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Cloning and DNA Sequence Analysis of a *Serpulina* (*Treponema*) *hyodysenteriae* Gene Encoding a Periplasmic Flagellar Sheath Protein

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A *Serpulina* (*Treponema*) *hyodysenteriae* expression library was constructed in vector λZAP and screened with a polyclonal antiserum raised against *S. hyodysenteriae* periplasmic flagella. A single immunoreactive plaque was chosen for further analysis. The recombinant phage from this plaque contained a gene encoding the 44-kDa protein that is on the outer layer (or sheath) of the periplasmic flagella. DNA sequence analysis showed that the gene encodes a protein of 320 amino acids. The protein is homologous to the flagellar sheath proteins of *Treponema pallidum* and *Spirochaeta aurantia* but not to any other flagellar proteins. We designated the cloned *S. hyodysenteriae* flagellar sheath protein gene *flaA* and the encoded protein FlaA. The 19 N-terminal amino acid residues of FlaA constitute a signal peptide that is cleaved from the protein before assembly onto the flagella in the periplasm. Amino acid residues 20 to 38 correspond to the N-terminal amino acid sequence of the native protein. Upstream from the gene, DNA motifs that are similar to the consensus *Escherichia coli* −35 and −10 promoter sequences and a ribosome binding site were identified. Downstream from the gene, two inverted repeat sequences that may serve as a rho-independent transcription termination signal are present.

The spirochete *Serpulina* (*Treponema*) *hyodysenteriae* is the etiologic agent of swine dysentery. The disease occurs primarily in pigs between 8 and 16 weeks of age (10, 29). After infection with *S. hyodysenteriae*, pigs frequently develop mucohemorrhagic diarrhea, which results in dehydration, emaciation, rapid weight loss and, in severe cases, death (1). *S. hyodysenteriae* possesses two bundles of periplasmic flagella that are attached subterminally to the ends of the protoplasmic cylinder and overlap each other in the middle. The flagella are completely contained within the outer envelope and are essential for motility (5). They may hence be an important virulence factor.

Natural immunity against swine dysentery has been demonstrated in convalescent animals (15), and partial resistance to the disease can be induced by vaccination (8, 9, 24, 25). Although the mechanism of protection is unknown, the results of some studies suggest that (part of) the protective immune response is directed against a flagellar protein. Studies with sera from convalescent pigs and pigs protected by vaccination revealed a predominant humoral response to a group of proteins of 29 to 45 kDa that is conserved among serotypes (6). These proteins are probably components of the flagella (17). Furthermore, Boyden et al. (2) showed that a cloned flagellar protein of *S. hyodysenteriae* was protective in a mouse model of swine dysentery.

The flagella of *S. hyodysenteriae* consist of multiple proteins (17). We report here the cloning and DNA sequence analysis of the gene encoding the largest flagellar protein of *S. hyodysenteriae* C5 and show that this protein is on the sheath of the periplasmic flagella.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. *S. hyodysenteriae* C5 is a hemolytic Dutch field isolate originating from the colon of a pig severely affected by swine dysentery and was cultured at 40°C in an anaerobic hood by use of either 250 ml of Trypticase soy broth supplemented with 10% fetal calf serum, 0.05% RNA core type IIC from *Candida utilis* (Sigma Chemical Co., St. Louis, Mo.), and 400 μg of spectinomycin per ml or Trypticase soy agar (Becton Dickinson, Cockeysville, Md.) plates supplemented with 10% sheep blood, 400 μg of spectinomycin per ml, and 0.06% yeast extract (Oxoid, Basingstoke, England). *Escherichia coli* Y1090 (35) was used for library construction and screening. *E. coli* DH5αF′ (Bethesda Research Laboratories Life Technologies, Inc., Gaithersburg, Md.) was used as the recipient for plasmid transformations. *E. coli* was grown in LB medium or on LB agar plates (28).

Isolation of periplasmic flagella. Bacterial cultures (250 ml) containing 10^9 bacteria per ml were harvested by centrifugation at 15,000 × *g* for 30 min at 4°C and washed once with phosphate-buffered saline (PBS; 0.14 M NaCl, 2.7 mM KCl, 0.01 M Na_2HPO_4, 1.76 mM KH_2PO_4 [pH 7.3]). Pellets were suspended in 20 ml of PBS, and outer envelopes were removed by the addition of 10% sodium dodecyl sulfate (SDS) to a final concentration of 0.1% and incubation of the suspensions for 30 min at room temperature with gentle shaking. The organisms were collected by centrifugation at 25,000 × *g* for 30 min at 4°C and resuspended in PBS. Periplasmic flagella were removed from the cells by shearing in a blender for 10 min. Pauses in which the suspensions were cooled on ice to prevent overheating of the samples were included. Periplasmic flagella were separated from the bacterial bodies by collective pelleting of the latter at 30,000 × *g* for 30 min at 4°C. Sodium lauroyl sarcosinate (Sarkosyl) was added (0.2%) to the supernatant, and flagella were

sedimented by centrifugation at 94,000 × *g* for 60 min at 4°C, resuspended in water, and stored at −20°C.

Electron microscopy. Samples of flagellar preparations were applied to pioloform (Wacker-Chemie, Munich, Germany)-coated copper grids (200 mesh; Bio-Rad) for 10 min. After the grids were rinsed twice with water, the samples were negatively stained with phosphotungstic acid and examined with a Philips 201 electron microscope operating at 60 kV and with an objective aperture of 30 μm.

Antisera. Rabbit antisera directed against flagella were raised in New Zealand White rabbits by an intramuscular injection with 130 μg of purified flagellar protein in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) followed by an intramuscular boost with 50 μg of antigen in incomplete Freund's adjuvant (Difco) 29 days later. Sera were collected 1 day before immunization and at 7-day intervals after immunization. Antibodies were affinity purified by adsorption to and elution from immunoreactive plaques. Approximately 50,000 plaques, grown on 14-cm LB agar plates at 42°C for 2.5 h, were overlaid with nitrocellulose filters (Schleicher and Schuell, Dassel, Germany) and incubated at 37°C for an additional 2 h. Subsequently, the filters were removed and blocked for 1 h in 0.5% gelatin–0.5% Tween 20 in PBS (PBS^+). They were then incubated for 3 h in rabbit immune serum diluted 1:50 in PBS^+ and washed three times for 15 min each time in PBS^+ before elution of the bound antibodies in 10 ml of 0.2 M glycine–0.15 M NaCl (pH 2.8) for 5 min. The pH of the eluate was immediately readjusted to 8.0 with 5 ml of 1 M Tris-HCl (pH 8.0). A 1:10 dilution of the eluate in PBS^+ was used to probe immunoblots.

SDS-PAGE and immunoblotting (Western blotting). Proteins were separated by 12.5% SDS-polyacylamide gel electrophoresis (PAGE) as described by Laemmli (20) and visualized by Coomassie brilliant blue R-250 staining. Molecular weights were estimated by comparison with low-molecular-weight markers (Pharmacia, Uppsala, Sweden). After electrophoretic transfer to nitrocellulose filters (30), proteins were reacted with antiserum and bound antibodies were visualized with alkaline phosphatase-conjugated secondary antibodies by development in Nitro Blue Tetrazolium (0.37 mM)–5-bromo-4-chloro-3-indolyl phosphate (0.34 mM) solubilized in 100 mM Tris-HCl–100 mM NaCl–5 mM $MgCl_2$ (pH 9.5).

DNA and cloning techniques and screening of the genomic library. Unless stated otherwise, standard cloning procedures were used for all DNA manipulations (28). Plasmid pBluescript II KS^+ (Stratagene, San Diego, Calif.) was used in subcloning experiments and to prepare the template for DNA sequence analysis. A genomic DNA library was constructed in phage λZAP (Stratagene) essentially as described by Young and Davis (34). In brief, *S. hyodysenteriae* C5 was collected from liquid cultures and washed twice with PBS. Chromosomal DNA was prepared and partially digested with *Sau*3AI. Fragments ranging in size from 6 to 9 kbp were fractionated by agarose gel electrophoresis, ligated to *Eco*RI adaptors and then to *Eco*RI-digested λZAP arms, and subsequently packaged in phage particles. The genomic library was screened with the flagellar antiserum diluted 1:500 in PBS^+. Bound antibodies were visualized as described for Western blotting.

For Southern blotting, DNA fragments were separated on agarose gels, transferred to Hybond-N nylon filters (Amersham Corp., Arlington Heights, Ill.), and fixed by exposure to UV. Filters were prehybridized in 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7])–5× Denhardt's reagent–0.5% SDS–100 μg of heat-denatured salmon sperm DNA per ml for 6 h at 45°C. A radiolabeled probe was added, and hybridization was performed at 45°C for 16 h. Blots were washed in 2× SSPE–0.1% SDS at 45°C and autoradiographed with intensifying screens at −70°C and with Fuji XR film (Fuji Photo Film Co., Ltd., Tokyo, Japan). DNA fragments to be used in Southern experiments were isolated with Gene Clean (Bio 101, Inc., La Jolla, Calif.) and labeled with [α^{32}P]dATP (Amersham, Buckinghamshire, England) by use of a random-primer labeling kit (Boehringer, Mannheim, Germany).

Double-stranded DNA sequencing was done with the T7 sequencing kit from Pharmacia in accordance with the manufacturer's instructions. As a template, either CsCl-purified plasmid DNA or DNA amplified by the polymerase chain reaction (PCR) and subsequently purified with Gene Clean was used.

PCR DNA amplification was performed with *Taq* polymerase (Promega) in accordance with the instructions of the manufacturer. Each PCR cycle consisted of 1 min at 95°C, 1 min at 48°C, and 2 min at 72°C. For amplification of a DNA fragment containing the entire sheath flagellin gene and its regulatory sequences, the T7 primer was used in combination with oligonucleotide A (5′-CCGCTTCCTCCTGAGC-3′), derived from partial sequence data obtained from DNA upstream of the flagellin gene (see Fig. 3).

In vitro transcription and translation of DNA were performed with linear DNA purified from agarose gels with Gene Clean in the presence of L-[^{35}S]methionine (Amersham) by use of a prokaryotic DNA-directed translation kit from Amersham. The molecular weights of the synthesized labeled proteins were estimated by comparison with ^{14}C-methylated protein molecular weight markers (Amersham).

Computer analysis of DNA sequences. Nucleotide sequences were analyzed with the PC/Gene computing programs (release 6.50; Genofit S.A., Geneva, Switzerland). The FASTA program, release 1.3 (21, 26), was used to compare nucleotide and amino acid sequences with the following data bases: EMBL (release 27.0), GenBank (release 67.0), NBRF/PIR (release 28.0), Swiss-Prot (release 18.0), and Brookhaven (release 4.0). Homologous sequences were aligned with the Clustal computer program (11, 12) by use of the default values.

Nucleotide sequence accession number. The DNA sequence for the sheath flagellin gene (see Fig. 4) has been submitted to the EMBL data library and assigned accession number X63006.

RESULTS

Cloning of the periplasmic flagellar sheath protein gene. Periplasmic flagella were isolated from *S. hyodysenteriae* C5. Electron microscopy confirmed the flagellar nature of the obtained material (data not shown). Rabbit antisera raised against these isolated flagella were used to screen an *S. hyodysenteriae* library constructed in phage λZAP.

The recombinant λZAP phage from a single immunoreactive plaque (designated λfla6) was analyzed in further detail. Western blot analysis demonstrated that λfla6 directed the synthesis of a 41-kDa polypeptide that reacted strongly with the polyclonal rabbit antiflagellar serum (Fig. 1). The synthesis of immunoreactive proteins did not require isopropyl-D-β-thiogalactopyranoside (IPTG) (Fig. 1, lanes A and B), suggesting that *S. hyodysenteriae* expression signals are present on λfla6 and function in *E. coli*. Antibodies that bound to the proteins expressed by λfla6 were affinity

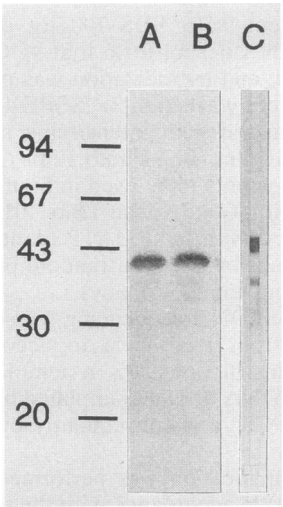

FIG. 1. Western blot analysis of whole-cell extracts of *E. coli* containing λfla6 cultured with (A) and without (B) IPTG and reacted with polyclonal rabbit antiserum to *S. hyodysenteriae* flagella. (C) Reaction of antibodies affinity purified from the expressed proteins with purified flagella. The numbers at the left indicate molecular masses in thousands.

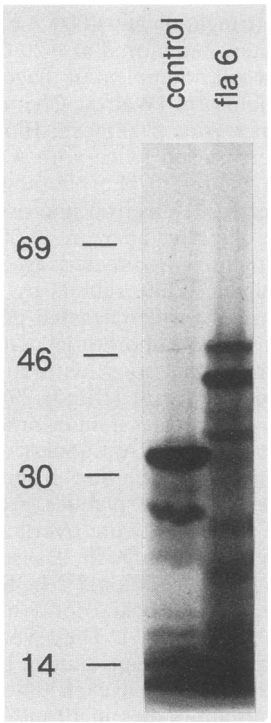

FIG. 2. Autoradiograph of an SDS-polyacrylamide gel of ^{35}S-labeled proteins synthesized from plasmid pAT153 (control) and the 1,750-bp *Eco*RI fragment from λfla6 (fla6). The numbers at the left indicate molecular masses in thousands.

purified from rabbit immune serum and used to probe Western blots of purified *S. hyodysenteriae* periplasmic flagella (Fig. 1, lane C). The affinity-purified antibodies bound strongly to the 44-kDa protein and weakly to the 35-kDa protein present in the periplasmic flagellar preparations.

In vitro transcription and translation. The DNA from phage clone λfla6 was isolated and digested with restriction endonuclease *Eco*RI. Agarose gel electrophoresis revealed the presence of two *S. hyodysenteriae*-specific *Eco*RI fragments of 1,500 and 1,750 bp. Whereas the 1,500-bp fragment could be subcloned in pBluescript II KS$^+$, all attempts to subclone the 1,750-bp fragment failed. This result suggests that the protein encoded by the 1,750-bp fragment is toxic for *E. coli*. For examination of whether the *S. hyodysenteriae*-specific *Eco*RI fragments indeed directed the synthesis of protein, DNA fragments were isolated from an agarose gel and tested in an in vitro transcription and translation system. Transcription of only the 1,750-bp fragment resulted in the synthesis of several labeled bands, among which was a prominent band of approximately 44 kDa (Fig. 2). This result indicates that the 1,750-bp fragment contains an entire flagellin gene with its transcription signals.

Nucleotide sequence analysis of the cloned flagellin gene. To obtain sufficient template DNA for sequence analysis, we PCR amplified the 1,750-bp DNA fragment from λZAP as outlined in Fig. 3. The nucleotide sequence was determined directly from the PCR products purified from agarose gels, instead of from subcloned PCR products, to avoid PCR-induced base-pair substitutions in the obtained nucleotide sequence. Nucleotide sequence analysis of the PCR-amplified DNA revealed the presence of a single open reading frame. The nucleotide sequence of this open reading frame and the derived amino acid sequence are shown in Fig. 4. The encoded protein consists of 320 amino acid residues and has a calculated molecular mass of 36.0 kDa. Residues 20 to 38 correspond to the N-terminal sequence of the purified 44-kDa *S. hyodysenteriae* flagellin (18). This fact indicates that the first 19 amino acid residues encoded by the gene are cleaved from the flagellin before assembly onto the flagella. Indeed, the 19 N-terminal amino acid residues have the characteristics of a signal peptide (31, 32). Hydrophilicity analysis by the algorithm of Kyte and Doolittle (19) (data not shown) demonstrated that this region is the only prominent hydrophobic area of the protein. Without the signal peptide, the protein has a calculated molecular mass of 33.8 kDa. This mass is considerably smaller than the apparent molecular mass of 44 kDa of this flagellin in flagellar preparations, as determined by SDS-PAGE. The difference suggests that the flagellin is posttranslationally modified. The complete absence of cysteine residues in the protein is consistent with

FIG. 3. Strategy used to obtain the DNA sequence of the *flaA* gene in λfla6. The 1,500-bp *Eco*RI fragment was subcloned in pBluescript II KS$^+$ and partially sequenced. From the obtained sequence, two oligonucleotides, A and B, were deduced. Oligonucleotide A was used in combination with the universal M13 primer to obtain a PCR product. Oligonucleotide B, the T7 primer, and oligonucleotides derived in the course of sequencing were used to determine the complete DNA sequence of the PCR product on both strands, encompassing the 1,750-bp *Eco*RI (E) fragment. The open reading frame on the fragment and its orientation are indicated by the large arrow. The solid boxes represent vector sequences.

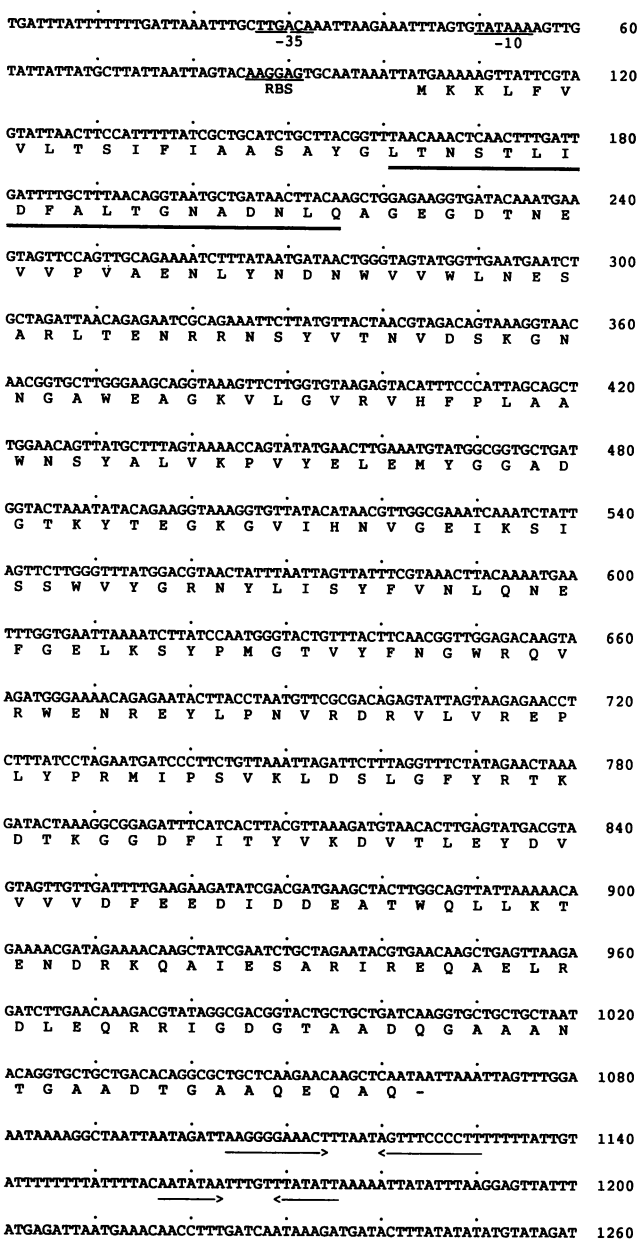

FIG. 4. Nucleotide sequence and deduced amino acid sequence of the *S. hyodysenteriae flaA* gene. Sequences similar to *E. coli* −35 and −10 promoter sequences and a ribosome binding site (RBS) are underlined. Amino acids confirmed by N-terminal sequencing of the mature protein are doubly underlined. Inverted repeats are indicated by arrows underneath the sequence.

the situation in other bacterial flagellar proteins. The G+C content of the *flaA* gene is 36%, a value slightly higher than the overall G+C contents of 28.5 to 30% reported for the *S. hyodysenteriae* genome (7). As expected in an organism with such a low G+C content, codon usage shows a strong bias for codons with A or T in the second (62%) and third (81%) positions. Upstream from the coding region, sequences similar to *E. coli* −35 and −10 promoter sequences and a ribosome binding site are present. Downstream from the termination codon, two inverted repeats that may serve as a transcription terminator are present (Fig. 4).

Homology analysis of the *S. hyodysenteriae* flagellin. A comparison of both the nucleotide and the deduced amino acid sequences of the cloned *S. hyodysenteriae* flagellin gene with the sequences present in the data bases revealed significant homology between the *S. hyodysenteriae* flagellar sheath protein and the flagellar sheath proteins of *Treponema pallidum* (13, 14) and *Spirochaeta aurantia* (4) but not other flagellar proteins. The alignment of the amino acid sequence of the *S. hyodysenteriae* flagellin with those of these sheath flagellins is shown in Fig. 5. When both identical amino acids and conserved amino acid substitutions are included, the overall sequence similarities between the *S. hyodysenteriae* flagellin and these sheath flagellins are 30.5% for *T. pallidum* and 32.1% for *S. aurantia*. These similarities indicate that the cloned *S. hyodysenteriae* flagellin is on the sheath of the periplasmic flagella. In accordance with the nomenclature used for other spirochetal flagellins (3, 13, 23), we designated the cloned *S. hyodysenteriae* flagellar sheath protein gene *flaA* and the encoded protein FlaA.

Determination of the copy number of *flaA*. DNA hybridization studies were performed to determine the number of genes identical or homologous to *flaA* present in the genome of *S. hyodysenteriae* (results not shown). The probe used represents the coding region of *flaA* (nucleotides 193 to 1212). On Southern blots with chromosomal DNA digested with *Hae*II, *Hha*I, or *Hin*dIII, one fragment hybridized (length of the fragment approximately 10, 6.5, or 8 kbp, respectively). On Southern blots with chromosomal DNA digested with *Taq*I, an intense band hybridized at approximately 1.5 kbp and a faint band was visible at approximately 0.8 kbp. As the probe used does not contain recognition sites for restriction endonucleases *Hae*II, *Hha*I, and *Hin*dIII and contains two recognition sites for *Taq*I (at nucleotides 1075 and 1319), these results indicate that there is a single *flaA* gene present in the *S. hyodysenteriae* genome and no homologs.

DISCUSSION

The periplasmic flagella of *S. hyodysenteriae* C5 are composed of multiple proteins (17). We isolated the gene encoding the largest flagellar protein when screening an *S. hyodysenteriae* expression library with an antiserum against purified periplasmic flagella of *S. hyodysenteriae* C5. DNA data base searches revealed significant homology of the encoded protein with the flagellar sheath proteins of *T. pallidum* (13, 14) and *S. aurantia* (4) but not with other flagellar proteins, indicating that the cloned *S. hyodysenteriae* gene encodes a sheath flagellin. In accordance with the nomenclature used for other spirochetal sheath flagellins (3, 13, 23), the gene was designated *flaA* and the protein that it encodes was designated FlaA. Nucleotide sequence analysis showed that *flaA* encodes a protein of 320 amino acid residues. A signal peptide of 19 amino acids is cleaved from the flagellin before assembly onto the flagella. The calculated molecular mass of the flagellin without its signal sequence is 33.8 kDa, whereas the molecular mass of the flagellin in flagellar preparations is 44 kDa, as determined by SDS-PAGE. The discrepancy between the calculated molecular mass of the protein and its apparent mobility in SDS-PAGE may be the result of aberrant migration of the protein on SDS-polyacrylamide gels. Alternatively, the native protein may be posttranslationally modified, as described for flagellar proteins from a number of bacteria (16, 22, 33). We are

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SHFLAA          MKKLFVVLTSIFIAASAYGLTN-STLIDFA------LTGNADNLQAGEGDTNEVVPVAEN    53
TPFLAA          MKKA-VVLSAVALLSGVCAVADESVLIDFAKLNADIMADKSGGMTHNRRTVLDYASLADT    59
SAFLAA          MKRFFAILGAALFVGNSGAFAEQATLIDFSKL----VGEGNTGL--HAPTTIDYSRQAGS    54
                **.  ..*..  . ..  . .. ..****.      ... ....     .. .  . *..

SHFLAA          LYNDN-------------WVVWLNESARLTENRRNSYVTNVDSKGNNGAWEAGKVLGVRV   100
TPFLAA          SYTDEQKALMRSSLAVAQWEVVLNSSARNPVAHAASRVIEAPVSEGAKSFAGERVLGVRV   119
SAFLAA          AYSAEDKAAMKISLAIPSWEIELASSSQTVENQTLSLVTAAPVKQDAARYGGETVMGVRI   114
                 *...             * . *..*..   ..  * *...  . ..  .... *.***.

SHFLAA          HFPLAAWNSYALVKPVYELEMYGGADGT-------------------KYTEGKGVIHNVG   141
TPFLAA          LFPTWDSNANAMIKPAFVIPAYEVMAQVDDQGNVQAPTEEEKASGKGRFEDGYGVVKNVG   179
SAFLAA          HFPSFGINSFAVIKPPFTIPAYATLGDATAQNAVAG----------GQF-DGFGVLKNVG   163
                 **  . *. *..** . .  *.  . .                    .. .* **..***

SHFLAA          EIKSISSWVYGRNYLISYFVNLQNEFGELKSYPMGTVYFNGWRQVRWENREYLPNVRDRV   201
TPFLAA          VLKSIAVNTYGMNYPHGLYVMMRDQDGEVHRYFMGYLLFDSWKELVWNNPSYISDVRSRE   239
SAFLAA          VIKSIQINILGRNYLNRLSLLLEDQNGDEREIVMGYLNFDGWKSLQWNNPNYQTEVRNRD   223
                 .***   . *.**     . . .. *. .   ** . *..*. . *.*..* ..**.*

SHFLAA          LVREPLYPRMIPSVKLDSLGFYRTKDTKGGDFITYVKDVTLEYDVVVVDFEEDIDDEATW   261
TPFLAA          VRLYPVYPASTPHVVFEGFMVTRDAAHAGGDYVGYFKDVKIIYDKAVLSTVRDFADEDLW   299
SAFLAA          LQIVPLYPRSAPLIKLKGIKIHRDGSQEGGDIVSYIKDIKVIYDQAVVDRNSDVDDEAIW   283
                .   *.**   * . ....   *. .  *** ..* **... ** .*..   * .**. *

SHFLAA          QLLKTENDRKQAIESARIREQAELRDLEQRRIGDGTAADQGAAANTGAADTGAAQEQAQ   320
TPFLAA          GIQARREAERKRVEVARFGQQQVLRYIEQEKLATEVGF---TPS-----GGAQRQEEQQ   350
SAFLAA          GILRQREEQYRNFELAKLGNLQVLRSLEKKKMAKEADFDQAAPA-----AAAARAPATN   337
                .    ..  .  * *.. . . ** .*. .......    ...     .... . . .
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FIG. 5. Comparison of sheath flagellin FlaA of *S. hyodysenteriae* (SHFLAA) with sheath flagellins of *T. pallidum* (TPFLAA) and *S. aurantia* (SAFLAA). Identical amino acids are indicated by asterisks, and conserved substitutions are indicated by dots. Spaces (dashes) were added to obtain maximum alignment.

currently investigating potential posttranslational modifications of the *S. hyodysenteriae* FlaA flagellar sheath protein.

Estimates from SDS-PAGE profiles of the total protein content of *S. hyodysenteriae* indicated that the flagellar proteins make up 5 to 10% of the total protein content. Therefore, the corresponding genes must be expressed at a high level. Upstream from the *flaA* gene, sequences that are similar to a consensus ribosome binding site and putative *E. coli* −35 and −10 promoter sequences were identified. Accordingly, the gene is expressed in *E. coli*. Downstream from the TAA stop codon, two self-complementary sequences that may serve as a potential termination signal are present (27). DNA hybridization studies demonstrated that the *S. hyodysenteriae* genome contains only one copy of the *flaA* gene and no detectable homologs.

A comparison of the *S. hyodysenteriae* FlaA sheath flagellin with the sheath flagellins of *T. pallidum* and *S. aurantia* revealed several conserved domains. Hydrophaticity analysis of FlaA demonstrated several sequences that may represent surface-exposed epitopes (data not shown). Whether the conserved regions are structurally important or whether the putative exposed epitopes are immunogenic is not known. The cloning and sequencing of the *flaA* sheath flagellin gene open new possibilities for studying its structure and function and its role in the pathogenesis of infection.

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