INFECTION AND IMMUNITY, Oct. 1997, p. 4355–4359 Vol. 65, No. 10
0019-9567/97/$04.00+0

# Identification of a 35-Kilodalton Serovar-Cross-Reactive Flagellar Protein, FlaB, from *Leptospira interrogans* by N-Terminal Sequencing, Gene Cloning, and Sequence Analysis

MIN LIN,* OM SURUJBALLI, KLAUS NIELSEN, SUSAN NADIN-DAVIS, AND GEOFFREY RANDALL

*Animal Diseases Research Institute, Nepean, Ontario, Canada K2H 8P9*

Received 2 April 1997/Returned for modification 16 June 1997/Accepted 29 July 1997

**During the screening of antibodies to pathogenic leptospires, a murine monoclonal antibody (designated M138) was found to react with various serovars. An antigen of approximately 35 kDa from *Leptospira interrogans* serovar pomona, which reacted strongly with M138, was characterized by N-terminal amino acid sequencing and identified as a flagellin, a class B polypeptide subunit (FlaB) of the periplasmic flagella. The gene encoding the FlaB protein, *flaB*, was amplified from the genomic DNA of several pathogenic serovars by PCR with a single pair of oligonucleotide primers, suggesting that FlaB is highly conserved among these serovars. Cloning and sequence analysis of *flaB* from serovar pomona revealed that it contains an 849-bp open reading frame with a G + C content of 46.88% which encodes a 283-amino-acid protein with a calculated molecular mass of 31.297 kDa and a predicted pI of 9.065. A sequence comparison of flagellin proteins revealed that the amino acid sequence is most variable in the central portion of the serovar pomona FlaB, which is believed to contain specific sequence information and which may thus be useful in the design of DNA or synthetic peptide probes suitable for the detection of infection with pathogenic leptospires.**

The pathogenic species of the *Leptospira* genus are the etiologic agents of leptospirosis, which is a worldwide zoonotic disease with a wide spectrum of clinical manifestations in animals (9) and infrequently in humans (7). Infection in humans is often a result of occupational or accidental contact with infected animals or due to ingestion of water contaminated with their urine (7, 9). All pathogenic leptospires were formerly classified as *Leptospira interrogans*. Recently, DNA homology studies have led to the reclassification of *L. interrogans* into seven pathogenic *Leptospira* species: *L. borgpetersenii*, *L. inadai*, *L. interrogans*, *L. kirschneri*, *L. noguchii*, *L. santarosai*, and *L. weilii* (24, 34). Leptospires, because of their terminal hooks, are unique among spirochetes. Motility in leptospires is dependent on the rotation of two periplasmic flagella (PF) (8), which are situated in the periplasmic space between the cytoplasmic membrane and the outer membrane sheath and which are composed of a basal body, hook, and filament (26). The distinct morphological features such as the thin helically shaped cell cylinder and the PF enable the leptospires to burrow into tissue (8). The serology of pathogenic *Leptospira* species is remarkably complicated, with more than 200 serovars within 23 serogroups identified to date (7). Development of a single specific reagent suitable for the serological detection of infections with all serovars remains a great challenge.

During the recent screening of monoclonal antibodies (MAbs) to various serovars of *Leptospira*, we demonstrated by enzyme-linked immunosorbent assay that a MAb (designated M138) reacted with many pathogenic serovars examined but not with saprophytic *Leptospira biflexa* serovar patoc. The antigenic structure recognized by M138, however, was unknown. It was thought that M138 may recognize a conserved cross-reactive protein antigen (epitope) expressed by all pathogenic leptospires. This antigen and its gene could contain sequence information necessary for the design of a specific peptide or DNA probe to detect infection with leptospires. This consideration has prompted us to identify, clone, and sequence analyze the gene encoding the antigen recognized by M138 from *L. interrogans* serovar pomona (strain Pomona) as described in this report.

**Identification of a leptospiral 35-kDa protein.** *L. interrogans* was cultured at 29°C in SPL 5× *Leptospira* medium (Scientific Protein Laboratories, Waunakee, Wis.). Whole-cell antigens were prepared from *L. interrogans* serovar pomona and analyzed by Western blotting with M138 (2 μg/ml) as the probe. The results show immunoreactive bands of various molecular sizes (Fig. 1a, lane 2). A strong immunoreactive band of approximately 35 kDa (Fig. 1a, lanes 2 and 3), which also reacted strongly with the antisera from naturally *L. interrogans*-infected cattle (17), was notable and may play an important immunological role in an infected host. Protease K treatment of the serovar pomona antigens eliminated the immunoreaction between M138 and the antigens (Fig. 1a, lane 4), suggesting that M138 recognized a protein epitope in nature. Although the reason for the reaction of M138 with many leptospiral antigens on Western blots was unclear, a 35-kDa protein that reacted strongly with M138 was targeted for further characterization. As shown in Fig. 1b, the 35-kDa antigen was highly purified from the outer sheath preparation (1) through a high-performance liquid chromatography size exclusion column (TSK G2000 SWG; 21.5 by 600 mm; Phenomenex, Torrance, Calif.) with 50 mM ammonium acetate as the elution buffer. The purified serovar pomona protein antigens were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (16), electroblotted onto a polyvinylidene difluoride membrane, and excised for the determination of the N-terminal amino acid sequence. Amino acid sequencing was performed on a model 470A gas phase sequencer equipped with an on-line model 120A PTH analyzer (Applied Biosystems, Foster City, Calif.). A similarity search of the N-terminal

* Corresponding author. Mailing address: Canadian Food Inspection Agency, Animal Diseases Research Institute, 3851 Fallowfield Road, Nepean, Ontario, Canada K2H 8P9. Phone: (613) 228-6698. Fax: (613) 228-6667.

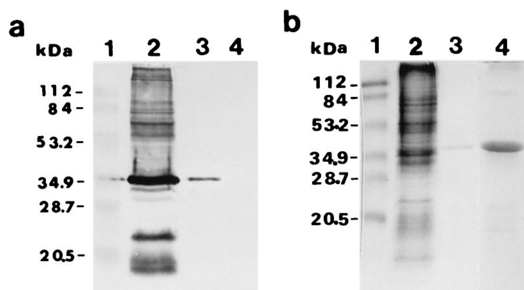


FIG. 1. Analysis of the antigens from *L. interrogans* serovar pomona by Western blotting with MAb 138 (a) or by SDS-PAGE with Coomassie staining (b). (a) Lane 1, molecular mass standards; lane 2, whole-cell antigen extract; lane 3, partially purified 35-kDa antigen; lane 4, protease K-treated whole-cell antigen extract. (b) Lane 1, molecular mass standards; lane 2, whole-cell antigen extract; lanes 3 and 4, partially purified 35-kDa antigen (2 and 15 μg, respectively).

amino acid sequence of the 35-kDa protein was performed against the sequence databases through the National Center for Biotechnology Information (NCBI) BLAST E-mail server. A striking homology (61.5 to 92.2% identity) between the N-terminal amino acid sequence (13 amino acids) of the serovar pomona protein and those of the flagellins from other bacteria was found (Fig. 2). This considerably high level of homology suggested that the 35-kDa protein is a constituent polypeptide of PF. The PF from several different spirochetes have been found to be complex structures consisting of several different polypeptide subunits (2, 5, 6, 13, 28). The PF filaments consist of a core structure surrounded either by one outer sheath layer as in *Spirochaeta aurantia* (2) and *Treponema pallidum* (6) or by two outer layers as in *L. interrogans* (28). Sequence analyses of the PF genes and proteins from several spirochetal sources have shown that there are two distinct classes of proteins (2, 3, 11, 18, 20–22, 32), namely FlaA and FlaB, which form the outer sheath and core layers of the PF filament, respectively. The 35-kDa protein described here is a FlaB protein based upon its N-terminal amino acid sequence, and its gene is therefore referred to as *flaB*.

**Cloning and sequence analysis of the serovar pomona flagellin gene.** The total genomic DNA was extracted from the cell cultures with a Rapidprep Macro Genomic DNA isolation kit (Pharmacia Biotech, Baie d'Urfe, Quebec, Canada). To analyze the gene encoding the leptospiral flagellin that reacted strongly with M138, PCR was employed to amplify the gene homolog from the serovar pomona genomic DNA with a pair of oligonucleotide primers: 5′GTGGAGCTCATGATTATCA A(T/C)CA(C/T)AA(C/T)CT3′ and 5′ACAGGATCCTCAGA T(A/G)TGCTGCAGAAG(C/T)TT3′. The two PCR primers, which contain the *Sac*I and *Bam*HI recognition sequences at their 5′ ends, were derived, respectively, from the N-terminal amino acid sequence of the serovar pomona 35-kDa protein and the conserved C-terminal amino acid sequence of a leptospiral periplasmic flagellar subunit (18). PCR was performed with *Taq* DNA polymerase (Life Technologies, Burlington, Ontario, Canada). Following a hot start (85°C for 5 min) and denaturation at 94°C for 30 s, 40 cycles of amplification at 94°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min were performed. The PCR resulted in a single DNA fragment of approximately 850 bp, which presumably corresponds to the open reading frame of the flagellin. With the same pair of primers, an 850-bp fragment was also amplified by PCR from the genomic DNA of serovars hardjo, autumnalis, and copenhageni. DNA sequence analysis revealed that these PCR products were the flagellin gene homologs (17). These results suggest that the FlaB protein is highly conserved among pathogenic serovars of *Leptospira*. The highly conserved FlaB in leptospires may explain the observation that M138 cross-reacted with various pathogenic serovars. The cross-reactivity of periplasmic flagellar proteins among various strains of *Leptospira* has been previously described (4, 13). Our data are consistent with the finding that the class B protein is highly conserved among spirochetes (3, 18, 20–22, 32).

The *Sac*I- and *Bam*HI-digested 850-bp amplified gene fragment from the serovar pomona genomic DNA was ligated into pUC 118 at *Sac*I and *Bam*HI sites and cloned in *Escherichia coli* TG1. Recombinant plasmids pUC118pomFla-1 and pUC118pomFla-2, prepared from the cultures of two selected white colonies, were restriction analyzed with *Sac*I and *Bam*HI. Both recombinant plasmids contain an insert with an apparent molecular size corresponding to that of the 850-bp amplified gene. DNA sequencing was performed on an ABI model 373 automatic sequencer with an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Foster City, Calif.) according to the manufacturer's instruction. The recombinant plasmid DNA was used as sequencing templates. Both strands of the DNA insert were initially sequenced with pUC/M13 forward and reverse primers, 5′GTAAAACGACGGCCAGT3′ and 5′CAGGAAACA GCTATGAC3′, respectively, and completed with an additional pair of internal primers, 5′TTATAATAAGCTCCCA TATC3′ and 5′TACTGAAGACGGAATGAGTT3′, synthesized according to the initial nucleotide sequence obtained with the pUC/M13 primers. The inserted DNA fragment contains an open reading frame of 849 bp with a G + C content of 46.88% which codes for a 283-amino-acid (aa) protein. The encoded protein contains 31 (10.95%) acidic (D and E), 34 (12.0%) basic (K and R), and 102 (36.0%) hydrophobic (A, I, L, F, W, and V) amino acids. The deduced protein sequence contains no cysteine residue and only one tryptophan residue. The calculated molecular mass and predicted pI value of the encoded protein were 31.297 kDa and 9.065, respectively. The G + C content of the serovar pomona *flaB* gene is significantly lower than the 54.7% found for serovar hardjo *flaB* (18) but higher than the reported 39% G + C content of the leptospiral genome (15). The calculated molecular mass of the serovar pomona FlaB is remarkably similar to that reported for the

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lp (this study) | M | I | I | N | H | N | L | S | A | V | N | A | H |
| Lpk1 | M | I | I | N | H | N | L | A | A | I | N | S | H |
| Lpk2 | M | I | I | N | H | N | I | S | A | I | F | A | H |
| Lh | M | I | I | N | H | N | L | S | A | V | N | S | H |
| Bs | M | I | I | N | H | N | L | P | A | M | N | A | H |
| Bb | M | I | I | N | H | N | T | S | A | I | N | A | S |
| Bh | M | I | I | N | H | N | T | S | A | I | N | A | S |
| Sa | M | I | I | N | H | N | M | S | A | I | N | A | Q |
| Tpa | M | I | I | N | H | N | M | S | A | M | F | S | Q |
| Tph | M | I | I | N | H | N | M | S | A | M | F | A | Q |

FIG. 2. N-terminal amino acid sequence analysis of the 35-kDa protein from *L. interrogans* serovar pomona. The sequence of 13 aa residues was determined for the N terminus of the serovar pomona 35-kDa protein by Edman degradation. A similarity search of this sequence was performed against the sequence databases through the NCBI BLAST E-mail server; the search showed that the flagellar filament proteins (flagellins) from other bacteria contained homologous sequences in the N termini. Flagellins from the following sources were selected here for comparison: the serovar pomona 35-kDa protein (Lp) (this study); *L. interrogans* serovar pomona (type kennewicki) 34- and 35.5-kDa proteins (Lpk1 and Lpk2, respectively) (28); *L. borgpetersenii* serovar hardjo (Lh) (18); *Bacillus* sp. strain C-125 (Bs) (25); *B. burgdorferi* (Bb) (29); *B. hermsii* (Bh) (23); *S. aurantia* (Sa) (22); *T. pallidum* 33-kDa protein (Tpa) (21); and *Treponema phagedenis* (Tph) (20). Amino acid residues that match exactly with those of the serovar pomona 35-kDa protein are shaded.

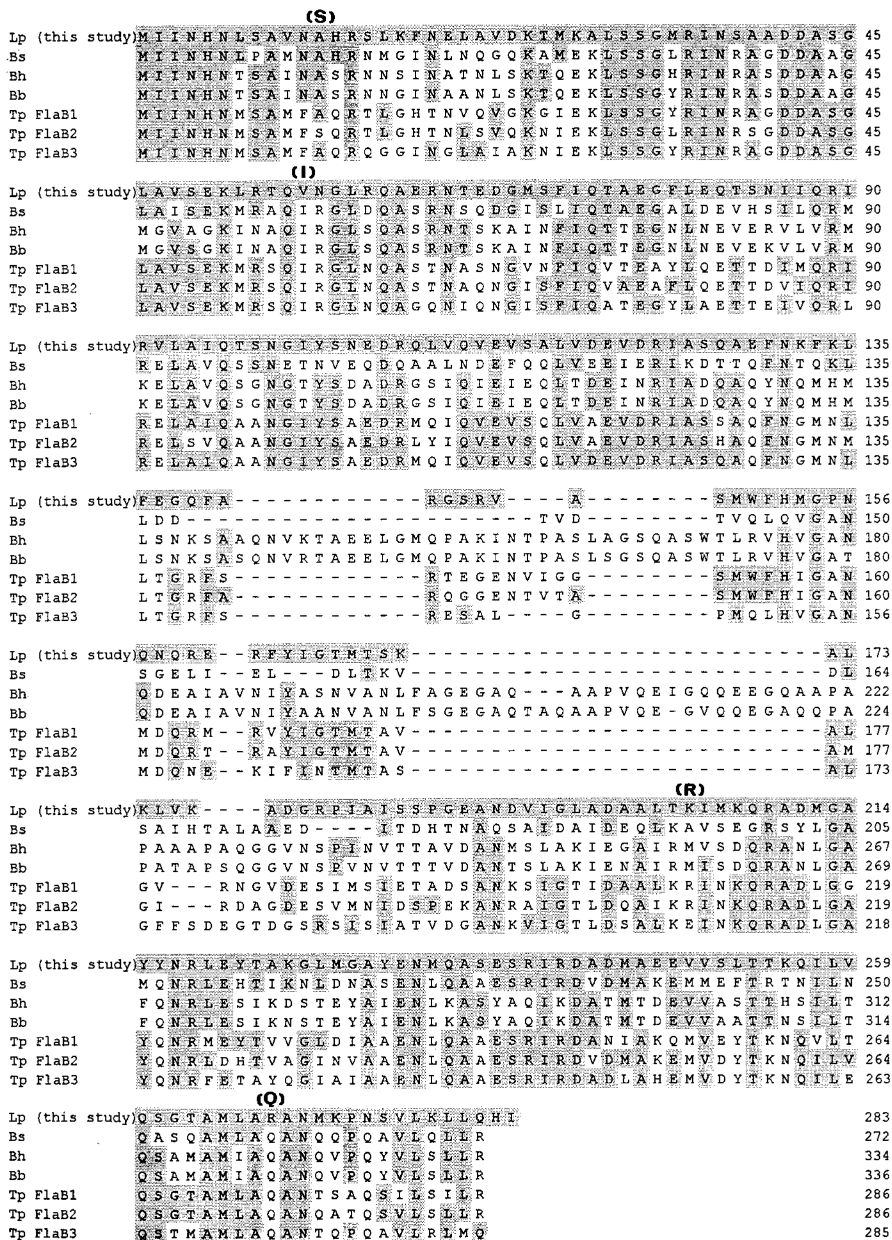

FIG. 3. Alignment of the deduced amino acid sequence of the serovar pomona flagellin with the sequences of other flagellin proteins. The protein sequences selected here for comparison are from *L. interrogans* serovar pomona (Lp) (this study), *Bacillus* sp. strain C-125 (Bs) (25), *B. burgdorferi* (Bb) (29); *B. hermsii* (Bh) (23), and *T. pallidum* (Tp) (FlaB1, FlaB2, and FlaB3) (3, 21). Amino acid residues that match exactly with those of the serovar pomona flagellin (this study) are shaded. Gaps are indicated by dashes. The serovar hardjo flagellin sequence (18) is displayed in parentheses, with only the residues not identical to those of the serovar pomona protein shown.

serovar hardjo FlaB (18) but smaller than the size of the native protein (35 kDa) as determined by SDS-PAGE. A similar difference between the deduced and apparent molecular masses of other flagellins has also been found (18, 29). There are two possibilities which may account for this difference. Molecular mass estimation with SDS-PAGE may not be precisely accurate. Alternatively, there may be a posttranslational modification of the serovar pomona FlaB protein in vivo. As found with the FlaB polypeptides of other spirochetes (5), the serovar pomona protein contains neither a cysteine residue nor a putative consensus sequence (33) necessary for the attachment of a lipid moiety. However, the serovar pomona FlaB protein contains an N-linked glycosylation site (14), Asn-X-Ser/Thr, at aa 6 to 8 in the N terminus. Glycosylation of flagellins has been reported for *Halobacterium halobium* (31). Therefore, the addition of a carbohydrate group to the serovar pomona flagellin is possible, although we have no direct experimental evidence for N-linked glycosylation.

**Sequence comparison of flagellin proteins.** Nucleotide and protein sequences were analyzed and aligned with a Lasergene software package (DNASTAR, Inc., Madison, Wis.). The serovar pomona flagellin sequence, deduced from the nucleotide sequence, was aligned with the sequences of flagellins from several spirochetes and one *Bacillus* sp. (Fig. 3). The *Bacillus*

species was included in the comparison as the representative of bacteria with exoflagella. The aligned sequences do not include all published amino acid sequences of flagellins but are limited to several of many sequences found when performing a similarity search of the N-terminal amino acid sequence of the serovar pomona 35-kDa protein. The serovar pomona flagellin shows a close relationship with the serovar hardjo protein, with a homology (identity) of 98.6%. In comparison with other bacterial species, the serovar pomona protein has an overall 42.0% identity with the *Bacillus* sp. strain C-125 flagellin, 39.6% identity with the flagellins from two *Borrelia* species (*B. hermsii* and *B. burgdorferi*), and 55.5 to 57.2% identity with the *T. pallidum* flagellins (FlaB1, FlaB2, and FlaB3). The N-terminal (aa 1 to 131) and C-terminal (aa 191 to 283) regions of the serovar pomona flagellin have 48.9 to 51.9% and 43.0 to 46.2% identity, respectively, to the comparable regions in the flagellins from the species of *Bacillus* and *Borrelia*, whereas the central region (aa 132 to 190) has only 10.2 to 13.6% identity. Similarly, the serovar pomona flagellin shows higher homology in the N-terminal and C-terminal regions (61.1 to 64.1% and 57.0 to 60.2%, respectively) and a reduced level of homology in the central region (33.9 to 44.1%) compared to the counterpart regions of the *T. pallidum* flagellins.

An amino acid sequence comparison showed that the serovar pomona FlaB protein had a considerable degree of overall homology with the FlaB polypeptides or flagellins from other bacterial species. The homology (identity) was even more remarkable in the N-terminal and C-terminal regions but was significantly reduced in the central region. This indicated that the central region of the leptospiral FlaB protein is highly variable in amino acid sequence and could be species specific. A similar sequence pattern was also found with other flagellins (25, 29). The information contained in the central region may be useful for the design of a specific peptide or DNA probe to detect infection with leptospires. A structural role has been proposed for the conserved N-terminal and C-terminal regions of a flagellin; that is, they point into the interior of the filament to form a hairpin loop structure with exposure of the central part on the outer surface of the filament (10, 19). The conserved regions are also believed to be involved in filament assembly (12, 27, 30). In contrast, the variable central region of a flagellin has not been related to any structural or functional roles in the filament. Based on the structural model proposed by Homma et al. (10) and Namba et al. (19), it may be possible that the variable central region exposed on the outer surface of the filament is involved in the induction of a species-specific immune response in an infected host. To test this hypothesis, a truncated polypeptide corresponding to the variable central region of *L. interrogans* FlaB may be expressed and used to assess the immune response. Such a truncated polypeptide could also be a useful reagent in the serological diagnosis of infection with leptospires.

**Nucleotide sequence accession number.** The serovar pomona *flaB* nucleotide sequence has been deposited in the GenBank database under accession number AF014114.

We acknowledge the technical assistance of Yvon Barbeau and Andre Bergern. We also thank C. Lutze-Wallace, S. Vydelinggum, and F. C. Thomas for comments and suggestions.

## REFERENCES

1. **Auran, N. E., R. C. Johnson, and D. M. Ritzi.** 1972. Isolation of the outer sheath of *Leptospira* and its immunogenic properties in hamsters. Infect. Immun. **5:**968–975.
2. **Brahamsha, B., and E. P. Greenberg.** 1989. Cloning and sequence analysis of *flaA*, a gene encoding a *Spirochaeta aurantia* flagellar filament antigen. J. Bacteriol. **171:**1692–1697.
3. **Champion, C. I., J. N. Miller, M. A. Lovett, and D. R. Blanco.** 1990. Cloning, sequencing, and expression of two class B endoflagellar genes of *Treponema pallidum* subsp. *pallidum* encoding the 34.5- and 31.0-kilodalton proteins. Infect. Immun. **58:**1697–1704.
4. **Chang, A., S. Faine, and T. Williams.** 1974. Cross-reactivity of the axial filament antigen as a criterion for classification of *Leptospira*. Aust. J. Exp. Biol. Med. Sci. **52:**549–568.
5. **Charon, N. W., E. P. Greenberg, M. B. H. Koopman, and R. J. Limberger.** 1992. Spirochete chemotaxis, motility, and the structure of the spirochaetal periplasmic flagella. Res. Microbiol. **143:**597–603.
6. **Cockayne, A., M. J. Bailey, and C. W. Penn.** 1987. Analysis of sheath and core structures of the axial filament of *Treponema pallidum*. J. Gen. Microbiol. **133:**1397–1407.
7. **Farr, R. W.** 1995. Leptospirosis. Clin. Infect. Dis. **21:**1–8.
8. **Goldstein, S. F., and N. W. Charon.** 1988. Motility of the spirochete *Leptospira*. Cell Motil. Cytoskeleton **9:**101–110.
9. **Heath, S. E., and R. Johnson.** 1994. Leptospirosis. J. Am. Vet. Med. Assoc. **205:**1518–1523.
10. **Homma, M., H. Fujita, S. Tamaguchi, and T. Iino.** 1987. Regions of *Salmonella typhimurium* flagellin essential for its polymerization and excretion. J. Bacteriol. **169:**291–296.
11. **Isaacs, R. D., J. H. Hanke, L.-M. Guzman-Verduzco, G. Newport, N. Agabian, M. V. Norgard, S. A. Lukehart, and J. D. Radolf.** 1989. Molecular cloning and DNA sequence analysis of the 37-kilodalton endoflagellar sheath protein gene of *Treponema pallidum*. Infect. Immun. **57:**3403–3411.
12. **Joys, T. M.** 1985. The covalent structure of the phase-1 flagellar filament protein of *Salmonella typhimurium* and its comparisons with other flagellins. J. Biol. Chem. **260:**15758–15761.
13. **Kelson, J. S., B. Adler, A. J. Chapman, and S. Faine.** 1988. Identification of leptospiral flagellar antigens by gel electrophoresis and immunoblotting. J. Med. Microbiol. **26:**47–53.
14. **Lechner, J., and F. Wieland.** 1989. Structure and biosynthesis of prokaryotic glycoproteins. Annu. Rev. Biochem. **58:**173–194.
15. **LeFebvre, R. B., A. B. Thiermann, and J. Foley.** 1987. Genetic and antigenic differences of serologically indistinguishable leptospires of serovar *hardjo*. J. Clin. Microbiol. **25:**2094–2097.
16. **Lin, M., E. A. Sugden, M. E. Jolley, and K. Stilwell.** 1996. Modification of the *Mycobacterium bovis* extracellular protein MPB70 with fluorescein for rapid detection of specific serum antibodies by fluorescence polarization. Clin. Diagn. Lab. Immunol. **3:**438–443.
17. **Lin, M.** Unpublished data.
18. **Mitchison, M., J. I. Rood, S. Faine, and B. Adler.** 1991. Molecular analysis of a *Leptospira borgpetersenii* gene encoding an endoflagellar subunit protein. J. Gen. Microbiol. **137:**1529–1536.
19. **Namba, K., I. Yamashita, and F. Vonderviszt.** 1989. Structure of the core and central channel of bacterial flagella. Nature (London) **342:**648–654.
20. **Norris, S. J., N. W. Charon, R. G. Cook, M. D. Fuentes, and R. J. Limberger.** 1988. Antigenic relatedness and N-terminal sequence homology define two classes of periplasmic flagellar proteins of *Treponema pallidum* subsp. *pallidum* and *Treponema phagedenis*. J. Bacteriol. **170:**4072–4082.
21. **Pallesen, L., and P. Hindersson.** 1989. Cloning and sequencing of a *Treponema pallidum* gene encoding a 31.3-kilodalton endoflagellar subunit (FlaB2). Infect. Immun. **57:**2166–2172.
22. **Parales, J., Jr., and E. P. Greenberg.** 1991. N-terminal amino acid sequences and amino acid compositions of the *Spirochaeta aurantia* flagellar filament polypeptides. J. Bacteriol. **173:**1357–1359.
23. **Picken, R. N.** 1992. Polymerase chain reaction primers and probes derived from flagellin gene sequences for specific detection of the agents of Lyme disease and North American relapsing fever. J. Clin. Microbiol. **30:**99–114.
24. **Ramadass, P., B. D. W. Jarvis, R. J. Corner, D. Penny, and R. B. Marshall.** 1992. Genetic characterization of pathogenic *Leptospira* species by DNA hybridization. Int. J. Syst. Bacteriol. **42:**215–219.
25. **Sakamoto, Y.-I., K. J. Sutherland, J. Tamaoka, T. Kobayashi, T. Kudo, and K. Horikoshi.** 1992. Analysis of the flagellin (*hag*) gene of alkalophilic *Bacillus* sp. C-125. J. Gen. Microbiol. **138:**2159–2166.
26. **Smibert, R. M.** 1973. Spirochaetales, a review. Crit. Rev. Microbiol. **2:**491–552.
27. **Trachtenberg, S., and D. J. DeRosier.** 1988. Three-dimensional reconstruction of the flagellar filament of *Caulobacter crescentus*. J. Mol. Biol. **202:**787–808.
28. **Trueba, G. A., C. A. Bolin, and R. L. Zuerner.** 1992. Characterization of the periplasmic flagellum proteins of *Leptospira interrogans*. J. Bacteriol. **174:**4761–4768.
29. **Wallich, R., S. E. Moter, M. M. Simon, K. Ebnet, A. Heiberger, and M. D. Kramer.** 1990. The *Borrelia burgdorferi* flagellum-associated 41-kilodalton antigen (flagellin): molecular cloning, expression, and amplification of the gene. Infect. Immun. **58:**1711–1719.
30. **Wei, L., and T. M. Joys.** 1985. Covalent structure of three phase-1 flagellar

filament proteins of Salmonella. J. Mol. Biol. **186:**791–803.
31. **Wieland, F., P. Gerhard, and M. Sumper.** 1985. Halobacterial flagellins are sulfated glycoproteins. J. Biol. Chem. **260:**15180–15185.
32. **Woodward, M. J., and J. S. Redstone.** 1994. Deoxynucleotide sequence conservation of the endoflagellin subunit protein gene, flaB, within the genus *Leptospira*. Vet. Microbiol. **40:**239–251.
33. **Wu, H. C., and M. Tokunaga.** 1986. Biogenesis of lipoprotein in bacteria. Curr. Top. Microbiol. Immunol. **125:**127–157.
34. **Yasuda, P. H., A. G. Steigerwalt, K. R. Sulzer, A. F. Kaufmann, F. Rogers, and D. J. Brenner.** 1987. Deoxyribonucleic acid relatedness between serogroups and serovars in the family *Leptospiraceae* with proposals for seven new *Leptospira* species. Int. J. Syst. Bacteriol. **37:**407–415.

*Editor:* J. G. Cannon