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Sequencing, Expression, and Genetic Characterization of the *Helicobacter pylori ftsH* Gene Encoding a Protein Homologous to Members of a Novel Putative ATPase Family

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In this study, we isolated and sequenced a *Helicobacter pylori* gene, designated *ftsH*, coding for a 632-amino-acid protein which displayed striking similarity throughout its full length to FtsH proteins identified in *Escherichia coli*, *Lactococcus lactis*, and *Bacillus subtilis*. *H. pylori* FtsH also possessed a ~200-amino-acid region containing a putative ATPase module which is conserved among members of the AAA protein family (AAA, ATPase associated with diverse cellular activities). The *H. pylori ftsH* product was overexpressed in *E. coli* and reacted immunologically with an anti-*E. coli* FtsH serum (T. Tomoyasu, K. Yamanaka, K. Murata, T. Suzaki, P. Bouloc, A. Kato, H. Niki, S. Hiraga, and T. Ogura, J. Bacteriol. 175:1352–1357, 1993). FtsH was also shown to be present in the membrane fraction of *H. pylori*, suggesting that it is membrane bound. Disruption of the *ftsH* gene led to the loss of viability of *H. pylori*, demonstrating that this gene is essential for cell growth. Overproduction of both *H. pylori* FtsH and *E. coli* FtsH together tremendously reduced the growth rate of the *E. coli* host cells, whereas the growth of the *E. coli* cells carrying the wild-type *E. coli ftsH* operon on the chromosome was not significantly affected by overproduction of *H. pylori* FtsH itself. This result suggests that the abnormal growth of cells results from interaction between *H. pylori* FtsH and *E. coli* FtsH.

Helicobacter pylori is an important cause of gastritis and has been associated with duodenal ulcer, gastric ulcers, and gastric cancer in some individuals (6, 7). Several factors have been implicated in the pathogenesis of *H. pylori*, including urease (12), flagella (20, 26, 36), a cytotoxin-associated gene A protein (CagA) (10, 42), an adhesin (14), and a vacuolating toxin (VacA) (11, 29, 33, 37). These proteins are either surface exposed (for example, flagella, CagA, and adhesin) or extracellular (for example, ureases and VacA). Hence, the process of how these proteins are either translocated into the membrane or secreted from the cells is essential for *H. pylori* pathogenesis. Recently, it has been reported that two *H. pylori* genes, *picA* and *picB*, play a role in the induction of an inflammatory response (43). On the basis of the sequence similarity between the *picB* gene product and the *Bordetella pertussis* toxin secretion protein, it was suggested that the *picB* gene product is involved in the induction of interleukin 8 expression in gastric epithelial cells by secreting an interleukin 8 inducer present in the *H. pylori* cells (43). However, the general processes of membrane translocation or protein secretion by *H. pylori* are poorly understood at present.

The FtsH (filamentation-temperature-sensitive) protein of *Escherichia coli* is membrane bound and essential for the viability of *E. coli* (28, 40, 41). This protein is thought to be involved in secretory protein export (1, 2) and has been demonstrated to play an important role in degradation of transcriptional regulatory factors, σ^{32} and λ CII (21, 22, 39), and SecY (an essential protein translocase subunit) (23). In addition, the *ftsH* gene, which is involved in tolerance to both high temperature and salt, has also been identified in *Bacillus subtilis* (13, 18) and *Lactococcus lactis* (27). Data indicating that the FtsH proteins have multiple functions which are essential for the cell survival have accumulated increasingly. Moreover, these bacterial FtsH proteins belong to a new family of putative ATPases, known as the AAA (ATPases associated with diverse cellular activities) protein family (8, 9, 24). Members of this AAA protein family, found in both prokaryotes and eukaryotes, are believed to have diverse cellular activities (8, 24).

The goals of this study were (i) to clone and sequence an *H. pylori ftsH* gene which encodes a protein homologous to members of the AAA family, (ii) to study gene expression in *E. coli*, (iii) to localize FtsH in *H. pylori* by using an anti-*E. coli* serum, and (iv) to determine if this gene product is essential for the viability of *H. pylori.*

MATERIALS AND METHODS

Bacterial strains and media. A clinical *H. pylori* isolate, UA802, was used in this study and cultured as described previously (16). *E. coli* strains are listed in Table 1.

LB medium (31), M9 medium (31), and a supplemented M9 medium (32) were used for growth of bacteria. Ampicillin (100 μg/ml), chloramphenicol (20 μg/ml), and kanamycin (20 μg/ml) were added to these media for growth of plasmid-containing cells and for screening transformants.

Techniques for DNA manipulations. Chromosomal DNA from *H. pylori* was extracted as described by Ezaki et al. (15). Plasmid DNA was prepared by the method of Birnboim and Doly (5). Sequential deletions of recombinant plasmid DNA were then made with the Erase-a-Base system as described by the supplier (Promega, Madison, Wis.). Nucleotide sequence was determined by a thermocycling sequencing method with *Taq* DNA polymerase as described in the supplier's manual (Gibco BRL, Gaithersburg, Md.). Primers were also employed for such sequencing. PCR amplification was used to detect the *H. pylori ftsH* gene within other *H. pylori* strains as described previously (16).

Plasmid constructs. A genomic DNA library of *H. pylori* UA802 chromosomal DNA prepared with the λDASH vector (Stratagene, La Jolla, Calif.) was detailed previously (16). A clone, designated P9, containing a fragment of ~20 kb from *H. pylori* UA802 is shown in Fig. 1. A ~4.6-kb fragment of interest was then subcloned into pBluescript SK^+ (Stratagene) by digestion with *Xba*I and *Eco*RI, followed by ligation with the appropriately digested vector. A plasmid clone, pBSHpC10, carrying the *H. pylori ftsH* gene and the partial *H. pylori copA* gene was selected for further study. Various plasmid constructs derived from pBSHpC10 are listed in Table 1.

Expression of the *H. pylori ftsH* gene in *E. coli*. Expression of the *H. pylori ftsH* gene in CSR603 was carried out as described previously (32) but in CSRDE3

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Characteristics	Source or reference
E. coli		
DH10B	F$^-$ *mcrA* Δ[*mrr-hsdRMS-mcrBC*] ϕ80d*lacZ*ΔM15 Δ*lacX74 deoR ecA1 ara*Δ*139* Δ(*ara leu*)7697 *galU galK* λ$^-$ *rpsL endA1 nupG*	Gibco BRL
CSR603	*rescA1 uvrA6 phr1*	32
CSRDE3	CSR603 with DE3, λ prophage carrying the T7 RNA polymerase under the *lac* promoter control	48
YJ430	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150* (Str^r) *relA1 flbB5301 deoC1 ptsF25 rbsR* Δ(*srl-recA*) *306*::Tn*10 ftsH3*::*kan* [F′ *lac*$^+$ *lacI*^{q}/pSTD401 *ftsH* Cm^r]	35
Plasmid		
pSTD401	pHSG575 with *E. coli ftsH* under the *lac* promoter control	35
pBluescript SK$^+$	Cloning vector (Ap^r)	Stratagene
pBluescript KS$^-$	Cloning vector (Ap^r)	Stratagene
pBSHpC10	pBluescript SK$^+$ with a ~4.6-kb *Xba*I-*Eco*RI fragment (*H. pylori ftsH copA*)	This study
pBSHpC10Nm	pBSHpC10 with the insertion of a CAT cassette at a *Nae*I site	This study
pBSHpC10Bm	pBSHpC10 with the insertion of a CAT cassette at a *Bam*HI site	This study
pBSHpC10Nhm	pBSHpC10 with the insertion of a CAT cassette at a *Nhe*I site	This study
pBSHpC10H	pBluescript SK$^+$ with a ~2.6-kb *Xba*I-*Hin*dIII fragment containing *H. pylori ftsH* under the *lac* promoter control	This study
pBKHpC10	pBluescript KS$^-$ with a ~4.6-kb *Xba*I-*Eco*RI fragment containing *H. pylori ftsH* and *copA* under the T7 promoter control	This study
pBKHpC10H	pBluescript KS$^-$ with a ~2.6-kb *Xba*I-*Hin*dIII fragment containing *H. pylori ftsH* under the T7 promoter control	This study
pBKHpC10HN	pBKHpC10 with a deletion of a ~0.6-kb *Nhe*I-*Hin*dIII fragment	This study

with some modifications. CSRDE3 cells harboring a plasmid carrying the *H. pylori ftsH* gene (pBKHpC10H, pBKHpC10NH, or pBKHpC10) were grown in LB broth supplemented with 100 μg of ampicillin per ml to an optical density at 620 nm and then collected. For [^{35}S]methionine-labeled *H. pylori* FtsH synthesis, cells were washed twice with 1× M9 medium and resuspended in 1 ml of supplemented M9 medium. The cells were incubated for 1 h at 37°C with agitation and induced by IPTG (isopropyl-β-D-thiogalactopyranoside; 1 mM) for 30 min. Subsequently, 10 μl of a 20-mg/ml concentration of rifampin was added to the respective samples, and after 30 min, 5 μl of [^{35}S]methionine was added to the respective samples and cell growth was continued for 1 h. For the synthesis of nonradioactively labeled FtsH protein, cells were induced with 1 mM IPTG for 1 h in the presence of 50 μg of cold methionine per ml instead of [^{35}S]methionine and then harvested. The cells were boiled for 5 min in 1× cracking buffer containing 50 mM Tris-HCl (pH 6.8), 1% sodium dodecyl sulfate (SDS), 20 mM disodium ethylenediaminetetraacetic acid, 1% mercaptoethanol, and 10% glycerol and stored at −20°C.

Insertional mutagenesis and natural transformation. Plasmid mutants carrying the disrupted *H. pylori ftsH* gene were created by inserting the chloramphenicol acetyltransferase (CAT) cassette (47) at *Nae*I and *Bam*HI sites (Table 1 and Fig. 1). An additional mutant was constructed by the insertion of the CAT cassette at a *Nhe*I site located immediately downstream of the *ftsH* gene. Subsequently, plasmid mutants were introduced into *H. pylori* UA802 by a natural transformation procedure (46).

Preparation of membrane and soluble fractions of *H. pylori* cells. Cells were grown to an optical density of 0.3 and harvested by centrifugation. The cells were then washed three time with 1× MOPS buffer (50 mM morpholinepropanesulfonic acid [pH 7.0], 5 mM EDTA, 1 mM dithiothreitol) and resuspended in the same buffer. The cells were cracked with a French press at 8,000 lb/in^2. The cell extracts were centrifuged at 12,900 × *g* at 4°C for 10 min. The supernatants were collected and recentrifuged in a Beckman TL 100/(rotor 100.2) ultracentrifuge at 60,000 rpm at 4°C for 1 h. The supernatant was collected as the cytoplasmic fraction. The membrane fraction was treated with 1 mM NaCl as described previously (27).

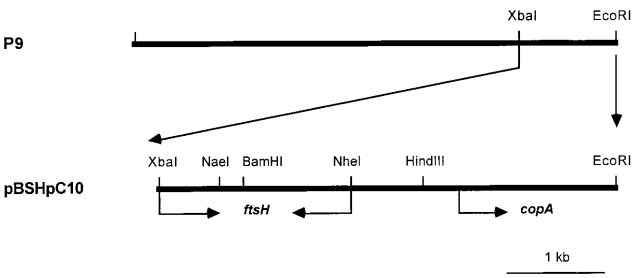

FIG. 1. Cloning of the *H. pylori ftsH* gene. A plasmid recombinant, pBSHpC10, was generated by subcloning the *Xba*I-*Eco*RI fragment derived from a λDASH clone P9 into the vector pBluescript SK$^+$. The corresponding locations of the gene *ftsH* (this study) and the copper-exporting gene *copA* (16, 17) are indicated as are the restriction enzymes used for constructing various plasmids.

Immunoblotting. The protein samples were separated by a 10% SDS polyacrylamide gel (31) and electrophoretically blotted onto a nitrocellulose membrane (MSI). The membrane was probed with antiserum against *E. coli* FtsH with an enhanced chemiluminescence system (Amersham Canada Ltd., Ontario, Canada). The antiserum was kindly provided by Y. Akiyama and K. Ito (Institute for Virus Research, Kyoto University, Kyoto, Japan). Protein bands were visualized on X-ray film.

Analysis and accession number of the nucleotide sequence. The nucleotide sequence reported in this study was analyzed with the software package (version 8) of Genetics Computer Group (Madison, Wis.) and has been deposited in GenBank under accession number U59452.

RESULTS

Cloning and nucleotide sequence of the *H. pylori ftsH* gene. A λDASH clone P9 was demonstrated to contain the *H. pylori copA* gene (16, 17) and its upstream sequence. Subsequently, the *Eco*RI-*Xba*I fragments derived from P9 were cloned into pBluescript SK$^+$. A subclone, namely pBSHpC10, was obtained and sequenced (Fig. 1). Figure 2 depicts the sequence containing an open reading frame (ORF) and its downstream 162 nucleotides. The ORF coding for 632 amino acid residues starts at nucleotide 59 and terminates at nucleotide 1938. A putative ribosome binding site or Shine-Dalgarno sequence (SD) was identified (34). A putative −10 region of a promoter was located in the upstream sequence of the start codon of the predicted ORF. A potential stem-loop structure followed by a string of T's (nucleotides 2056 to 2091) was located downstream of this ORF, which may represent a rho-independent transcription terminator (30).

Since the amino acid sequence deduced from the predicted ORF was highly homologous to various bacterial FtsH proteins as described below, this ORF was designated the *H. pylori ftsH* gene. A hydropathy profile of the deduced amino acid sequence of *H. pylori ftsH* calculated by the method of Kyte and Doolittle (25) showed that *H. pylori* FtsH contains two highly hydrophobic regions (Fig. 3, underlined residues 9 to 31 and 121 to 140). These regions could form two transmembrane

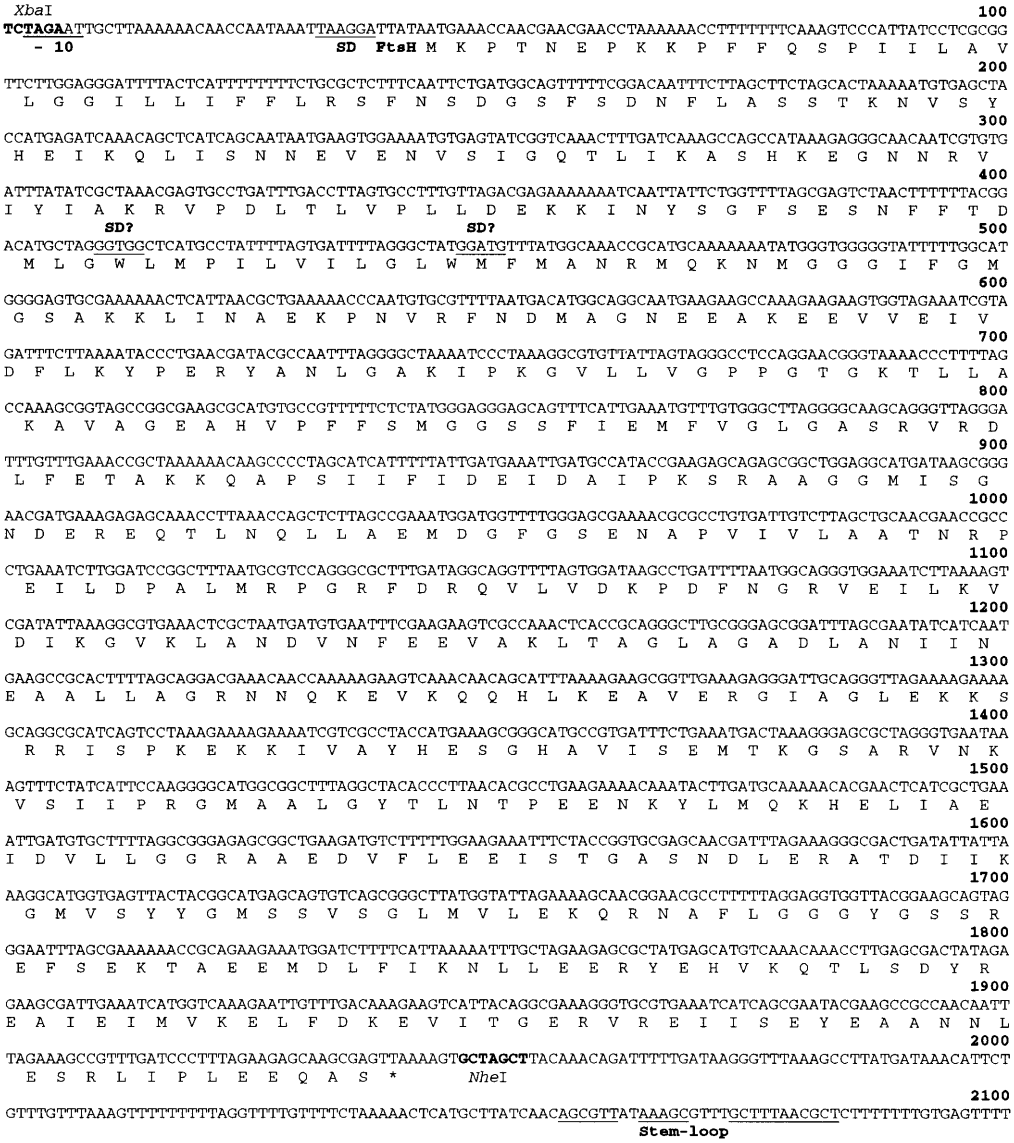

FIG. 2. Nucleotide sequence and the predicted amino acid residues of the cloned *H. pylori ftsH* gene. Several putative transcription and translation elements, including the −10 region of a promoter, ribosome binding sites (SD), and a transcription terminator, are underlined. The nucleotides are numbered on the right. The restriction recognition sites of *Xha*I and *Nhe*I are indicated in bold letters.

segments, which would correspond to the transmembrane segments found in the FtsH proteins of *E. coli*, *L. lactis*, and probably *B. subtilis* (Fig. 3) (27, 40, 41). In addition, a model of the membrane topology of *H. pylori* FtsH was generated by a method described previously (44, 45). In this model, two transmembrane helices were connected by a periplasmic loop and the C-terminal domain containing about 480 residues was exposed to the cytoplasm (data not shown). The cytoplasmic domain carries the putative ATP-binding motifs (19) and the conserved region of the AAA protein family (9, 24). This predicted membrane topology of *H. pylori* FtsH is consistent with those of the *E. coli* and *L. lactis* FtsH proteins (27, 40).

Sequence similarity of *H. pylori* FtsH protein to other proteins. The amino acid sequence of *H. pylori* FtsH was applied to search homologous proteins available in the databases. *H. pylori* FtsH displays a striking sequence identity (50 to 60%) to the AAA protein family (ranging from bacterial to human) within a ~200-amino-acid ATPase module (8, 9, 24). Several members of this proposed protein family have been identified in bacteria including *E. coli*, *L. lactis*, *B. subtilis*, *Haemophilus influenzae*, *Synechocystis* sp. PCC6803, *Mycoplasma genitalium*, and *Mycobacterium leprae* (nucleotide sequence accession numbers P28691, P46469, P37476, U32758, D64000, U39732 and U00017, respectively). Among these known bacterial AAA proteins, *E. coli*, *L. lactis*, and *B. subtilis* FtsH proteins have been functionally characterized and the others were deduced from their nucleotide sequences. Therefore, in Fig. 3, only a sequence comparison of *H. pylori* FtsH with the *E. coli*, *L. lactis*, and *B. subtilis* FtsH proteins was presented. *H. pylori* FtsH exhibits a similar degree of sequence identity to each of these three FtsH proteins, namely, 41% identity and 60% similarity to *B. subtilis* FtsH, 41% identity and 58% similarity to *L. lactis* FtsH, and 40% identity and 61% similarity to *E. coli* FtsH. It is apparent that the amino acid residues in the putative

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HpFtsH    -MKPTNEPKK PFFQSPIILA VLGGILLIFF LRSFNSDGSF SDNFLASSTK NVSYHEIKQL  59
LlFtsH    MNNNKQPKQG NFVKNILMWV ILAIVVVVGF NFFFSSN--- -----QSSVD KISYSQLMTK  52
EcFtsH    ---------- --MAKNLILW LVIAVVLMSV FQSFGPS--- -----ESNGR KVDYSTFLQE  40
BsFtsH    ---------M NRVFRNTIFY LLILLVVIGV VSYFQTS--- -----NPKTE NMSYSTFIKN  43
                         .    ..   ....     *                     . *

HpFtsH    ISNNEVENVS IGQTLIKASH KEGNNRVIYI AKR---VPD- ---------- -------LTL  98
LlFtsH    LDGNKIENVT MQPSDSLITV TGEYKEPVKV KGTNNFPLLG NSSSEVKNFQ AYIIPTDSVV 112
EcFtsH    VNNDQVREAR INGREINVTK KDSNRYTTYI PVQ---DPK- ---------- --------LL  78
BsFtsH    LDDGKVDSVS VQPVRGVYEV KGQLKNYDKD Q----YFLTH VPEG------ -------KGA  86
           .    .     .

HpFtsH    VPLLDEK--- --KINYSGFS ESNFFTDMLG WLMPILVILG LWMFMANRMQ KN---MGGGI 150
LlFtsH    KDIQNAAKSN DVKLSVVQAS SSGMWVQLIS YIIPMLLFVG IFWLMMGGMG ARGGGGGGNP 172
EcFtsH    DNLLTKN--- -VKVVGEPPE EPSLLASIFI SWFPMLLLIG VWIFFMRQMQ G---GGGKGA 131
BsFtsH    DQIFNALKKT DVKVEPAQET SG--WVTFLT TIIPFVIIFI LFFFLLNQAQ G----GGSRV 140
            .          *.                 *  ..     .                 *

HpFtsH    FGMG-SAKKL INAEKPNVRF NDMAGNEEAK EEVVEIVDFL KYPERYANLG AKIPKGVLLV 209
LlFtsH    MSFGKSRAKQ QDGKTSKVRF ADVAGSEEEK QELVEVVDFL KNPKKYHDLG ARIPAGVLLE 232
EcFtsH    MSFGKSKARM LTEDQIKTTF ADVAGCDEAK EEVAELVEYL REPSRFQKLG GKIPKGVLMV 191
BsFtsH    MNFGKSKAKL YTEEKKRVKF KDVAGADEEK QELVEVVEFL KDPRKFAELG ARIPKGVLLV 200
             * *  .            *  *.** .* *  .*. *.*..*  . *  ..  **   .** ***.
          motif A                                           motif B
HpFtsH    GPPGTGKTLL AKAVAGEAHV PFFSMGGSSF IEMFVGLGAS RVRDLFETAK KQAPSIIFID 269
LlFtsH    GPPGTGKTLL AKAVAGEAGV PFYSISGSDF VEMFVGVGAS RVRDLFENAK KTAPSIIFID 292
EcFtsH    GPPGTGKTLL AKAIAGEAKV PFFTISGSDF VEMFVGVGAS RVRDMFEQAK KAAPCIIFID 251
BsFtsH    GPPGTGKTLL AKACAGEAGV PFFSISGSDF VEMFVGVGAS RVRDLFENAK KNAPCLIFID 260
          ********** *** **** * **...  ** * .*****.*** ****.** ** * **..****

HpFtsH    EIDAIPKSRA AGGMISGNDE REQTLNQLLA EMDGFGSENA PVIVLAATNR PEILDPALMR 329
LlFtsH    EIDAVGRQRG AGLGG-GNDE REQTLNQLLV EMDGFQDDGN SVIVIAATNR SDVLDPALLR 351
EcFtsH    EIDAVGRQRG AGLGG-GHDE REQTLNQMLV EMDGFEGN-E GIIVIAATNR PDVLDPALLR 309
BsFtsH    EIDAVGRQRG AGLGG-GHDE REQTLNQLLV EMDGFSAN-E GIIIIAATNR ADILDPALLR 318
          ****. . *  **     *.** *******.*  *****        .*..***** ..*****.*

HpFtsH    PGRFDRQVLV DKPDFNGRVE ILKVDIKGVK LANDVNFEEV AKLTAGLAGA DLANIINEAA 389
LlFtsH    PGRFDRKVLV GAPDVKGREA VLKVHAKNKP LASDVDLHNV ATQTPGYVGA DLENVLNEAA 410
EcFtsH    PGRFDRQVVV GLPDVRGREQ ILKVHMRRVP LAPDIDAAII ARGTPGFSGA DLANLVNEAA 369
BsFtsH    PGRFDRQITV DRPDVIGREA VLKVHARNKP LDETVNLKSI AMRTPGFSGA DLENLLNEAA 378
          ******.. *    **   **  .***    .     *   .    . *  * *  ** ** *..****

HpFtsH    LLAGRNNQKE VKQQHLKEAV ERGIAGLEKK SRRISPKEKK IVAYHESGHA VISEMTKGSA 449
LlFtsH    LVAARQNKKE INAADIDEGM DRAMAGPAKK DRIQSMRERE IVAYHEAGHA IVGLVLENGS 470
EcFtsH    LFAARGNKRV VSMVEFEKAK DKIMMGAERR SMVMTEAQKE STAYHEAGHA IIGRLVPEHD 429
BsFtsH    LVAARQNKKK IDARDIDEAT DRVIAGPAKK SRVISKKERN IVAYHEGGHT VIGLVLDEAD 438
          * * * *..  .          .. . *  ..     .  ..     **** **. ..  .

HpFtsH    RVNKVSIIPR GMAALGYTLN TPEENKYLMQ KHELIAEIDV LLGGRAAEDV FLE--EISTG 507
LlFtsH    TVRKVTVVPR -GRIGGYMLA LPDEEIMQPT NFHLQDQLAS LMGGRLGEEI VFG--VATPG 527
EcFtsH    PVHKVTIIPR -GRALGVTFF LPEGDAISAS RQKLESQIST LYGGRLAEEI IYGPEHVSTG 488
BsFtsH    MVHKVTIVPR -GQAGGYAVM LPREDRYFQT KPELLDKIVG LLGGRVAEEI IFG--EVSTG 495
           * **...**      *      *            *   .  * ***  *..          . *

HpFtsH    ASNDLERATD IIKGMVSYYG MSSVSGLMVL EK--QRNAFL GGGYGSSREF SEKTAEEMDL 565
LlFtsH    ASNDIEKATH IARSMVTEYG MSKKLGMVSY EG--DHQVFI GRDYGQTKTY SEATAVMIDD 585
EcFtsG    ASNDIKVATN LARNMVTQWG FSEKLGPLLY AE-EEGEVFL GRSVAKAKHM SDETARIIDQ 547
BsFtsH    AHNDFQRATN IARRMVTEFG MSEKLGPLQF GQSQGGQVFL GRDFNNEQNY SDQIAYEIDQ 555
          * **   **   . . **.  *   *   * .              *. *     .     *.  *  .*

HpFtsH    FIKNLLEERY EHVKQTLSDY REAIEIMVKE LFDKEVITGE RVREIISEYE AANN------ 619
LlFtsH    EVRRLIGEAY DRAKEAIETH REQHKAIAEA LLKYETLDAK QIMSLFKTGK MPDEAAAAEV 645
EcFtsH    EVKALIERNY NRARQLLTDN MDILHAMKDA LMKYETIDAP QIDDLMARRD VRPPAGWEEP 607
BsFtsH    EIQRIIKECY ERAKQILTEN RDKLELIAQT LLKVETLDAE QIKHLIDHGT LPERNFSDDE 615
            .. ..   *   . ... .      .     .     *    * .    .. .

HpFtsH    ------LES- ---------- ---RLIPLEE QAS------- ---------            632
LlFtsH    PEPKTFEESL KDANANVDDF SNINIYNGDE KTDSKPEENK EKSEDETAE            695
EcFtsH    GASNNSGD-- --------NG SPKAPRPVDE PRTPNPGNTM SEQLGDK--            644
BsFtsH    KN----DD-- ---------- VKVNILTKTE EKKDDTKE-- ---------            637
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FIG. 3. Sequence comparison of *H. pylori* FtsH with three other bacterial FtsH proteins. Amino acid sequences were aligned with the program of CLUSTAL W (38). Hp, *H. pylori*; Ll, *L. lactis*; Ec, *E. coli*; Bs, *B. subtilis*. The identical residues at the corresponding positions of these proteins are indicated by asterisks, whereas the conservative replacements are marked by dots. Gaps are introduced to optimize alignment. Two putative ATP-binding motifs (A and B) are indicated by letters in bold type (19). The putative transmembrane segments of *H. pylori* FtsH corresponding to those of the other FtsH proteins are underlined. The ~200-amino-acid ATPase module conserved by the AAA protein family is indicated by arrows.

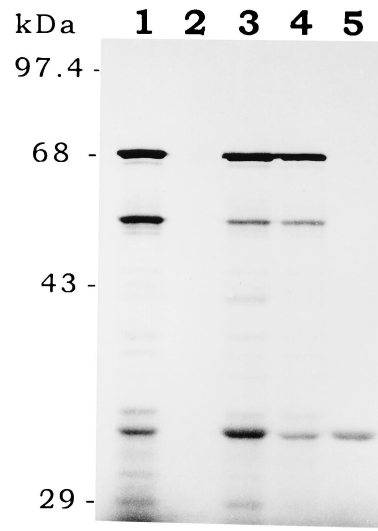

FIG. 4. Expression of *H. pylori* FtsH in *E. coli* CSRDE3. The plasmid-encoded proteins were synthesized as detailed in Materials and Methods. Equal amounts of the protein extracts adjusted by the turbidity of the cultures were separated on a 10% SDS-polyacrylamide gel. Lanes: 1, pBKHpC10H; 2, no plasmid DNA; 3, pBKHpC10; 4, pBKHpC10NH; 5, pBluescript KS⁻. *H. pylori* FtsH (~68 kDa) is present in lanes 1, 3, and 4. Molecular mass markers (BRL) are labeled on the left.

ATPase module (Fig. 3) (~200 amino acid residues), which are well conserved by the AAA protein family (9, 24), are highly homologous among these four FtsH proteins, with up to 57% identity and 73% similarity. In addition, the putative Zn-binding motif HEXXH located in the C-terminal region of *E. coli* FtsH (39) was identified in *H. pylori* FtsH (residues 434 to 438) (Fig. 3).

Expression of *H. pylori ftsH* in *E. coli* and localization of *H. pylori* FtsH in *H. pylori*. A maxicell system (32) was used to detect a protein encoded by the *H. pylori ftsH* gene. By using a conventional CSR603 strain as a host, a protein with the molecular mass of approximately 68 kDa was produced in cells harboring plasmid pBSHpC10H containing the *H. pylori ftsH* gene, but the protein was poorly expressed (data not shown). This result could be due to the fact that the upstream sequence of the *H. pylori ftsH* gene is too short to contain complete transcription elements (Fig. 2). Subsequently, a modified CSR603 strain (designated CSRDE3), which carries a p*lac*-controlled T7 DNA polymerase gene (48) on the chromosome, was used for *H. pylori* FtsH expression. Various plasmids in which the *H. pylori ftsH* gene was controlled by a T7 promoter were constructed (Table 1). The expression results are shown in Fig. 4. The plasmid constructs pBKHpC10H (Fig. 4, lane 1), pBKHpC10NH (lane 3), and pBKHpC10 (lane 4), which carried the entire *H. pylori ftsH* gene (detailed in Table 1), all gave rise to a specific protein band of ~68 kDa, whereas there was no such protein expressed in the cells with pBluescript KS⁻ (Fig. 4, lane 5) or without any plasmids (lane 2). The molecular mass of this protein is in agreement with that (67.5 kDa) of the deduced *H. pylori* FtsH. In addition, a protein of about 50 kDa was also weakly produced from the cells containing the *H. pylori ftsH* gene. This protein may result from nonspecific translation initiation in the region of nucleotides 510 to 550, where two possible translation signals exist (Fig. 2, SD? and AUG). Two lines of evidence support this possibility: (i) translation initiation in this region is capable of encoding ~500 amino acid residues, which is consistent with the size of the ~50-kDa protein; and (ii) this protein reacted immunologically with the anti-FtsH serum as described below. Furthermore, a protein of ~35 kDa was present in the cells harboring both a pBluescript plasmid and its derivatives but not in the cells without the plasmid (Fig. 4, lane 2), indicating that this likely is β-lactamase.

Since putative *H. pylori* FtsH and *E. coli* FtsH exhibit a striking degree of sequence similarity, they should contain some identical antigenic epitopes. An antiserum, which was raised against a synthetic peptide corresponding to amino acid residues 297 to 312 of *E. coli* FtsH (40), was applied to detect the *H. pylori* FtsH protein in the maxicells. The *H. pylori* FtsH was expressed with cold methionine instead of [^{35}S]methionine, separated on a SDS-polyacrylamide gel, and blotted onto a nitrocellulose membrane. Although there are three substitutions in the 15 residues used to produce the anti-FtsH

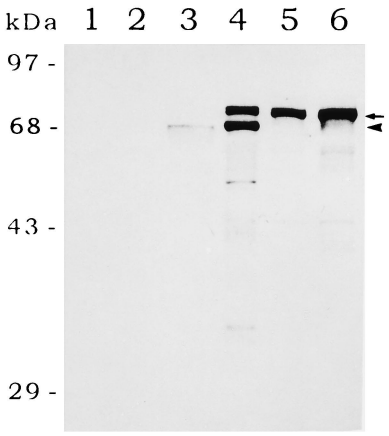

FIG. 5. Immunological detection of *H. pylori* FtsH in the cell fractions of *H. pylori* and in the *H. pylori ftsH*-containing *E. coli* cells with the anti-*E. coli* FtsH serum. Ten micrograms of proteins prepared from *H. pylori* cells (lanes 1 to 3) were subjected to immunoblotting, whereas fixed amounts (determined by the turbidity of the cells) of protein extracts prepared from *E. coli* cells (lanes 4 to 6) were used. The protein samples were separated on a 10% SDS-polyacrylamide gel. Lanes: 1, cytoplasmic proteins; 2, salt-treated membrane supernatant; 3, membrane proteins; 4, pBKHpC10H; 5, pBluescriptSK$^-$; 6, pSTD401. The positions of *H. pylori* FtsH (~68 kDa) and *E. coli* FtsH (~72 kDa) are indicated by an arrowhead and an arrow, respectively. Molecular mass markers (Gibco-BRL) are labeled on the left.

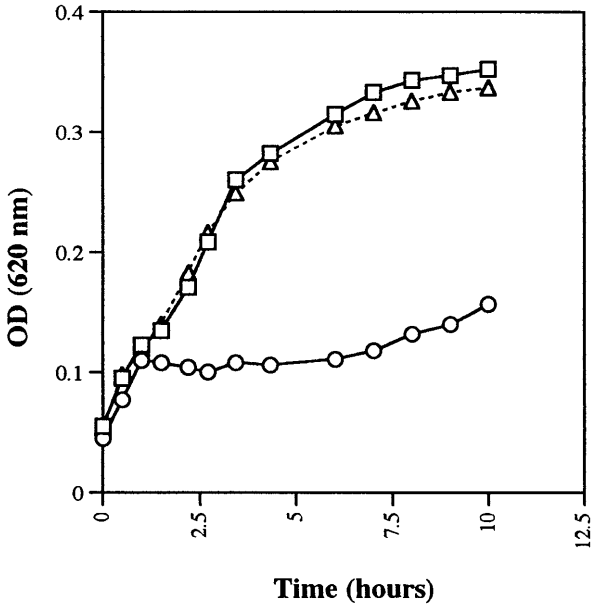

FIG. 6. Effects of overexpressing *H. pylori* FtsH and *E. coli* FtsH on growth of cells. Cells of YJ430 (Δ*ftsH*::*kan*/pSTD401) with or without an additional plasmid were grown at 37°C with agitation overnight in M9 glucose medium supplemented with Casamino Acids (0.2%), thiamine (2 μg/ml), chloramphenicol (20 μg/ml), ampicillin (50 μg/ml), if appropriate, and IPTG (1 mM). Cells were then treated as described previously (35). Thereafter, the cells were grown in the same medium in the presence of 1 mM IPTG, and the turbidity of the cells was measured as optical densities (at 620 nm) with a microtiter plate reader. Symbols: squares, YJ430; triangles, YJ430 with pBluescript KS$^-$; circles, YJ430 with pBSHpC10H.

serum (Fig. 3, D by E, V by I, and L by M) between *H. pylori* and *E. coli* FtsH proteins, the antiserum still strongly reacted with the ~68-kDa *H. pylori* FtsH (Fig. 5, lane 4), whereas cells harboring pBluescript KS$^-$ without the insert did not give rise to a band of the corresponding protein (Fig. 5, lane 5). Another protein, whose molecular mass appeared larger (~72 kDa in this gel) than that of *H. pylori* FtsH, was detected in all the *E. coli* cells by using this antiserum (Fig. 5, lanes 4 to 6). This 72-kDa protein must be *E. coli* FtsH, since this protein is identical to that generated from *E. coli* YJ430 harboring the plasmid pSTD401 (Fig. 5, lane 6). The minor protein of ~50 kDa expressed in cells containing the *H. pylori ftsH* gene was also detected by the same antiserum, indicating that this protein could represent a portion of the *H. pylori* FtsH protein as proposed above.

To investigate the localization of FtsH in the *H. pylori* cells, membrane and cytoplasmic fractions were prepared and probed with anti-*E. coli* FtsH serum. A protein of ~68 kDa was identified in the membrane fraction (Fig. 5, lane 3) but not in either the cytoplasmic fraction (Fig. 5, lane 1) or the salt-treated membrane supernatant (Fig. 5, lane 2). This result demonstrated that *H. pylori* FtsH is membrane bound, which is in agreement with that of *L. lactis* (27) and *E. coli* FtsH (40) proteins. The molecular mass of the *H. pylori* FtsH is consistent with that of the protein encoded by the predicted ORF (Fig. 5, lanes 3 and 4), indicating that the cloned gene represents the *H. pylori ftsH* locus.

***ftsH* is essential for the viability of *H. pylori*.** Three plasmid mutants, pBSHpC10Nm, pBSHpC10Bm, and pBSHpC10Nhm, were constructed as detailed in Table 1. pBSHpC10Nm and pBSHpC10Bm were constructed by *H. pylori ftsH* inactivation with the CAT cassette, and pBSHpC10Nhm was constructed by the insertion of the CAT cassette at a *Nhe*I site located immediately downstream of *ftsH*. By using a procedure for natural transformation as described previously (46), *H. pylori* mutants with the disrupted *ftsH* gene were not obtained, whereas the plasmid pBSHpC10pNhm was integrated into the UA802 chromosome and an *H. pylori* mutant was obtained with a Cm^r phenotype. In addition, various *copA*-disrupted mutants of *H. pylori* have been created by the same technique (16, 17), and the *copA* gene is located at about 1 kb downstream of *ftsH* (Fig. 1). Therefore, our failure to generate the *H. pylori ftsH*-disrupted mutants is likely due to the fact that the normal function of *ftsH* is essential for the viability of *H. pylori*. In addition, conservation of the *FtsH* gene was investigated by PCR. Two primers were generated from the regions corresponding to nucleotides 1479 to 1499 and 1944 to 1962 (Fig. 2), respectively. Eighteen strains of *H. pylori* were subjected to PCR, and all gave rise to a specific product of ~640 nucleotides (data not shown). This result indicated that this gene is common to all *H. pylori* strains.

Cooverexpression of *H. pylori* and *E. coli ftsH* genes interferes with the normal growth of the *E. coli* host cells. Since *H. pylori* FtsH displays very strong sequence similarity to *E. coli* FtsH, we were interested in whether the *H. pylori ftsH* gene is functional in *E. coli* cells. The plasmids pBSHpC10H and pBKHpC10H were introduced into *E. coli* DH10B and CSRDE3, respectively, which carry a normal *ftsH* gene on their chromosome. Overproduction of *H. pylori* FtsH in these strains did not significantly affect the growth of the host cells (data not shown). In contrast, pBSHpC10H was introduced into *E. coli* YJ430 harboring a compatible pSC101-based plasmid, pST401, containing the *E. coli ftsH* gene under the *lac* promoter control. It was evident that such cells grew much more slowly than the cells harboring either pST401 alone or pST401 and pBluescript KS$^-$ on LB agar plates supplemented with 1 mM IPTG. Growth rates of cells containing the respective plasmids in the presence of 1 mM IPTG, therefore, were monitored (Fig. 6). In comparison with cells harboring pST401, the pST401- and pBHpC10pH-containing cells grew well in the first hour and

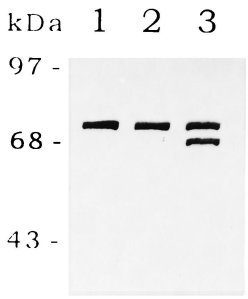

FIG. 7. Abundance of *H. pylori* FtsH and *E. coli* FtsH in cells induced with 1 mM IPTG as described in the legend to Fig. 6. Cells were harvested 8 h after the addition of IPTG. Fixed amounts of cell extracts adjusted by the turbidity of cultures were subjected to electrophoresis and then detected with the anti-*E. coli* FtsH antiserum. Lanes: 1, YJ430; 2, YJ430 with pBluescript KS$^-$; 3, YJ430 with pBSHpC10H.

then began to grow very slowly. In contrast, the growth curve of cells harboring pST401 and pBluescript KS$^-$ was similar to that of cells containing pSTD401.

The levels of *H. pylori* FtsH and *E. coli* FtsH in these plasmid-containing cells were detected immunologically with the anti-*E. coli* FtsH serum as shown in Fig. 7. Approximately equal amounts of *E. coli* FtsH existed in the cells harboring pST401, pST401-pBSHpC10pH, and pST401-pBluescript KS$^-$. However, *H. pylori* FtsH of ~68 kDa was also accumulated in cells containing a *H. pylori ftsH* gene (Fig. 7, lane 3). The decrease of the cell growth rate is therefore associated with the overproduction of both of the FtsH proteins.

DISCUSSION

The *H. pylori ftsH* gene appears to encode a membrane-bound protein (FtsH) of 632 amino acid residues with the predicted two transmembrane segments. *H. pylori* FtsH exhibits a striking full-length sequence similarity to known bacterial FtsH proteins and carries a ~200-amino-acid ATPase module which has been highly conserved by the AAA protein family (8). As a result, the *H. pylori* FtsH protein belongs to this protein family.

The *H. pylori ftsH* gene plays an essential role in the growth of *H. pylori*, since disruption of this gene leads to a loss in viability of *H. pylori* cells. Similarly, the *E. coli ftsH* gene has been shown to be essential for cell viability (41). However, the *ftsH* genes isolated from *L. lactis* and *B. subtilis* seem dispensable for growth since their *ftsH*-knockout mutants are still viable (13, 18, 27). It should be noted that the sites used for the inactivation of the *L. lactis* and *B. subtilis ftsH* genes were close to their C termini so that the truncated FtsH proteins could still remain functional for cell growth. Thus, the results obtained from the knockout mutagenesis of the *L. lactis* and *B. subtilis ftsH* genes did not completely rule out the possibility that the *ftsH* genes are essential for the growth of *L. lactis* and *B. subtilis.*

Precise functions of *H. pylori* FtsH are unclear at present. Lethality associated with *ftsH* inactivation indicates that FtsH plays a crucial role in the life cycle of *H. pylori*. A high degree of sequence identity and similar patterns of the putative functional domains such as transmembrane segments and putative ATP-binding motifs between *H. pylori* FtsH and other bacterial FtsH proteins suggest that they have similar functions in these organisms. In *E. coli*, FtsH has been implicated in multiple cellular activities. Accumulation of penicillin-binding protein 3 and β-lactamase precursors in the *E. coli ftsH1* mutant indicates that the maturation of these proteins is dependent on FtsH (4, 41). It was suggested that FtsH is involved in folding and assembly of proteins into and through the membrane, since mutations in FtsH lead to a dominant Std (stop-transfer-defective) phenotype, reduced retention of a cytoplasmic reporter SecY-PhoA fusion protein, and translocation retardation of some export proteins (1, 2). Recently, it has been shown that *E. coli* FtsH plays a crucial role in rapid degradation of three proteins, including the heat shock transcription factor σ^{32} (22, 39), the λ CII transcriptional activator (21), and the uncomplexed forms of SecY (3). In addition, the proteolytic activity of *E. coli* FtsH as an ATP- and Zn-dependent metalloprotease was demonstrated in vitro (39). On the other hand, *L. lactis* FtsH is able to functionally replace the *E. coli* FtsH to some extent and is involved in stress responses such as increased temperature and salt level (27). Similarly, an increased amount of the *B. subtilis* FtsH is essential for the cells in response to osmotic and temperature upshift (13).

Our results showed that overproduction of both *H. pylori* FtsH and *E. coli* FtsH significantly reduced the growth rate of host cells (Fig. 7). *E. coli* CSRDE3 cells containing the *E. coli ftsH* gene under its own promoter control are able to grow normally when *H. pylori* FtsH is overexpressed. How can this result be explained? It has been found that the high-level overexpression of *E. coli* FtsH is toxic to host cells (1, 3). A similar effect on cell viability may, therefore, be produced by the overaccumulation of the FtsH proteins encoded by both *H. pylori ftsH* and *E. coli ftsH*. This might be expected, since *H. pylori* FtsH is structurally and probably functionally related to *E. coli* FtsH. Alternatively, the decrease in growth rate could result from interference of the *H. pylori* FtsH protein with the formation of an FtsH complex, possibly located in the cytoplasmic membrane of *E. coli* (3). The previous study has shown that expression of the mutated *E. coli* FtsH even in the presence of the chromosomal wild-type *ftsH* gene reduces cytoplasmic retention of the alkaline phosphatase moiety attached to cytoplasmic regions of the membrane protein SecY, which is defined as a dominant Std phenotype (2). Recently, formation of the *E. coli* FtsH protein complex in the cytoplasmic membrane of *E. coli* has been demonstrated, indicating that *E. coli* FtsH may function as such a complex (3). Hence, it is possible that the *H. pylori* FtsH proteins in the cells are heterologously associated with *E. coli* FtsH to form inactive or malfunctional complexes which interfere with the growth of the host cells. On the other hand, excess *H. pylori* FtsH in the *E. coli* cells may tend to form homomultimeric complexes. The host cell could maintain normal growth, which is regulated by the *E. coli* FtsH complexes and probably by the *H. pylori* FtsH multimeric complexes. Additional analysis of components of the FtsH complexes in the cells cooverproducing *H. pylori* FtsH and *E. coli* FtsH would be required to shed light on this hypothesis.

In summary, we cloned the *H. pylori ftsH* gene which has been highly conserved in *H. pylori* strains and is essential for the viability of *H. pylori*. More importantly, *H. pylori* FtsH could, like *E. coli* FtsH, have essential and multiple roles such as protein assembly, secretory protein export, and proteolytic activity. As a result, *H. pylori* FtsH would be involved in the export of some extracellular virulence factors so that this may greatly contribute to the pathogenesis of *H. pylori*. This point will be able to be addressed by using *H. pylori* Std and temperature-sensitive mutants created by mutations within the *H. pylori ftsH* gene.

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