Molecular analysis of a flagellar core protein gene of *Serpulina (Treponema) hyodysenteriae*

MARCEL B. H. KOOPMAN, ELLEN BAATS, OLAV S. DE LEEUW, BERNARD A. M. VAN DER ZEIJST and JOHANNES G. KUSTERS*

Department of Bacteriology, Institute of Infectious Diseases and Immunology, School of Veterinary Medicine, University of Utrecht, PO Box 80.165, 3508 TD Utrecht, The Netherlands

(Received 5 October 1992; revised 2 March 1993; accepted 9 March 1993)

The *flaB2* gene encoding a protein located in the core of the periplasmic flagella of *Serpulina hyodysenteriae* was cloned and sequenced. The FlaB2 protein consists of 285 amino acids and has a calculated molecular mass of 31·1 kDa. Southern blot analysis indicated that at least one, and possibly two genes related to *flaB2* are present in the genome of *S. hyodysenteriae*. Comparison of the amino acid sequence of FlaB2 to sequences present in data banks showed significant similarity with the core flagellins of other spirochaetes, in particular with a FlaB2 protein from *Treponema phagedenis*.

Introduction

The spirochaete *Serpulina hyodysenteriae* (formerly *Treponema hyodysenteriae*) is the aetiological agent of swine dysentery (Harris *et al.*, 1972; Taylor & Alexander, 1971). It is a spiral-shaped bacterium that preferentially colonizes the mucus gel covering the epithelium and the mucus-filled crypts of Lieberkühn in dysenteric pigs (Kennedy *et al.*, 1988). To remain in the colon the bacteria must withstand the flow of the intestinal contents. There is no evidence that specific adhesion mechanisms are used for this *in vivo*, although *in vitro* *S. hyodysenteriae* adheres to epithelial cells (Knoop *et al.*, 1979; Bowden *et al.*, 1989). Most bacteria are immobilized in the viscous intestinal mucus but *S. hyodysenteriae* is highly motile in this environment (Kennedy *et al.*, 1988). This association with the mucus may be the predominant mechanism of mucosal colonization used by *S. hyodysenteriae* (Kennedy *et al.*, 1988) and hence motility may contribute to virulence of this organism.

The characteristic corkscrew-like movement by which *S. hyodysenteriae* and other spirochaetes propel themselves through viscous solutions is mediated by flagella that are located in the periplasmic space, completely

surrounded by the outer membrane of the cell (Canale-Parola, 1978; Paster & Canale-Parola, 1980). *S. hyodysenteriae* has two bundles of seven to nine periplasmic flagella that are each attached subterminally to one cell end and overlap in the middle of the cell cylinder (Harris *et al.*, 1972). Whereas most bacterial flagella have only one major flagellar protein, the periplasmic flagella of *S. hyodysenteriae* are complex and contain multiple major proteins. They consist of a core composed of three proteins (molecular masses 37, 34 and 32 kDa, designated FlaB1, FlaB2 and FlaB3, respectively) and a sheath containing a 44 kDa (FlaA1) and 35 kDa (FlaA2) protein (Koopman *et al.*, 1992a). With the exception of *Borrelia burgdorferi* (Coleman & Benach, 1989) and *Treponema zuelzera* (Bharier & Rittenberg, 1971), whose periplasmic flagella contain a single protein species, other spirochaetes have a similar flagella composition (Brahamsha & Greenberg, 1988; Canale-Parola, 1978; Charon *et al.*, 1992; Cockayne *et al.*, 1987; Kelson *et al.*, 1988; Nauman *et al.*, 1969; Norris *et al.*, 1988). In this paper we report the cloning and sequencing of a gene, *flaB2*, encoding the 34 kDa flagellar core protein of *S. hyodysenteriae* and provide evidence that at least one, and possibly two, genes related to *flaB2* are present in the genome of *S. hyodysenteriae*.

* Author for correspondence. Tel. 31 30 534987; fax 31 30 540784.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number X63513.

Methods

Bacterial strains, culture conditions and cloning vectors. *S. hyodysenteriae* strain C5 is a haemolytic Dutch field isolate, isolated from the colon of a pig severely affected by swine dysentery. *Escherichia*

Fig. 1. Nucleotide sequence and deduced amino acid sequence of the *S. hyodysenteriae* core flagellar gene *flaB2*. Amino acid residues that are in agreement with the N-terminal sequence of native flagellar protein are underlined. The putative ribosome-binding site (RBS) and sequences that show similarity to the *E. coli* consensus -10 and -35 sequences are also underlined.

Fig. 2. Hybridization of the *flaB2* gene with chromosomal DNA of *S. hyodysenteriae* C5. Chromosomal DNA fragments obtained after digestion with various restriction endonucleases were separated in a 0.6% agarose gel, blotted onto nitrocellulose and hybridized with a PCR-amplified *flaB2* sequence. Size markers are indicated on the left. (a) Ethidium-bromide-stained agarose gel; (b) autoradiograph after stringent washing of the Southern blot; (c) autoradiograph after low-stringency washing of the Southern blot.

al., 1990; Harshey *et al.*, 1989; Joys, 1985; Kuwajima *et al.*, 1986; Lavallie & Stahl, 1989; Martin & Savage, 1988; Nuijten *et al.*, 1990; Totten & Lory, 1990). This revealed the presence of an amino acid sequence (MLAQANQ) that is not only present at the carboxy-terminal end of most spirochaete core flagellar proteins but also at the carboxy-terminal ends of other flagellar proteins of remotely related bacteria. We expected that a highly similar amino acid sequence would be present at the carboxyl-terminal end of core flagellar proteins of *S. hyodysenteriae*. Thus, a mixture of oligodeoxynucleotides covering all possible sequences coding for the amino acid sequence MLAQANQ was used in conjunction with mixed oligonucleotides derived from the known N-terminus (MINNNI) of the *S. hyodysenteriae* core

proteins (Koopman *et al.*, 1992a) to amplify core flagellar gene sequences from the chromosome of *S. hyodysenteriae* by PCR. This resulted in a PCR product of approximately 800 bp, corresponding to the size of the expected PCR products of other spirochaetes. Partial sequencing of the amplified DNA fragment confirmed that it encoded part of a flagellar core gene (data not shown).

Cloning of the *flaB2* gene

The radiolabelled PCR product was used to screen an *S. hyodysenteriae* genomic library. This screening was designed to obtain clones containing an entire core flagellar gene and to correct PCR errors. Approximately

50 000 plaques were screened. Twenty-eight of these reacted positively with the probe. The insert DNA of these clones was isolated and restricted with *Eco*RI and *Hind*III. Agarose gel electrophoresis of the DNAs revealed four different, but related, restriction patterns (results not shown). Some of the restriction fragments were common to all clones, indicating that the clones contain DNA from the same area of the *S. hyodysenteriae* chromosome. The smallest of the 28 plasmids (designated pC1) containing 2 kb of insert DNA was chosen for nucleotide sequence analysis.

Nucleotide sequence and deduced amino acid sequence of the *flaB2* gene

The nucleotide sequence of part of the *S. hyodysenteriae* insert DNA in pC1 was determined. The sequence data in Fig. 1 show an 855 bp open reading frame, corresponding to 285 amino acid residues. The first 23 amino acid residues are in agreement with the previously determined N-terminal amino acid sequence of the 34 kDa core flagellin of *S. hyodysenteriae* (Koopman *et al.*, 1992a). Since this is the second-largest core flagellin the gene is designated *flaB2* and the protein it encodes is called FlaB2. The calculated molecular mass of FlaB2 is 31.1 kDa, which is slightly less than the apparent molecular mass of the native protein on SDS-PAGE gels. This discrepancy may be the result of aberrant migration of the protein on SDS-PAGE gels. Alternatively, the native FlaB2 core protein may be post-translationally modified, as has been described for flagellar proteins from a number of bacteria (Joys & Kim, 1979; Logan *et al.*, 1989; Wieland *et al.*, 1985). The G + C percentage of *flaB2* is 36 mol%, which is higher than the reported 28 mol% G + C content of the *S. hyodysenteriae* genome (Coene *et al.*, 1989). As in flagellins of other bacteria, there are no cysteine residues present. A consensus *E. coli* Shine-Dalgarno sequence is present upstream from the initiation codon. Although sequences that show some similarity to *E. coli* -10 or -35 promoter consensus sequences can be discerned, *E. coli* transformed with plasmid pC1 did not produce amounts of flagellin that could be detected on immunoblots with specific antibodies (results not shown). Perhaps the *flaB2* gene forms part of an operon. Alternatively, it may be transcribed from an unusual promoter that is not recognized by *E. coli*. In many bacteria the expression of flagellar genes and chemotaxis and motility genes is regulated by a σ factor that resembles the *B. subtilis* σ^{28} factor (Helmann, 1991). A σ^{28} -like promoter sequence has also been identified upstream of the *flaB* flagellar genes of *T. pallidum* (Champion *et al.*, 1990; Pallesen & Hindersson, 1989). However, a σ^{28} recognition sequence can not be discerned with the *flaB2* gene of *S. hyodysenteriae*.

Probing *S. hyodysenteriae* DNA with the *flaB2* gene

The N-terminal amino acid sequences of the three core proteins of *S. hyodysenteriae* are highly similar, but not identical (Koopman *et al.*, 1992a), suggesting that the core proteins are encoded by separate genes. DNA hybridization studies were therefore performed to determine whether genes related to *flaB2* are present in the *S. hyodysenteriae* chromosome. Chromosomal DNA was restricted with three endonucleases that do not cut in the *flaB2* gene (*Bgl*II, *Hae*II, *Hha*I, or *Ssp*I), or restricted with *Hind*III, for which one site is present. The DNA was then blotted to nitrocellulose and hybridized to a radiolabelled PCR fragment representing the entire *flaB2* gene. After high-stringency washing of the blot the probe hybridized to a single band in *Bgl*II, *Hae*III, *Hha*I or *Ssp*I-cut DNA and to two bands in chromosomal DNA cut with *Hind*III (Fig. 2b). The hybridizing *Hae*III and *Hha*I bands are large enough to accommodate more than one *flaB2* gene, but the single *Ssp*I fragment (approximately 1 kb) that hybridizes to the *flaB2* probe is not. These results show that there is a single *flaB2* gene in the *S. hyodysenteriae* chromosome. When blots were washed under conditions of lower stringency, additional bands were observed (Fig. 2c). These extra bands may represent *flaB* genes. The extra bands observed in the lane containing chromosomal DNA cut with *Ssp*I indicate that a second *flaB* gene resides completely or for the most part on the 800 bp fragment and perhaps partly on the fragment represented by the faint 2.2 kbp band. Alternatively, the latter band may contain (part of) a third *flaB* gene. In any case, the third *flaB* gene that should be present on the chromosome of *S. hyodysenteriae* will be weakly related to the other two *flaB* genes. A similar situation exists in *T. pallidum* (Champion *et al.*, 1990; Pallesen & Hindersson, 1989).

Homology analysis of FlaB2 with other flagellins

The deduced amino acid sequence of the *S. hyodysenteriae* FlaB2 protein was aligned with other spirochaete class B proteins cloned and sequenced so far. Significant homology was observed (Fig. 3). This alignment reveals an amino acid identity of 61.2%, 59.8% and 59.3%, respectively, with the FlaB1, the FlaB2 and the FlaB3 flagellar core proteins of *Treponema pallidum* (Champion *et al.*, 1990; Pallesen & Hindersson, 1989). The *S. hyodysenteriae* FlaB2 protein also showed 58.5% identity with the recently cloned FlaB2 protein of *Leptospira borgpetersenii* (Mitchison *et al.*, 1991), 62.9% identity with the FlaB2 of *Treponema phagedenis* (Limberger *et al.*, 1992) and (although not a 'true' core protein) 39.3% identity with the flagellin protein of *Borrelia burgdorferi* (Gassmann *et al.*, 1989). The *S. hyodysenteriae* FlaB2 protein and other spirochaete

SHYOFLAB2	MIINMISAINAORTLKFRMVDLSKDMAALSSGMRINRAGDDASGLAVSEKMRQIRGLR	
TPHAFLAB2	MIINMISAMFAQRTLGHTLNVQKNEKLSGLRINRAGDDASGLAVSEKMRQIRGLN	
TPALFLAB1	MIINMISAMFAQRTLGHTNVQVQKIEKLSGGRINRAGDDASGLAVSEKMRQIRGLN	
TPALFLAB2	MIINMISAMFAQRTLGHTLNVQKNEKLSGLRINRAGDDASGLAVSEKMRQIRGLN	
TPALFLAB3	MIINMISAMFAQRTLGHTLNVQKNEKLSGGRINRAGDDASGLAVSEKMRQIRGLN	
LEPFLAB	MIINHL SAVWSHRLKFNELAVOKTKKLSGGRINRAGDDASGLAVSEKMRQIRGLR	
BBFLA	MIINHTSAINASRNNGINAANL SKTQKLSGGRINRAGDDAAGMVGSKINAQIRGLS	**** * * * * *
SHYOFLAB2	QAERNNSGSIQITTEGYLQESQDILQRIELAVQSANGIYTDADRMLTQVEVSQVDE	
TPHAFLAB2	QASTNAQNGISFIQVAESYLQETTDVQIRREL SVOSANGIYSAEDRMHYIQVEVSQVAE	
TPALFLAB1	QASTNASNGVNFQVTEAYLQETTDVQIRREL SVOSANGIYSAEDRMHYIQVEVSQVAE	
TPALFLAB2	QASTNAQNGISFIQVAESYLQETTDVQIRREL SVOSANGIYSAEDRMHYIQVEVSQVAE	
TPALFLAB3	QAGQTIQNGISFIQATEGYLAETTEIVQRLRELATQAANGIYSAEDRMHYIQVEVSQVDE	
LEPFLAB	QAEINTEDEGNSFIQTAEGLEQTSNIIQIRVLATQTSNGIYSNEDRLQVQVEVSQVDE	
BBFLA	QASRNTSKAINFIQTTGHLNEVEKLVVKKELAVQSGNGIYSDADRGSTQIEIQLTDE	** * * * * *
SHYOFLAB2	VNRIASHAQNTLMLTGRFSNPNEGAPVASH-----H-----FHIGAN	
TPHAFLAB2	IDRIASHAQNGNMLTGRFARETGENTYTASH-----H-----FHIGAN	
TPALFLAB1	VDRIASAQNGNMLTGRFSRTGENTVIGGSM-----H-----FHIGAN	
TPALFLAB2	VDRIASAQNGNMLTGRFAQGGENTYTASH-----H-----FHIGAN	
TPALFLAB3	VDRIASAQNGNMLTGRFSRESA-----LGHVCA	
LEPFLAB	VDRIASAQNGNMLTGRFSRESA-----LGHVCA	
BBFLA	VDRIASAQNGNMLTGRFSRESA-----LGHVCA	
SHYOFLAB2	MDERR-----RVYIGTMTAAALGL-----QTAEG-----TGISI	
TPHAFLAB2	MDQRT-----RAYIGTMTAAALGV-----RDVGE-----SILNI	
TPALFLAB1	MDQRM-----RVYIGTMTAAALGV-----RNGVDE-----SINSI	
TPALFLAB2	MDQRT-----RAYIGTMTAAALGV-----RDVGE-----SILNI	
TPALFLAB3	MDQNE-----KIFINTMTASALGFFSDEGTGDS-----RSTSI	
LEPFLAB	ONORE-----RFYIGTMTSKALKLVK-----ADG-----RPIAI	
BBFLA		
SHYOFLAB2	SSIDKANSAGTIDEALTKVSKQRSLPAYQNRLELTAQGLMIAYENTAASESRIRDTH	
TPHAFLAB2	DDPEKANRAIGTLDEATKK INKQADL GAYQNRLEYTVGVNVAENLQAAESRIRDVM	
TPALFLAB1	ETADSAKNSIGTIDAALKR INKQADL GGYQNRMEYTVVGLDTAAENLQAAESRIRDVM	
TPALFLAB2	DDPEKANRAIGTLQDAIKR INKQADL GAYQNRLEHTVAGINVAENLQAAESRIRDVM	
TPALFLAB3	ATVDGANKVIGTLQDAIKR INKQADL GAYQNRLEYTVAGIATAENLQAAESRIRDVM	
LEPFLAB	SSPGGANVIGTLQDAALTR INKQADL GAYQNRLEYTAKGLNGAYENHQAASESRIRDVM	
BBFLA	TTVDANTSLAKIENATRIISQANL GAFQNRLESTKSTAYATENLKASYAQIKDATH	.. * * * * *
SHYOFLAB2	AETSVKFAQDKQILSQANLMLAQANTMQGALRLIQ--	
TPHAFLAB2	AKEMVDYTKNQILVQSGTAMLAQANQATQSVLSLLR--	
TPALFLAB1	AKQVVEYTKNQILVQSGTAMLAQANQATQSVLSLLR--	
TPALFLAB2	AKEMVDYTKNQILVQSGTAMLAQANQATQSVLSLLR--	
TPALFLAB3	AKEMVDYTKNQILVQSGTAMLAQANQATQSVLSLLR--	
LEPFLAB	TDEVAATNTSILTSQSAHANIAQANQVPPVLSLLR--	
BBFLA	.. * * * * *	.. * * * * *

Fig. 3. Comparison of the core flagellin FlaB2 (SHYOFLAB2) to the flagellin of *L. borgpetersenii* (LEPFLAB), the *T. phagedenis* FlaB2 core flagellin (TPHAFLAB2), the three core flagellins of *T. pallidum* (TPALFLAB1-3) and the flagellin of *B. burgdorferi* (BBFLA). Identical amino acids are indicated with an asterisk, conserved substitutions by single dots. Spaces (dashes) are included to obtain maximum alignment.

flagellar core proteins share several characteristics with flagellins from a wide range of bacteria: the amino- and carboxyl-terminal regions of the proteins are conserved and they do not have a consensus signal sequence at their amino-terminus. Hence, spirochaete core flagellar proteins may be secreted through the centre of the flagellar filament to polymerize at its tip, a mechanism similar to that suggested in *E. coli* and *Salmonella typhimurium*. The terminal regions of the flagellins have an important role in this transport and assembly (Homma *et al.*, 1987; Kuwajima, 1988; Kuwajima *et al.*, 1989; Lavallie & Stahl, 1989).

All three core flagellar genes of *T. pallidum* have been sequenced. When comparing the *S. hyodysenteriae* FlaB2 protein with these three proteins, the highest degree of homology is not with the *T. pallidum* FlaB2 protein but with the FlaB1 protein. Since the differences in homology between FlaB1 and FlaB2 are very small, this finding

does not necessarily imply that *S. hyodysenteriae* FlaB2 is phylogenetically most closely related to the *T. pallidum* FlaB1. It is more probably a reflection of the strong conservation of functional domains of the spirochaetal flagellar core proteins. The latter is substantiated by the finding that all core proteins show approximately 60% identity with the *S. hyodysenteriae* FlaB2. Because this conservation is probably imposed by functional and structural demands on the core proteins we feel that, until we know more about the functional relationship of the three different spirochaetal flagellar core proteins, phylogenetic relationships based on these proteins are merely speculative. The phylogenetic relationships as calculated from the DNA sequence alignment of the Fla genes (not shown) are well in line with those for the rRNA sequences as reported by Stanton *et al.* (1991). Further studies are required to find out what the function of the periplasmic flagella of *S. hyodysenteriae* is and why *S. hyodysenteriae* and most other spirochaetes have multiple flagellar proteins.

This study was supported by Solvay Duphar BV Weesp, The Netherlands. We gratefully acknowledge the use of the services and facilities of the Dutch national NWO/SURF Expertise Center CAOS/CAMM, under grant numbers SON 326-052 and STW NCH99.1751. We thank C. Buskens for experimental assistance.

- BHARIER, M. A. & RITTENBERG, S. C. (1971). Chemistry of axial filaments of *Treponema zuelzeri*. *Journal of Bacteriology* **105**, 422-429.
- BOWDEN, C. A., JOENS, L. A. & KELLY, L. M. (1989). Characterization of the attachment of *Treponema hyodysenteriae* to Henle intestinal epithelial cells in vitro. *American Journal of Veterinary Research* **50**, 100-104.
- BRAHAMSHA, B. & GREENBERG, E. P. (1988). A biochemical and cytological analysis of the complex periplasmic flagella from *Spirochaeta aurantia*. *Journal of Bacteriology* **170**, 4023-4032.
- CANALE-PAROLA, E. (1978). Motility and chemotaxis of spirochetes. *Annual Review of Microbiology* **32**, 69-99.
- CHAMPION, C. I., MILLER, J. N., LOVETT, M. A. & BLANCO, D. R. (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. *Infection and Immunity* **58**, 100-104.
- CHARON, N. W., GREENBERG, E. P., KOOPMAN, M. B. H. & LIMBERGER, R. J. (1992). Spirochete chemotaxis, motility, and the structure of the spirochaetal periplasmic flagella. *Research in Microbiology* **143**, 100-104.
- COCKAYNE, A., BAILEY, M. J. & PENN, C. W. (1987). Analysis of sheath and core structures of the axial filament of *Treponema pallidum*. *Journal of General Microbiology* **131**, 1397-1407.
- COENE, M., AGLIANO, M., PAQUES, A. T., CATTANI, P., DETTORI, G., SANNA, A. & COCITO, C. (1989). Comparative analysis of the genomes of intestinal spirochetes of human and animal origin. *Infection and Immunity* **57**, 138-145.
- COLEMAN, J. L. & BENACH, J. L. (1989). Identification and characterization of an endoflagellar antigen of *Borrelia burgdorferi*. *Journal of Clinical Investigation* **84**, 322-330.
- GASSMANN, G. S., KRAMER, M., GOEBEL, U. B. & WALLICH, R. (1989). Nucleotide sequence of a gene encoding the *Borrelia burgdorferi* flagellin. *Nucleic Acids Research* **17**, 3590.

- Genomic organization and expression of *Campylobacter* flagellin genes. *Journal of Bacteriology* **172**, 1853–1860.
- HARRIS, D. L., GLOCK, R. D., CHRISTENSEN, C. R. & KINYON, J. M. (1972). Swine dysentery: inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. *Veterinary Medicine and Small Animal Clinician* **67**, 61–64.
- HARSHEY, R. M., ESTEPA, G. & YANAGI, H. (1989). Cloning and nucleotide sequence of a flagellin-coding gene (*hag*) from *Serratia marcescens* 274. *Gene* **79**, 1–8.
- HELMANN, J. D. (1991). Alternative sigma factors and the regulation of flagellar gene expression. *Molecular Microbiology* **5**, 2875–2882.
- HIGGINS, D. G. & SHARP, P. M. (1988). CLUSTAL: a package for performing multiple alignment on a microcomputer. *Gene* **73**, 237–244.
- HIGGINS, D. G. & SHARP, P. M. (1989). Fast and sensitive multiple sequence alignments on a microcomputer. *CABIOS Communication* **5**, 151–153.
- HOMMA, M., FUJITA, H., YAMAGUCHI, S. & IINO, T. (1987). Regions of *Salmonella typhimurium* flagellin essential for its polymerization and excretion. *Journal of Bacteriology* **169**, 291–296.
- JOYS, T. M. (1985). The covalent structure of the phase-1 flagellar filament protein of *Salmonella typhimurium* and its comparison with other flagellins. *Journal of Biological Chemistry* **60**, 15758–15761.
- JOYS, T. M. & KIM, H. (1979). Identification of *N*-methyl-lysine residues in the phase-1 flagellar protein of *Salmonella typhimurium*. *Microbios Letters* **7**, 65–68.
- KELSON, J. S., ADLER, B., CHAPMAN, A. J. & FAINE, S. (1988). Identification of leptospiral flagellar antigens by gel electrophoresis and immunoblotting. *Journal of Medical Microbiology* **26**, 47–53.
- KENNEDY, M. J., ROSNICK, D. K., ULRICH, R. G. & YANCEY, R. J., JR (1988). Association of *Treponema hyodysenteriae* with porcine intestinal mucosa. *Journal of General Microbiology* **134**, 1565–1576.
- KNOOP, F. C., SCHRANK, G. D. & FERRARO, F. M. (1979). *In vitro* attachment of *Treponema hyodysenteriae* to mammalian epithelial cells. *Canadian Journal of Microbiology* **25**, 399–405.
- KOOPMAN, M. B. H., BAATS, E., VAN VORSTENBOSCH, C. J. A. H. V., VAN DER ZEIJST, B. A. M. & KUSTERS, J. G. (1992a). The periplasmic flagella of *Serpulina* (*Treponema*) *hyodysenteriae* are composed of two sheath proteins and three core proteins. *Journal of General Microbiology* **138**, 2697–2706.
- KOOPMAN, M. B. H., DE LEEUW, O. S., VAN DER ZEIJST, B. A. M. & KUSTERS, J. G. (1992b). Cloning and DNA sequence analysis of a *Serpulina* (*Treponema*) *hyodysenteriae* gene encoding a periplasmic flagellar sheath protein. *Infection and Immunity* **60**, 2920–2925.
- KUWAJIMA, G. (1988). Construction of a minimum-size functional flagellin of *Escherichia coli*. *Journal of Bacteriology* **170**, 3305–3309.
- KUWAJIMA, G., ASAKA, J.-I., FUJIWARA, T., FUJIWARA, T., NODE, K. & KONDO, E. (1986). Nucleotide sequence of the *hag* gene encoding flagellin of *Escherichia coli*. *Journal of Bacteriology* **168**, 1479–1483.
- KUWAJIMA, G., KAWAGISHI, I., HOMMA, M., ASAKA, J.-I., KONDO, E. & MACNAB, R. M. (1989). Export of an N-terminal fragment of *Escherichia coli* by a flagellum-specific pathway. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 4953–4957.
- LAVALLE, E. R. & STAHL, M. L. (1989). Cloning of the flagellin gene from *Bacillus subtilis* and complementation studies of an in vitro-derived deletion mutation. *Journal of Bacteriology* **171**, 3085–3094.
- LIMBERGER, R. J., SLIVIENSKI, L. L., YELTON, D. B. & CHARON, N. W. (1992). Molecular genetic analysis of a class B periplasmic flagellum gene of *Treponema phagedenis*. *Journal of Bacteriology* **174**, 6404–6410.
- LOGAN, S. M., TRUST, T. J. & GUERRY, P. (1989). Evidence for posttranslational modification and gene duplication of *Campylobacter* flagellin. *Journal of Bacteriology* **171**, 3031–3038.
- MARTIN, J. H. & SAVAGE, D. D. (1988). Cloning, nucleotide sequence and taxonomic implications of the flagellin gene of *Roseburia cecicola*. *Journal of Bacteriology* **170**, 2612–2617.
- MITCHISON, M., ROOD, J. I., FAINE, S. & ADLER, B. (1991). Molecular analysis of a *Leptospira borgpetersenii* gene encoding an endoflagellar subunit protein. *Journal of General Microbiology* **137**, 1529–1536.
- NAUMAN, R. K., HOLT, S. C. & COX, C. D. (1969). Purification, ultrastructure, and composition of axial filaments from *Leptospira*. *Journal of Bacteriology* **98**, 264–280.
- NORRIS, S. J., CHARON, N. W., COOK, R. G., FUENTES, M. D. & LIMBERGER, R. J. (1988). Antigenic relatedness and N-terminal sequence homology define two classes of periplasmic flagellar proteins of *Treponema pallidum* subsp. *pallidum* and *Treponema phagedenis*. *Journal of Bacteriology* **170**, 4072–4082.
- NUIJTEN, P. J. M., VAN ASTEN, F. J. A. M., GAASTRA, W. & VAN DER ZEIJST, B. A. M. (1990). Structural and functional analysis of two *Campylobacter jejuni* flagellin genes. *Journal of Biological Chemistry* **265**, 17798–17804.
- PALLESEN, L. & HINDERSSON, P. (1989). Cloning and sequencing of a *Treponema pallidum* gene encoding a 31·3-kilodalton endoflagellar subunit (FlaB2). *Infection and Immunity* **57**, 2166–2172.
- PASTER, B. J. & CANALE-PAROLA, E. (1980). Involvement of periplasmic fibrils in motility of spirochetes. *Journal of Bacteriology* **141**, 359–364.
- PEARSON, W. R. & LIPMAN, D. J. (1988). Improved tools for biological sequence comparison. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 2444–2448.
- SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. (1989). *Molecular Cloning, a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- STANTON, T. B., JENSEN, N. S., CASEY, T. A., TORDOFF, L. A., DEWHIRST, F. E. & PASTER, B. J. (1991). Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus, *Serpula* gen. nov., as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb. nov. *International Journal of Systematic Bacteriology* **41**, 50–58.
- TAYLOR, D. J. & ALEXANDER, T. J. L. (1971). The production of dysentery in swine by feeding cultures containing a spirochaete. *British Veterinary Journal* **127**, 58–61.
- TOTTEN, P. A. & LORY, S. (1990). Characterization of the type A flagellin gene from *Pseudomonas aeruginosa* PAK. *Journal of Bacteriology* **172**, 7188–7199.
- WIELAND, F., PAUL, G. & SUMPER, M. (1985). Halobacterial flagellins are sulfated glycoproteins. *Journal of Biological Chemistry* **260**, 15180–15184.
- YOUNG, R. A. & DAVIS, R. W. (1983). Efficient isolation of genes by using antibody probes. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 1194–1198.