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Cloning and Sequence Analysis of *flaA*, a Gene Encoding a *Spirochaeta aurantia* Flagellar Filament Surface Antigen

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Spirochaeta aurantia **DNA that coded for an antigenic determinant of the flagellin associated with the filament surface of the periplasmic flagella was isolated. When expressed in *Escherichia coli*, the antigenic polypeptide had an apparent molecular weight of 37,000. Sequence analysis of the antigen-encoding DNA revealed the presence of an open reading frame that determined a polypeptide with a predicted molecular weight of 31,241. This polypeptide showed a region of identity with the N-amino-terminal region of the 39,000- and 37,000-dalton flagellins of the distantly related spirochetes *Treponema phagedenis* and *Treponema pallidum*, respectively (S. J. Norris, N. W. Charon, R. G. Cook, M. D. Fuentes, and R. J. Limberger, J. Bacteriol. 170:4072–4082, 1988). The region of identity in the deduced *S. aurantia* polypeptide was preceded by a possible signal sequence and signal peptidase cleavage site.**

Spirochetes are thin, helical bacteria that are motile by means of flagella that are contained entirely within the periplasmic space (11). *Spirochaeta aurantia*, a facultatively anaerobic spirochete isolated from freshwater sediments (4), has two periplasmic flagella that are ultrastructurally and biochemically complex (3). The flagellar filament consists of a core and an outer layer and is composed of three major polypeptides with apparent molecular weights of 37,500, 34,000, and 31,500 and three minor polypeptides with apparent molecular weights of 36,000, 33,000, and 32,000 (3). This structural complexity of the filament appears to be a characteristic that is shared by many of the spirochetes (5, 11, 15, 17, 18, 24), and in this respect, these organisms differ from other flagellated eubacteria, which generally have flagellar filaments composed of one (28) or at most two (31) major polypeptide subunits. Furthermore, layered filaments have been described only in spirochetes (11). The reasons for this structural and biochemical complexity are not yet clear, but they may be related to the unusual location of the flagella and to the way that they function to propel cells.

As part of ongoing studies of the biochemical and genetic basis of *S. aurantia* motility, we sought to isolate the genes that code for the flagellar filament subunits. Analysis of such genes could provide insight into the localization of spirochete flagella. *Escherichia coli* and *Salmonella typhimurium* flagellins are secreted, yet analysis of the genes coding for the filament polypeptide subunit has revealed no signal sequence (12). Rather, sequences at the C terminus appear to be important for the secretion (12), and it is thought that the secretion process involves the migration of individual flagellin subunits through the core of a growing filament to the growing tip, where they polymerize (6). Since spirochete flagellins remain in the periplasmic space, a different secretion mechanism may have evolved. Furthermore, should the motility and chemotaxis genes of *Spirochaeta aurantia* be clustered on the chromosome, as they are in other bacteria that have been studied (21), a flagellin gene could serve as a probe for their retrieval.

We report here the isolation and sequence of *S. aurantia* DNA which codes for a major flagellar filament antigen that is associated with the surface of flagella (3). To our knowledge, this is the first report of the DNA sequence of a spirochete flagellin gene, and the data are consistent with the idea that secretion of this outer layer protein involves a signal peptide.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. aurantia* M1 (4) was grown in GTY medium as described elsewhere (8). The *E. coli* strains used in this study are listed in Table 1 and were grown in L broth as described previously (2). The cultures of *E. coli* used in Western blot (immunoblot) analyses were prepared by inoculating an isolated colony into 5 ml of L broth containing 100 μg of ampicillin per ml with or without 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), as indicated. Cultures were incubated with shaking overnight at 37°C.

Plasmids, DNAs, and transformations. The plasmids used in this study are listed in Table 1. Plasmids and *S. aurantia* chromosomal DNA were isolated and purified as described elsewhere (2). Phage M13 mp18 and mp19 replicative-form DNA was obtained either from Bethesda Research Laboratories (Gaithersburg, Md.) or New England BioLabs, Inc. (Beverly, Mass.). Transformations were done by the procedure described by Hanahan (9).

Cloning and subcloning procedures. A plasmid clone bank consisting of *S. aurantia* M1 chromosomal DNA that was partially digested with *Sau*3A-I and that was ligated into the *Bam*HI site of the *tac* promoter vector pDR540 was prepared essentially as described previously (2), with the exception that the chromosomal DNA was digested to give an average fragment size of 15 to 20 kilobase pairs (kbp). All subcloning procedures were performed by cutting the desired fragments out of low-melting-temperature agarose (Sea Plaque; FMC Corp., Rockland, Maine) and by ligating them either directly in agarose as described by Struhl (29) or after purification by silica gel chromatography (Gene Clean; Bio101, LaJolla, Calif.).

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
E. coli K-12 strains		
JM107	*endA1 gyrA96 thi hsdR17 supE44 relA1 traD36* Δ(*lac proAB*) F′ *proAB lacI*^{q}ZΔM15	Bethesda Research Laboratories (29)
JM109	JM107 *recA1*	M. Weiner (32)
Plasmids		
pDR540	*tac* promoter vector, Ap^r	Pharmacia Fine Chemicals (25)
pBR322	Ap^r Tet^r	C. Maina (1)
pBB190	pDR540 containing a *Sau*3A-I fragment of *S. aurantia* DNA	This study
pBB601	pBR322 with an 8.2-kbp *Hin*dIII fragment of pBB190	This study
pBB701	*Kpn*I deletion of pBB601	This study
pBB801	*Sac*II deletion of pBB601	This study
pBB901	*Xho*I deletion of pBB601	This study

Immunological screening of the genomic library. Cells from a transformation were spread onto sterile nitrocellulose filters (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) that were resting on plates of L agar containing 100 μg of ampicillin per ml. Following overnight incubation at 37°C, the filters were first replicated and then placed on fresh L agar plates that were spread with 100 μl of 100 mM IPTG in deionized water. Following a 3-h incubation at 37°C, the filters were processed as described by Howe et al. (14), with the following modifications. After the treatment with DNase, the filters were incubated for 90 min with adsorbed (see below) rabbit antiserum raised against purified *S. aurantia* flagella (1:100 dilution) in NE buffer (0.15 M NaCl, 5 mM EDTA, 0.25% gelatin in 50 mM Tris hydrochloride [pH 7.4]). The filters were then washed in NEW buffer (NE buffer containing 0.05% Tween 20) four times for 15 min each time and incubated for 90 min with goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase that was diluted 1:2,000 in NEW buffer. Following a 15-min wash in NEW buffer and a brief rinse in deionized water, the filters were developed with 4-chloro-1-naphthol (Sigma Chemical Co., St. Louis, Mo.) as described previously (3). Colonies giving a positive reaction were picked for further analysis.

SDS-polyacrylamide gel electrophoresis, Western blotting, and antibodies. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western blotting (immunoblotting) (30) were performed as described elsewhere (3). Both the rabbit antiserum directed against purified *S. aurantia* flagella and the monoclonal antibody (MAb) specific for the 37,500-molecular-weight flagellin (37.5K flagellin) (MAb.3E9F6) have been described previously (3). For Western blot analyses of *E. coli* containing recombinant plasmids, the rabbit antiserum was preadsorbed for 2 h with boiled *E. coli* JM109 carrying pDR540, as described elsewhere (10).

DNA-DNA hybridizations. The DNA probe used in the Southern blot analysis was prepared by excision of 1 μg of the desired fragment from a low-melting-temperature agarose gel. This fragment was purified by means of silica gel chromatography as described above. Nick translation of the purified DNA fragment as well as DNA transfer to nitrocellulose and hybridizations were performed as described previously (2).

DNA sequencing. Restriction fragments from either pBB601 or pBB701 were subcloned into the M13 phage vectors mp18 and mp19 (32) and sequenced by the chain-termination method described by Sanger et al. (26) with the Sequenase reagents (U.S. Biochemical Corp., Cleveland, Ohio). The following primers, which were synthesized by the Cornell University Biotechnology Institute DNA Synthesis Facility, were used in addition to the M13 primer: 5′-CCCAGACCGTCG-3′ (nucleotides 381 to 392), 5′-GGT GAACGGAGG-3′ (nucleotides 537 to 526), and 5′-GGG AGGTTCCCC-3′ (nucleotides 748 to 737). Nucleotide sequence data were analyzed for the presence of open reading frames (ORFs) with the DNA Inspector II$^+$ program (Textco, West Lebanon, N.H.).

RESULTS

Construction of an *S. aurantia* genomic library and isolation of immunoreactive clones. An *S. aurantia* genomic library was constructed by partially digesting chromosomal DNA with *Sau*3A-I and ligating the DNA fragments into the *Bam*HI site of the *tac* promoter vector pDR540. The library was used to transform *E. coli* JM109, and approximately 20,000 ampicillin-resistant colonies were obtained, 95% of which were recombinants, as judged by the ligation efficiency of the dephosphorylated vector. Following a 3-h induction with IPTG, the colonies were screened for reactivity to rabbit antiserum that was raised against purified *S. aurantia* flagella. One such screening resulted in the isolation of an *E. coli* colony carrying a 24-kbp plasmid, pBB190, which encoded an antigen that reacted with the polyclonal antiserum and with a MAb that was specific for the 37.5K *S. aurantia* flagellin (MAb.3E9F6) (3). As demonstrated by Western blot analyses, pBB190 directed the synthesis of a polypeptide with an apparent molecular weight of 37,000 that was reactive with both the polyclonal antiserum and MAb.3E9F6 (Fig. 1). Furthermore, synthesis of this polypeptide was inducible by IPTG (Fig. 1). Throughout the remainder of this report we refer to the DNA that encodes the antigenic protein as the *flaA* gene.

Localization of the *flaA* gene. The *flaA* gene was first localized to an 8.2-kbp *Hin*dIII fragment from pBB190 by subcloning this fragment into pBR322 to form pBB601 (Table 1 and Fig. 2). This cloned fragment contained 92 bp of pDR540, including the *tac* promoter. As shown by Western blot analysis (Fig. 1), pBB601 encoded the 37K antigen, the production of which was still inducible by IPTG.

In order to localize further the antigen-encoding region, pBB601 was used to construct a number of deletion derivatives (Table 1). A map showing the location and the extent of each deletion is shown in Fig. 2. The deletion plasmids were analyzed by Western blotting to determine whether they expressed the 37K antigen. pBB901, the plasmid carrying the largest deletion, directed the synthesis of a polypeptide with an apparent molecular weight of 32,000 which reacted with MAb.3E9F6 (Fig. 1 and 2), while the other two plas-

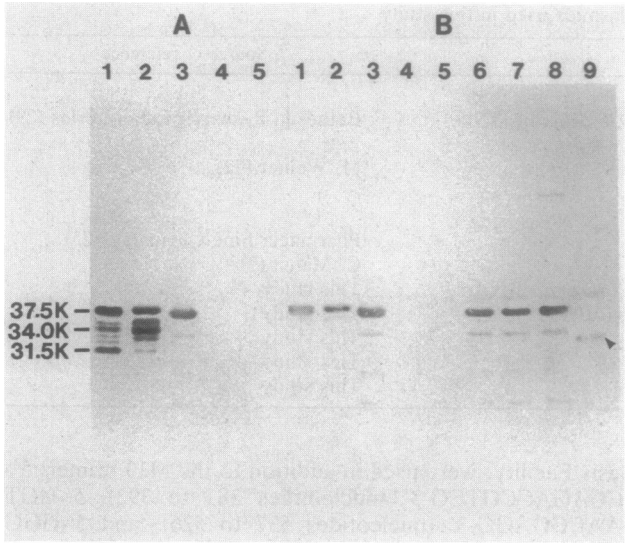

FIG. 1. Western blot analysis of whole-cell extracts of *E. coli* containing pBB190 and its derivatives. The filter shown in panel A was reacted with polyclonal antiserum raised against purified *S. aurantia* flagella, while the one shown in panel B was reacted with MAb.3E9F6. Whole-cell extract (lane 1) and purified flagella of *S. aurantia* (lane 2) were included as controls. Lane 3, *E. coli* JM109(pBB190) with IPTG; lane 4, *E. coli* JM109(pBB190) without IPTG; lane 5, *E. coli* JM109(pDR540) with IPTG; lane 6, *E. coli* JM109(pBB601) with IPTG; lane 7, *E. coli* JM107(pBB701) with IPTG; lane 8, *E. coli* JM107(pBB801) with IPTG; lane 9, *E. coli* JM107(pBB901) with IPTG (the arrowhead denotes the 32K antigen).

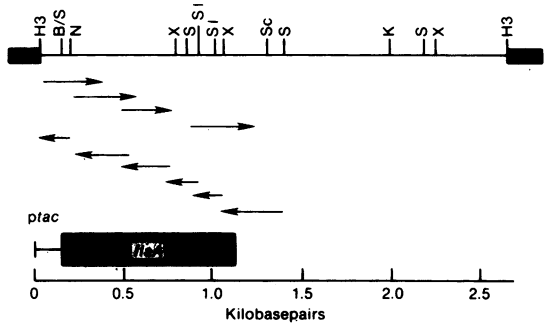

FIG. 3. Restriction map of pBB701 and sequencing strategy. The direction and extent of the sequencing reactions is indicated by the arrows. Abbreviations: H3, *Hin*dIII; B/S, *Bam*HI-*Sau*3A; N, *Nru*I; X, *Xho*I; S, *Sal*I; SI, *Sac*I; Sc, *Sac*II. The ORF is represented by the closed box. The region between nucleotides 961 and 1344 was completely sequenced (both strands) independently by C.-Y. Han and I. P. Crawford (unpublished data), and their sequence is consistent with that shown in Fig. 4.

mids that were analyzed, pBB801 and pBB701, expressed a 37K antigen (Fig. 1 and 2). On the basis of these results, it appears that the *flaA* gene begins after the left *Hin*dIII site of pBB601 and ends somewhere before the left *Sac*II site. This region contains 1.3 kbp of DNA, which should be sufficient to code for a 37K polypeptide. Furthermore, because the *tac* promoter lies between the left *Hin*dIII site of pBB601 and the *Bam*HI-*Sau*3A-I site and because the expression of the 37K antigen is under the control of IPTG, it appears that transcription proceeds from the left to the right.

Southern blot analysis of the antigen-encoding region. In order to determine whether the coding region defined above represented a contiguous stretch of DNA on the *S. aurantia* chromosome, a Southern blot analysis was performed in which a 1.1-kbp *Nru*I-*Sac*II fragment from pBB601 was used to probe a *Bcl*I digest of *S. aurantia* DNA. This probe hybridized to a 23-kbp *Bcl*I fragment (data not shown). Because there is no *Bcl*I site in the *S. aurantia* DNA inserted in pBB601 or its derivatives, the fact that the probe hybridized to only one *Bcl*I fragment in chromosomal digests of *S. aurantia* indicates that it represents DNA that is contiguous in the *S. aurantia* chromosome.

DNA sequence analysis. DNA extending from the left *Hin*dIII site to the second *Sal*I site in pBB701 (Table 1 and Fig. 3) was subcloned into M13 mp18 and mp19 phage vectors and was sequenced by the chain-termination method (26). The sequencing strategy is shown in Fig. 3. Analysis of this sequence revealed the presence of an ORF that coded for a polypeptide consisting of 301 amino acids (Fig. 4). The molecular weight calculated for this 301-residue polypeptide was 31,241. This is somewhat smaller than the M_r value of 37,000 obtained by SDS-polyacrylamide gel electrophoresis (Fig. 1). This disparity in molecular weights appears to be slightly greater than would be expected to result from the inaccuracy that is commonly experienced in measuring protein sizes by SDS-polyacrylamide gel electrophoresis; this suggests that the *flaA* gene product migrates anomalously on SDS-polyacrylamide gels. An analysis of the predicted polypeptide indicated the presence of a single region at the N terminus that was capable of spanning a membrane (Fig. 5). The amino acid sequence of the *flaA* product was compared with sequences in a data base of known proteins with the FASTA program developed by Pearson and Lipman (22). The survey did not detect any identities that we judged to be significant; however, as discussed below, the predicted protein did show a region of

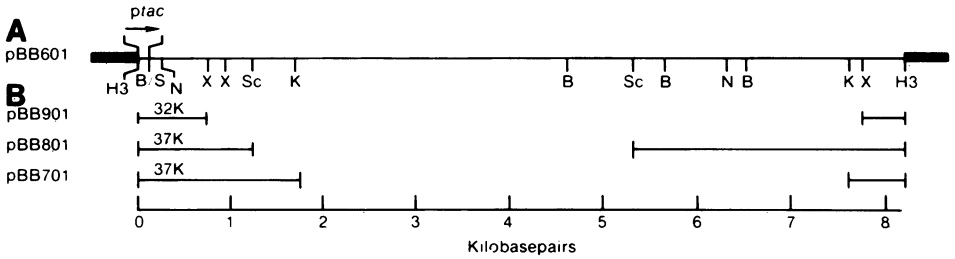

FIG. 2. Partial restriction map and deletion analysis of pBB601. (A) Partial restriction map of pBB601. The heavier line represents pBR322. Abbreviations: H3, *Hin*dIII; N, *Nru*I; B/S, *Bam*HI-*Sau*3A; X, *Xho*I; Sc, *Sac*II; K, *Kpn*I; B, *Bgl*II. There are four additional unmapped *Xho*I sites in the region between the left- and the rightmost *Xho*I sites. (B) Analysis of three deletion derivatives of pBB601. The size of the flagellar antigen encoded by each plasmid (Fig. 1) is indicated. The extent of each deletion is defined by the gap between segments.

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1    CCCCATCCCCCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACA   75
76   GGAAACAGGATCAAGCATCCATATAATGAATTTCAGCAGAATCCAAGGTAAATAAGGAGCACTGGATGAAAAGGT  150
                                                              M  K  R  F
151  TCTTCGCGATTCTGGGGGCTGCCCTGTTCGTGGGTAACAGTGGTGCCTTCGCAGAACAAGCTACTCTCATTGATT  225
       F  A  I  L  G  A  A  L  F  V  G  N  S  G  A  F  A  E  Q  A  T  L  I  D  F
226  TCTCTAAGTTGGTTGGCGAAGGTAACACCGGGTTGCACGCTCCGACGACGATCGACTACAGCCGCCAGGCTGGGT  300
       S  K  L  V  G  E  G  N  T  G  L  H  A  P  T  T  I  D  Y  S  R  Q  A  G  S
301  CGGCCTACAGTGCCGAAGATAAGGCAGCGATGAAGATTTCTCTGGCCATTCCCAGCTGGGAGATCGAGTTGGCTT  375
       A  Y  S  A  E  D  K  A  A  M  K  I  S  L  A  I  P  S  W  E  I  E  L  A  S
376  CTTCGTCCCAGACCGTCGAGAACCAGACTCTTTCGTTGGTGACCGCCGCTCCCGTGAAGCAGGACGCCGCCCGCT  450
       S  S  Q  T  V  E  N  Q  T  L  S  L  V  T  A  A  P  V  K  Q  D  A  A  R  Y
451  ACGGTGGTGAAACCGTCATGGGTGTTCGGATTCACTTCCCCTCGTTCGGCATCAACTCCTTTGCCGTCATCAAGC  525
       G  G  E  T  V  M  G  V  R  I  H  F  P  S  F  G  I  N  S  F  A  V  I  K  P
526  CTCCGTTCACCATCCCCGCCTACGCCACCCTCGGCGACGCCACGGCCCAGAACGCGGTGGCCGGTGGTCAGTTCG  600
       P  F  T  I  P  A  Y  A  T  L  G  D  A  T  A  Q  N  A  V  A  G  G  Q  F  D
601  ACGGCTTCGGCGTCCTCAAGAACGTCGGCGTCATCAAGTCCATCCAGATCGACCAGGGTCTTGAGGACCGCACCC  675
       G  F  G  V  L  K  N  V  G  V  I  K  S  I  Q  I  D  Q  G  L  E  D  R  T  Q
676  AGGAACCGGTCCACCGAGGGGCGACCCAGGGAGAGCACCCCGTCGGCCACGCCCCACTCGAGGGGAACCTCCCGG  750
       E  P  V  H  R  G  A  T  Q  G  E  H  P  V  G  H  A  P  L  E  G  N  L  P  G
751  GGCTGTCCCGTGGCCGAGAGTTCGGGGTAGGCGGCCAGGGCCGCCGACGAATGCCCGGGCAGGTCGACCTCGGGA  825
       L  S  R  G  R  E  F  G  V  G  G  Q  G  R  R  R  M  P  G  Q  V  D  L  G  N
826  ACGACGACGATGTGGCGGCGGGCGGCGAAGGCCACCACCCGGGACACATCGGCGGCGGTGTAGTACCCGCCGTAG  900
       D  D  D  V  A  A  G  G  E  G  H  H  P  G  H  I  G  G  G  V  V  P  A  V  G
901  GTCCCGTCGAGCTCTTGGCGCCAGGCTCCCACCTCGGTGAGCCTGGGCCAACCGGGAACCTCGAGACGCCACCCC  975
       P  V  E  L  L  A  P  G  S  H  L  G  E  P  G  P  T  G  N  L  E  T  P  P  L
976  TGGTCGTCGGTCAGGTGCCAATGAAAACGGTTGAGCTTGTGTTGCGCCATGAGCTCAAGGACCCATTCTAGGGTC  1050
       V  V  G  Q  V  P  M  K  T  V  E  L  V  L  R  H  E  L  K  D  P  F  *
1051 TCCACCGATTGAAAGTGGCGGCTGGAATCGACCATGAGTCCTCGCCAAGGGTACTTTGGACCGTCTTCGACCACC  1125
1126 CCGGTGGCCGCCGCGACGGTCTCCAGAGACGAGGCCTGGGGCGGAAGAAGCTGACCCAGGGTGACCAGGGCGTAG  1200
1201 AACCGACCCCGCGGGGTCGGCGGCCTCGACCCGGGCGCCCTGGGCGGAAATCACCAGCCGATACCCCTCGTCCCC  1275
1276 GGGGCCCTGGTCCGAGAAGGTCAACGTGGTCCGTGGCCCCTGAACCGGCGGGCCGAGGGCGTCGAC  1341
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FIG. 4. Nucleotide sequence analysis of the antigen-encoding region of pBB701. Nucleotides 1 to 83 represent the pDR540 sequence. The underlined GATC sequence represents the junction between the *Bam*HI site of pDR540 and the leftmost *Sau*3A-I site of the inserted *S. aurantia* DNA. The underlined sequence at nucleotides 129 to 134 represents a possible ribosome-binding site. Amino acids are represented by the single-letter code. The asterisk at position 1044 is the stop codon of the ORF.

identity with the N-terminal sequence of flagellar proteins from two other spirochetes.

DISCUSSION

An immunological procedure was used to screen an *S. aurantia* genomic library in *E. coli* for clones that reacted with an antiserum that was raised against purified *S. aurantia* flagella. A plasmid was isolated, pBB190, that directed the synthesis of a 37K polypeptide that was reactive with both a polyclonal antiserum raised against purified *S. aurantia* flagella and with a MAb specific for the *S. aurantia* 37.5K flagellin. Deletion analysis revealed that the responsible gene

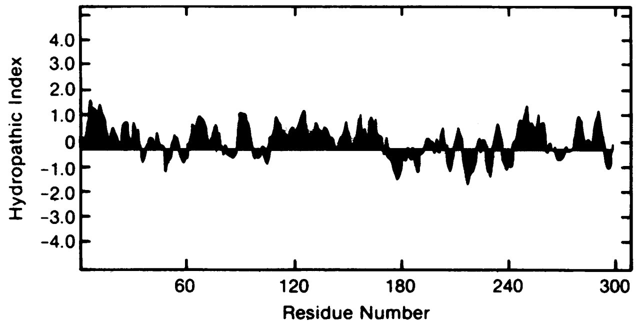

FIG. 5. Hydrophobicity (hydropathy) predicted by the SOAP program of Kyte and Doolittle (16). There was one region that was significantly hydrophobic (positions 2 to 21) and that was capable of spanning a membrane.

resided on an approximately 1.3-kbp region of DNA and that expression of the gene was under the control of the *tac* promoter. Southern hybridization analysis provided evidence that this 1.3-kbp region is a contiguous stretch of DNA on the *S. aurantia* chromosome. Analysis of the DNA sequence revealed an ORF beginning at nucleotide 141 that was preceded by a sequence conforming well to an *E. coli* consensus ribosome-binding site (7, 27) 6 nucleotides upstream from the initiation codon. The codon usage found in this ORF agreed well with that which we have found in the cloned *S. aurantia trpE* gene (2; I. Crawford, B. Brahamsha, and E. P. Greenberg, manuscript in preparation). In addition, the protein sequence predicted by this ORF showed regions of similarity with the N-terminal sequences of the 39K and 37K flagellins, respectively, of the distantly related spirochetes *Treponema phagedenis* and *Treponema pallidum* (19) (Fig. 6). In fact, amino acids 22 to 29 and 31 to 37 predicted by the ORF were identical to amino acids 1 to 8 and 10 to 11, respectively, of the *T. phagedenis* 39K flagellin (19) (Fig. 6). We consider this ORF to represent the gene that encodes the *S. aurantia* 37.5K flagellar filament outer layer protein (3). Thus, we refer to it as *flaA*.

It is of interest that the polypeptide produced by *E. coli* appeared to be slightly smaller than the native 37.5K *S. aurantia* flagellin. One possible explanation that could account for the difference in molecular weights is that in *E. coli* this polypeptide was posttranslationally modified. In fact, the first 20 amino acids predicted by the ORF had a striking similarity to a signal sequence (20, 23), including a possible signal peptidase cleavage site between the alanine at position 21 and the glutamic acid at position 22 (23). There is recent evidence that some spirochete flagellins may be processed. Norris et al. (19) have shown that the *T. phagedenis* 39K flagellin and the *T. pallidum* 37K flagellin lack an N-terminal methionine. Furthermore, the region of identity between the amino acid sequence predicted from the ORF and the *T. phagedenis* flagellin N-amino-terminal sequence begins after residue 22 of the predicted *S. aurantia* polypeptide (Fig. 6). Thus, it is possible that *E. coli* recognizes this sequence and processes it. This does not explain why it is larger in the spirochete, but it is possible that *E. coli* may process it differently than *S. aurantia*. Alternatively, it is possible that an *E. coli* protease acts on the foreign polypeptide. A number of what appear to be degradation products always occur on Western blots of *E. coli* strains expressing the 37K antigenic polypeptide (Fig. 1). Furthermore, it should be mentioned that Howe et al. (14) have noted that two outer membrane proteins of the spirochete *Borrelia burgdorferi* are also, for unknown reasons, expressed as slightly smaller polypeptides in *E. coli*.

The *S. aurantia* 37.5K flagellin is associated with the surface of the filament and, in this respect, is analogous to the 39K flagellin of *T. phagedenis* and the 37K flagellin of *T. pallidum* (5, 24). Recent evidence (19) reveals that the *T. phagedenis* and *T. pallidum* flagellar polypeptides can be grouped into two classes on the basis of their antigenicities and N-terminal amino acid sequences. The outer surface flagellins, which do not have an N-terminal methionine, belong to one class (class A), while the flagellar filament core polypeptides, which have an N-terminal methionine, belong to another (class B). It is thus possible that secretion of these two classes of polypeptides may occur by different pathways. It should also be noted that neither the predicted *S. aurantia flaA* gene product nor the N-terminal amino acid sequence of the class A flagellins of *T. phagedenis* and *T. pallidum* shows any homology to other flagellins that have been studied (19). The evidence presented here supports the hypothesis that the *S. aurantia* flagellar surface protein is secreted like *E. coli* periplasmic proteins, in this respect, differing from other flagellins that have been studied (6, 12, 13). It is possible that the interaction of this flagellin with the core polypeptides serves to localize them in the periplasm. Further work, including the cloning of the genes encoding the core polypeptides, should provide insights into this matter.

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S. aurantia	M K R F F A I L G A A L F V G N S G A F A E Q A T L I D F S K L V G E G N T G L H A P T T I D
T. phagedenis	E Q A T L I D F G K L N A D I V P D K N G
T. pallidum	D E S V L I D F A K L N A D I M A D K S G G M T H N

FIG. 6. Comparison and alignment of the deduced N-amino-terminal sequence of the *S. aurantia* 37.5K flagellin and the N-amino-terminal sequence of the *T. phagedenis* 39K flagellin and the *T. pallidum* 37K flagellin. Amino acids are represented by the single-letter code, and identical residues are indicated by boxes.

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