which may function as a rho-independent transcriptional terminator was located 37 bases downstream of the stop codon (Tinoco *et al.*, 1973). There were no identifiable promoters upstream of *flaB* within the sequenced region. The G + C content of *flaB* was 54.7 mol%, in contrast to the reported 39 mol% G + C content of serovar *hardjo* genomic DNA (LeFebvre *et al.*, 1987). The encoded protein contained 31 (10.9%) acidic and 33 (11.7%) basic amino acids. Eighty-nine (31%) of the amino acids were

hydrophobic, but there were no significant hydrophobic domains within the protein. There was a greater similarity of codon usage between the *flaB* gene and *E. coli* genes (Ikemura & Ozeki, 1982) than with codon usage observed with the sphingomyelinase C gene from serovar *hardjo* (Segers *et al.*, 1990), in particular for the amino acids Gln, His, Phe, Tyr and Val. Interestingly, CCG was the only codon used for the amino acid Pro.

Comparative amino acid sequence analysis of the flagellin proteins

The predicted amino acid sequence of the serovar *hardjo* L171 FlaB protein had significant sequence similarity with flagellar proteins from other bacteria (Fig. 4). The aligned sequences do not include *Salmonella typhimurium*, *Campylobacter coli*, *Rhizobium meliloti*, *Serratia marcescens* or *E. coli* flagellins due to the greater length of these amino acid sequences. However, these proteins are included in a dendrogram derived from an alignment of the amino acid sequences (Fig. 5). The alignment data revealed a close relationship between the

Fig. 2. Restriction maps of pLBA21 and derived subclones. The location of the *flaB* gene is shown. Arrows for each plasmid indicate the direction of transcription from the *lac* promoter. Proteins were detected with anti-flagellar serum R390 by Western blotting analysis of the recombinant strains harbouring the plasmids. A dash indicates that no proteins were detected.

T. pallidum FlaB1, B2 and B3 proteins and *L. borgpetersenii* FlaB, with homologies of 56–59%. The relationship was more evident if conserved amino acid changes were considered, resulting in similarities of 88–89%. Flagellins from *Roseburia cecicola*, *Borrelia burgdorferi* and *Bacillus subtilis* were less similar to *L. borgpetersenii*, having 38%, 40% and 41% homology, respectively. *Caulobacter crescentus* flagellin was the least related of those studied, having only 18% homology with FlaB. The dendrogram (Fig. 5) indicates that *L. borgpetersenii* is phylogenetically more distant from *Borrelia burgdorferi* than *Bacillus subtilis*. The close phylogenetic relatedness of the FlaB proteins from *L. borgpetersenii* and *T. pallidum* is apparent.

Discussion

The flagellin proteins from a diverse range of bacteria have previously been cloned, including those from *B. burgdorferi* and *T. pallidum* which, like leptospires, are members of the order *Spirochaetales*. Leptospiral flagellar components have been identified in SDS-

PAGE gels as four major protein bands of 31 kDa, 37 kDa and a 33–34 kDa doublet (Kelson *et al.*, 1988). The doublet observed in profiles of isolated leptospiral flagella has previously been shown to be conserved among the different genera of the family *Leptospiraceae* (Kelson *et al.*, 1988). A similar flagellar doublet profile was observed in flagella isolated from *T. pallidum* and shown to consist of two subunit proteins, FlaB1 and FlaB2. Here we report the cloning and expression in *E. coli* of a flagellin gene from *L. borgpetersenii*. The recombinant clone carrying pLBA21 produced two proteins, both of which were expressed under the control of the *lacZ* promoter. These consisted of the 31 kDa FlaB protein and a 28 kDa protein designated p28. Antisera raised against denatured flagellar proteins from an SDS-PAGE gel reacted with the cloned proteins. However, antisera raised against non-denatured leptospiral flagella did not react with the FlaB or p28 proteins, suggesting that the epitopes recognized by these antibodies were only exposed by the denaturation of flagellin. The gene encoding FlaB was localized to a central *NruI* region of pLBA21. A *PstI* site was present six nucleotides upstream of the *flaB* stop codon and yet the *PstI* subclone pLBA31 produced an 18 kDa protein that was much

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GATTGATAATTCATTGTTACTCCGTGATAGGAGGACGCCTGGTCTGTCAGGTGGTCTGAATATTGTCCAT
  10      20      30      40      50      60      70

CCCGGAGCACCGGACAGACCATGTCCGGCCGGCAGATAGGGAGATTTCGCGGTTTCATCTCTACAAAGGAG
  80      90     100     110     120     130     140
      M I I N H N L S A V N S H R S L K F N E L
TGTAAGATGATTATCAATCACAACCTGAGCGCGGTGAATTCTCACC GTTCTCTAAAGTTCAACAGAGCTTG
 150     160     170     180     190     200     210
      A V D K T M K A L S S G M R I N S A A D D A S G
CTGTGGACAAGACGATGAAGGCTTTGTCTTCCGGTATGCGGATCAATTCGCGCGCGGACGACGCTTCCGG
 220     230     240     250     260     270     280
      L A V S E K L R T Q I N G L R Q A E R N T E D
ACTCGCGGTTTCTGAAAAGCTAAGAACGACAGATCAACGGTCTGCGTCAGGCCGAAAGAACCCGAAGAC
 290     300     310     320     330     340     350
      G M S F I Q T A E G F L E Q T S N I I Q R I R
GGGATGAGCTTCATTCAAACCTGCCGAGGGTTCTCAGAACAGACGTCGAACATCATTTCAGAGAATCCGGG
 360     370     380     390     400     410     420
      V L A I Q T S N G I Y S N E D R Q L V Q V E V S
TGCTTGCCATCCAGACCTCGAATGGTATCTACAGCAACGAAGATAGGCAGCTTGTGCAGGTGGAAGTATC
 430     440     450     460     470     480     490
      A L V D E V D R I A S Q A E F N K F K L F E G
TGCGCTGGTGGACGAAGTCGACCGAATCGCTTCTCAGGCTGAATTTAATAAGTTCAAGCTTTTCGAGGGG
 500     510     520     530     540     550     560
      Q F A R G S R V A S M W F H M G P N Q N Q R E
CAATTCGCAAGAGGTTACGGGTCGCGTCCATGTGGTTTCATATGGGACCGAACCAAAATCAGCGAGAGA
 570     580     590     600     610     620     630
      R F Y I G T M T S K A L K L V K A D G R P I A I
GATTTTACATCGGCACGATGACTTCGAAAGCCCTGAAGCTTGTAAGGCGGACGGGAGACCGATCGCGAT
 640     650     660     670     680     690     700
      S S P G E A N D V I G L A D A A L T R I M K Q
TTCCTTCTCCGGGAGAAGCCAACGATGTTATCGGTTTAGCGGATGCGGCTCTTACGAGGATCATGAAGCAG
 710     720     730     740     750     760     770
      R A D M G A Y Y N R L E Y T A K G L M G A Y E
AGAGCGGATATGGGGCTTATTACAATAGGCTCGAGTATACCGCAAAAGGGCTGATGGGTGCATATGAAA
 780     790     800     810     820     830     840
      N M Q A S E S R I R D A D M A E E V V S L T T K
ATATGCAAGCATCGGAATCCAGAATTCGGGACGCCGATATGGCGGAGGAAGTTGTCTCGCTGACCACAAA
 850     860     870     880     890     900     910
      Q I L V Q S G T A M L A Q A N M K P N S V L K
ACAAATACGTTTCAGAGTGGTACGCAATGTAGCGCAGGCAAATATGAAACCGAATTCGGTCTCAAG
 920     930     940     950     960     970     980
      L L Q H I *
CTTCTGCAGCATATCTAAAGGAAGCCTTCCAGGAGGGAAGGTAGACAAAAAGGTTCCACCGGGAGGCATC
 990     1000     1010     1020     1030     1040     1050
      TCTTCCGGTGGATTTTTTATAAGAATTTTGAATTATAAAGTTTTAGAAAAACCTCCGTAGATAGTTCATA
1060     1070     1080     1090     1100     1110     1120
      ATCATTTTTTAAGAATATTCTAATTTTTGAATTTAAAGATTAAAGCCGGTTCCGGAATCGTCCAATGCCG
1130     1140     1150     1160     1170     1180     1190
      TATCTTACGGAGATTTT
1200

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Fig. 3. Nucleotide sequence of the 1207 bp *Nru*I fragment of pLBA38 and deduced amino acid sequence. An *E. coli* consensus Shine-Dalgarno sequence is underlined at position 136. A second potential ribosome-binding sequence is also underlined at position 213 followed by a putative internal initiation codon at position 225. The translation stop codon is indicated by the asterisk. A potential transcription termination signal sequence downstream of *flaB* is double underlined.

Fig. 4. Alignment of the predicted amino acid sequences of the flagellin proteins from *Leptospira borgpetersenii* (this work), *Treponema pallidum* FlaB1 and FlaB3 (Champion *et al.*, 1990), *T. pallidum* FlaB2 (Pallsen & Hindersson, 1989), *Borrelia burgdorferi* (Wallich *et al.*, 1990), *Bacillus subtilis* (LaValle & Stahl, 1989), *Roseburia cecicola* (Martin & Savage, 1988) and *Caulobacter crescentus* (Gill & Agabian, 1983). The gene products were aligned using the Treealign program. Identical amino acids are boxed.

Fig. 5. Dendrogram showing relatedness of the flagellins from the following organisms: *Escherichia coli* (Kuwajima *et al.*, 1986), *Salmonella typhimurium* (Joys, 1985), *Campylobacter coli* (FlaB) (Guerry *et al.*, 1990), *Serratia marcescens* (Harshey *et al.*, 1989), *Caulobacter crescentus* (Gill & Agabian, 1983), *Rhizobium meliloti* (FlaA) (Pleier & Schmitt, 1989), *Borrelia burgdorferi* (Wallich *et al.*, 1990), *Leptospira borgpetersenii* (this work), *Treponema pallidum* (FlaB1) (Champion *et al.*, 1990), *Bacillus subtilis* (LaVallie & Stahl, 1989) and *Roseburia cecicola* (Martin & Savage, 1988).

smaller than the predicted size of the truncated product (31 kDa). It is possible that the truncated product is less stable than the complete protein and is degraded to produce the 18 kDa protein seen in Western blots.

The FlaB protein and the p28 protein were antigenically related, as shown from the results of experiments in which specific antibodies eluted from each of the cloned proteins reacted with each other and with only the 33 kDa flagellar protein in leptospires. Interestingly, antibodies eluted from either FlaB or p28 did not react with the larger of the two leptospiral flagellar doublet proteins, indicating that these proteins were antigenically unrelated. This was in contrast to the findings in *T. pallidum*, where the two components of the doublet were both flagellin subunit proteins (FlaB1 and FlaB2) and were antigenically related. Further studies are required to determine whether the larger leptospiral protein constitutes a second flagellin subunit, which is antigenically distinct from FlaB.

The location of the gene encoding p28 is uncertain. Within the *flaB* gene coding region there is a potential in-frame internal initiation (nucleotide 225) codon preceded by a weak ribosome-binding site (Fig. 3). The calculated molecular mass of a protein initiating at this site is 28.3 kDa. The existence of a second gene encoding p28 is unlikely as none of the subclones expressed p28 (Fig. 2). Moreover, in Southern blots only the *NruI* fragments encoded by pLBA38 hybridized with the *flaB* gene. An alternative explanation for our data is that p28 is a breakdown product of FlaB, especially since additional bands of lower molecular mass were occasionally observed in profiles of strains carrying pLBA21.

The apparent molecular mass of the cloned FlaB protein in SDS-PAGE gels was approximately 32 kDa, close to that calculated from the deduced amino acid sequence (31.3 kDa). The size of the cloned polypeptide was smaller than the 33 kDa flagellar protein in

leptospires. The precise reason for this difference is unclear. One possibility may be post-translational modification of the protein in leptospires, such as the addition of a lipid moiety. However, the protein contains neither cysteine residues nor any identifiable putative signal peptide sequences which are necessary for the addition of lipids (Yamaguchi *et al.*, 1988). Although rarely reported in bacteria, the possibility nevertheless exists of the addition of sugar residues to the protein (Lechner, 1989). Indeed the flagellins of *Halobacterium halobium* are glycosylated and the amino acid sequence which surrounds *N*-glycosidic linkages is commonly of the formula Asn-X-Thr(Ser) (Wieland *et al.*, 1985). This sequence is present in the leptospiral FlaB protein six amino acids from the N-terminus. Previous comparisons of flagellins from different genera of bacteria have revealed extensive homology in the N-terminal and C-terminal regions and these regions in leptospiral flagellin are also highly conserved (Fig. 4). The amino acid sequence alignment also showed a high degree of homology between FlaB proteins from *L. borgpetersenii* strain L171 and *T. pallidum*. The G+C content of the leptospiral *flaB* gene is significantly higher than the reported 39 mol% G+C content of the leptospiral genome (LeFebvre *et al.*, 1987), but remarkably similar to the G+C content of the *T. pallidum* genome (Miao & Fieldsteel, 1978) and the *T. pallidum flab* genes (Pallesen & Hindersson, 1989; Champion *et al.*, 1990). Based on these results, we suggest that the FlaB protein from *L. borgpetersenii* and the FlaB proteins of *T. pallidum* may have a common ancestral gene. This hypothesis is consistent with the close phylogenetic relationship between the FlaB proteins from the two species (Fig. 5). The mechanism by which the gene may have been introduced into *L. borgpetersenii* is not known since genetic exchange has not been demonstrated with these organisms.

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