A Lactococcus lactis gene encodes a membrane protein with putative ATPase activity that is homologous to the essential Escherichia coli ftsH gene product

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INTRODUCTION

The facultative anaerobic Gram-positive bacterium Lactococcus lactis subsp. lactis is widely used as an industrial organism for homolactic fermentation. L. lactis has become a model organism for fundamental genetic research on plasmids and plasmid-encoded functions involved in the production of fermented food products. However, the genetic characterization of chromosomally encoded functions in L. lactis has only recently been started. We initiated a genetic approach to study purine metabolism in L. lactis and have characterized the hpt gene encoding hypoxanthine guanine phosphoribosyltransferase, an enzyme involved in the salvage of purine bases to the corresponding nucleotides (Nilsson & Lauridsen, 1992). During this work we detected part of an open reading frame adjacent to hpt, transcribed in the same direction, encoding a putative membrane protein.

In this report we describe the cloning and characterization of the L. lactis gene, previously named tma, adjacent to hpt and the tRNA operon trnA (Nilsson & Johansen, 1994). We show that the gene is homologous to the Escherichia coli ftsH gene, and that this gene is conserved in several Gram-positive bacteria, including lactic acid bacteria. We suggest that the gene be designated ftsH.

The ftsH genes of E. coli and L. lactis encode members of a new family of ATPases, the AAA-protein family (Kunau et al., 1993). The family includes both eukaryotic and prokaryotic members which have been reported to be involved in diverse cellular activities, e.g. cell cycle control, protein secretion, peroxisome biogenesis and proteolysis.

E. coli ftsH mutants show pleiotropic phenotypes: defects in cell growth and cell viability (Ogura et al., 1991;...
Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype/relevant features</th>
<th>Source or reference</th>
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<tr>
<td><em>Escherichia coli</em></td>
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<tr>
<td>XL1-Blue</td>
<td>endA1 hisR17 supE44 thi-1 recA1 gyrA96 relA1 Δ(lac) [F' proAB lacZΔM15 Tn10]</td>
<td>Stratagene, La Jolla</td>
</tr>
<tr>
<td>DH5a</td>
<td>Δ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hisR17 supE44 thi-1 gyrA96 relA1</td>
<td>Hanahan (1983)</td>
</tr>
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<td>SO609</td>
<td>ara thi rpsL Δ(pro-gpt-lac) hpt devD parD</td>
<td>Jochimsen et al. (1975)</td>
</tr>
<tr>
<td>CC118</td>
<td>araD139 Δ(ara-lac)7697 ΔlacX74 pboA Δ20 galE galK thi rpsE rpoB argE αm recA1</td>
<td>Manoil &amp; Beckwith (1986)</td>
</tr>
<tr>
<td>AR423</td>
<td>met gal supE bsdR sfiC Δ(wt-recA) 306::Tn10 ΔftsH1::kan [pAR171 ftsH rep&quot;&quot; CmR]</td>
<td>Akiyama et al. (1994a)</td>
</tr>
<tr>
<td>AR3120</td>
<td>met gal supE bsdR sfiC Δ(wt-recA) 306::Tn10 ΔftsH1::kan [pLN32 L. lactis ftsH]</td>
<td>This study</td>
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<tr>
<td><em>Lactococcus lactis</em></td>
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<tr>
<td>CHCC285</td>
<td>Wild-type</td>
<td>Nilsson &amp; Lauridsen (1992)</td>
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<tr>
<td>MG1363</td>
<td>Plasmid-free</td>
<td>Gasson (1983)</td>
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<tr>
<td>DN4302</td>
<td>MG1363 ftsH:ΔpLN43</td>
<td>This study</td>
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<tr>
<td><strong>Plasmids</strong></td>
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<tr>
<td>pBluescript KS+</td>
<td>Cloning vector (ApR)</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pBluescript SK−</td>
<td>Cloning vector (ApR)</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pVS2</td>
<td>EryR CmR</td>
<td>von Wright (1987)</td>
</tr>
<tr>
<td>pV2</td>
<td>pBluescript KS+ with a 1.3 kb <em>ClaI</em>-*HpaI fragment (<em>EryR</em>) from pVS2</td>
<td>Svend E. W. Hansen (personal</td>
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<td></td>
<td></td>
<td>communication)</td>
</tr>
<tr>
<td>pAR171</td>
<td>*ftsH rep&quot;&quot; CmR</td>
<td>Akiyama et al. (1994a)</td>
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<td>pBluescript SK− with a 1.9 kb <em>EcoRI</em>-MboI fragment (*ftsH&quot;) from CHCC285</td>
<td>Nilsson &amp; Lauridsen (1992)</td>
</tr>
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<td>pBluescript SK− with a 1.3 kb <em>HindIII</em>-MboI fragment (*ftsH&quot;) from CHCC285</td>
<td>Nilsson &amp; Lauridsen (1992)</td>
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<td>pLN2 ftsH:ΔTn5pboA1</td>
<td>This study</td>
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<td>pLN30</td>
<td>pBluescript KS+ with a 7.3 kb <em>SpeI</em> fragment (*ftsH&quot;) from CHCC285</td>
<td>This study</td>
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<td>pLN32</td>
<td>pBluescript KS+ with a 3.2 kb <em>EcoRI</em> fragment (*ftsH&quot;) from pLN30</td>
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<td>pLN39</td>
<td>pBluescript KS+ with a 1.0 kb <em>HindIII</em>-EcoRI fragment (*ftsH&quot;) from pLN32</td>
<td>This study</td>
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<tr>
<td>pLN43</td>
<td>pV2 with a 1.3 kb <em>EcoRV</em> fragment (*ftsH&quot;) from pLN32</td>
<td>This study</td>
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Tomoyasu et al., 1993a), defects in protein assembly into and through the membrane (Akiyama et al., 1994a, b; Tomoyasu et al., 1993a) and a defect in a proteolytic pathway (Herman et al., 1993). A *Bacillus subtilis* ftsH mutant shows salt sensitivity (Geisler & Schumann, 1993). However, it has not satisfactorily been demonstrated how *ftsH* mutations cause these diverse defects, and what the function of FtsH is. In this respect, it is interesting to know the structural similarity and functional relationship of FtsH in Gram-negative and Gram-positive bacteria. Comparative studies on the FtsH proteins in Gram-positive and Gram-negative bacteria will provide better understanding of the function of this highly conserved putative membrane ATPase.

**METHODS**

**Bacterial strains, plasmids and media.** The bacterial strains and plasmids used are listed in Table 1. *L. lactis* was grown in M17 medium (Oxoid), containing 0.5% glucose, routinely at 30°C. *E. coli* and *B. subtilis* were grown in L-broth (Miller, 1972) at 37°C unless otherwise stated. The phosphate-buffered minimal medium of Clark & Maaloe (1967) was used for selection of SO609 Hpt+ as described previously (Nilsson & Lauridsen, 1992). For growth of plasmid-containing cells, the
media contained appropriate antibiotics: *E. coli*, ampicillin (50 mg 1\(^{-1}\)), kanamycin (300 or 30 mg 1\(^{-1}\)), chloramphenicol (10 mg 1\(^{-1}\)); *L. lactis*, erythromycin (1 mg 1\(^{-1}\)). For identification of blue PhoA\(^+\) colonies on agar plates, 40 mg XP1 1\(^{-1}\) (5-bromo-4-chloro-3-indolyl phosphate, Sigma) was added.

**DNA manipulations and sequencing.** *L. lactis* chromosomal DNA was isolated according to Johansen & Kibbenich (1992). *E. coli* and *B. subtilis* chromosomal DNA was isolated as described by Silhavy et al. (1984) and *E. coli* plasmids as described by Birnboim & Doly (1979). The use of restriction enzymes, T4 DNA ligase and calf intestine alkaline phosphatase were as recommended by the suppliers (Boehringer Mannheim, Promega, Stratagene). Plasmid transformation of *E. coli* and *L. lactis* was performed according to Mandel & Higa (1970) and Holo & Nes (1989), respectively. The nucleotide sequence of both strands of DNA was determined by the dideoxy method (Sanger et al., 1977). Universal primers from Stratagene or customized primers were used as sequencing primers. All nucleotide sequence data were processed and the deduced amino acid sequences compared using the GCG software package, version 7.0 (Devereux et al., 1984) and EMBL nucleotide sequence database release 37.0.

**Cloning of ftsH.** A *L. lactis* strain CHCC285 chromosomal DNA library, containing 1-10 kb SpeI restriction fragments in the bacteriophage vector Z Zap II (Stratagene) was used. By selection for Pho\(^+\) colonies of *E. coli* strain SO609 (Nilsson & Lauridsen, 1992), we isolated clone 2LN2 containing a 7-3 kb and a 1-3 kb SpeI fragment. The 7-3 kb fragment, containing the bpt and ftsH genes, was subcloned in the plasmid vector pBluescript KS\(^+\) (Stratagene) resulting in pLN30 (Table 1).

**Construction of pLN2::Tn5phoA.** Plasmid pLN2 (Table 1), which contains the first 441 codons of the *ftsH* gene, was used to transform *E. coli* strain DC118. Following infection of DC118 (pLN2) with *Brevibacterium* sp. Tn5 phage (Gutterrez et al., 1987), blue colonies on LB XP agar plates (Pho\(^-\)) that were Kan\(^R\) and Ap\(^R\), were selected and pooled. Plasmids were isolated and used to transform DC118 to Pho\(^-\), Ap\(^R\) and Kan\(^R\). Six independent pLN2::Tn5phoA \(^R\) isolates were obtained.

**Identification of *L. lactis* FtsH in *E. coli*.** Various *E. coli* extracts from strains containing plasmids were used for SDS-PAGE and subsequently blotted semi-dry to nitrocellulose as described by Ilsen & Larsen (1988). The nitrocellulose was subsequently immunostained (Larsen et al., 1992) using the anti-*E. coli* FtsH serum described previously (Tomoyasu et al., 1993b).

**Cell-fractionation of *L. lactis*.** Cells were grown in 400 ml of M\(^1\) medium to an OD\(_{600}\) of 0.9-1.0, harvested and resuspended in 5 ml 30 mM sodium phosphate (pH 6.8) containing 0.4 M sucrose and 1 mg lysozyme ml\(^{-1}\). After incubation at 37\(^\circ\)C for 1 h, the cells were harvested, washed twice with 30 mM sodium phosphate containing 0.4 M sucrose, resuspended in 5 ml 10 mM sodium phosphate (pH 6.8) and sonicated. After ultracentrifugation of the cell extract (100000 g, 1 h at 4\(^\circ\)C), the supernatant (cytoplasmic) fraction was collected. The pellet (membrane) fraction was resuspended in 10 mM sodium phosphate containing 1 M NaCl and ultracentrifuged as above. The supernatant and pellet were collected separately. The supernatant was desalted by gel filtration (Sephadex G-25M, Pharmacia).

The collected fractions were used for SDS-PAGE, blotted to nitrocellulose and immunostained with anti-*E. coli* FtsH serum as described above.

**Southern hybridization analysis.** A 1-0 kb HindIII--KpnI (the KpnI site originates from the polylinker of pBluescript SK\(^-\)) restriction enzyme fragment of plasmid pLN3 (Table 1) contains an internal part of the *L. lactis* ftsH coding region. This fragment was used as probe in Southern hybridization analysis with SalI-digested chromosomal DNA from various bacterial strains, using the ECL Gene Detection System, version 2 (Amershams) as follows. In the hybridization buffer NaCl was added to a final concentration of 1-0 M; primary washes were in 2 x SSCE, 1 M urea, at 25\(^\circ\)C. All other procedures including probe labelling, DNA transfer and signal detection were as described by Amershams. The molecular mass markers were obtained from Gibco BRL.

To verify the integration of pLN43 (Table 1) into the *ftsH* gene of *L. lactis* strain DN4302, *ftsH*:pLN43, a 1-3 kb EcoRV internal fragment of *ftsH* was used as probe in Southern hybridization analysis with EcoRI-digested chromosomal DNA from DN4302 of *L. lactis* strain MG1363 wild-type. Standard conditions as recommended by Amershams were used.

**RESULTS**

**Nucleotide sequence of *L. lactis* ftsH.**

Adjacent to the bpt gene and trnA operon of *L. lactis*, a gene (*ftsH*) was found (Nilsson & Lauridsen, 1992; Nilsson & Johansen, 1994). The complete *ftsH* gene was cloned and the nucleotide sequence was determined. The sequence of the last 24 codons of bpt and the entire *ftsH* gene is shown in Fig. 1. A possible ribosome binding site (Ludwig et al., 1985; Shine & Dalgarno, 1974) and a putative promoter (Koivula et al., 1991) are located upstream of *ftsH* (Fig. 1). The putative promoter contains the sequence 5’-ATATG 3’ in the -16 region (consensus 5’-RTTG 3’ where R = purine), which is found in strong *B. subtilis* promoters (Henkin et al., 1988; Moran et al., 1982). A potential stem--loop structure followed by five Ts (nucleotides 82–101) was located between the bpt and *ftsH* coding regions. The translation stop codon of the *ftsH* gene was located in the loop of a potential transcription terminator structure (nucleotides 2352–2391).

Analysis of the deduced amino acid sequence of the *ftsH* gene revealed a purine nucleotide binding site motif (residues 233–240 and 288–293) (Walker et al., 1982). Two putative transmembrane sequences can be predicted (residues 1-29 and 136–158) (Kyte & Doolittle, 1982). The transmembrane sequence (residues 136–158) and the region containing the purine nucleotide binding site are separated by a very glycine rich sequence (residues 159–170, GGGMGARGGGGGG).

**Comparison of *L. lactis* FtsH with other proteins**

Database searches for genes encoding similar amino acid sequences revealed that the deduced amino acid sequence of *L. lactis* ftsH was 47% identical to that of *E. coli* ftsH (Tomoyasu et al., 1993a) and 36% identical to that of Saccharomyces cerevisiae Yme1 (Thorsness et al., 1993). The deduced *L. lactis* FtsH amino acid sequence contains a region of 200 amino acid residues that reveals similarity to conserved domains in the AAA-protein family of putative ATPases (Erdmann et al., 1991; Kunau et al., 1993). In this region the *L. lactis* FtsH amino acid
Fig. 1. For legend see facing page.
sequence is 71% identical to that of \( E. \ coli \) FtsH and 31 amino acid residues are identical among all sequences shown in Fig. 2.

**Localization of \( L. \ lactis \) FtsH in the membrane**

A system using \( Tn5phoA1 \) to generate protein fusions between the product of a target gene and alkaline phosphatase (AP) has been developed to detect genes encoding membrane and periplasmic proteins in \( E. \ coli \) (Manoil & Beckwith, 1985, 1986). Fusion of AP to a periplasmic protein or to a periplasmic domain of a membrane protein is essential for AP activity. Selection for \( Tn5phoA1 \) insertions in pLN2 (Table 1, contains the first 441 codons of \( L. \ lactis \) ftsH) resulted in six different plasmids (pLN22–pLN27, Table 1) expressing AP activity in \( E. \ coli \). The fusion points between \( Tn5phoA1 \) and pLN2 in pLN22–27 were determined by DNA sequencing. All the insertions were located in the ftsH coding region between nucleotide 467 and 663 (codons 64–129) (Fig. 1).

To further analyse the localization of the \( L. \ lactis \) FtsH protein in the cell, we carried out Western blotting analysis of fractionated \( L. \ lactis \) cell extracts with anti-\( E. \ coli \) FtsH serum, which also reacts with FtsH of \( L. \ lactis \). Sonicated cell extracts from \( L. \ lactis \) strain MG1363 and DN4302 ftsH::pLN43 (described below) were fractionated into cytoplasmic and membrane fractions by ultracentrifugation. The membrane fraction was washed with 1 M NaCl and separated into supernatant and pellet. In Fig. 3a and b (lanes 1–3) a SDS-polyacrylamide gel of the various fractions of cell extract of MG1363 and DN4302 blotted to nitrocellulose and gold-stained is shown. No significant differences in the protein patterns of the various fractions of cell extract of MG1363 and DN4302 differed in protein patterns (Fig. 3a and b, lanes 2). The anti-\( E. \ coli \) FtsH serum only detected FtsH from MG1363 and a truncated form of FtsH from DN4302 in the pellets of the salt-washed membrane fractions (Fig. 3a and b, lanes 6).

It is concluded from the results described above, that FtsH of \( L. \ lactis \) is an integral membrane protein spanning the membrane twice with the region flanked by these hydrophobic stretches protruding outside the cell, and that it has a large cytoplasmic carboxy-terminal part with a putative ATP-binding domain (Fig. 4). Such overall features of the \( L. \ lactis \) FtsH topology are consistent with those of the \( E. \ coli \) homologue, FtsH (Tomoyasu et al., 1993b), except that \( L. \ lactis \) FtsH has a larger outside domain than \( E. \ coli \) FtsH.

**Complementation of \( \Delta \)ftsH::kan in \( E. \ coli \) with ftsH of \( L. \ lactis \)**

Since the deduced amino acid sequences of ftsH from \( L. \ lactis \) and \( E. \ coli \) showed a high degree of identity, we were interested to know whether or not the \( L. \ lactis \) ftsH gene could complement a ftsH mutation in \( E. \ coli \).

The \( E. \ coli \) strain AR423 \( \Delta \)ftsH::kan (pAR171 ftsH.rep\( \text{R} \)) shows temperature-sensitive growth, because the replication of the plasmid pAR171, containing the essential ftsH gene and a chloramphenicol (Cm) resistance marker, is defective at 42 °C (Akiyama et al., 1994a).

The plasmid pLN32 contains the entire \( L. \ lactis \) ftsH gene, whereas the plasmids pLN2 and pLN39 contain various parts of the \( L. \ lactis \) ftsH gene, all cloned in the vector pBluescript KS+ or pBluescript SK− containing ampicillin resistance (Ap\( \text{R} \)) markers (Fig. 5a, Table 1). These plasmids were used to transform strain AR423 by selection for Ap\( \text{R} \) at 30 °C. The strains obtained were incubated in LB at 42 °C for 6 h, and then plated on LB agar at 30 °C. The plasmid content of the colonies was tested by streaking on LB agar containing Ap or Cm; and the possession of the \( \Delta \)ftsH::kan mutation was tested by streaking on LB agar containing Kan. From transformants containing the entire \( L. \ lactis \) ftsH gene on pLN32, Cm\( \text{R} \) colonies could be isolated with a frequency of approximately 40–50%, indicating that these had lost pAR171, containing the \( E. \ coli \) ftsH wild-type. Such colonies were all Ap\( \text{R} \) and Kan\( \text{R} \), indicating that these had retained pLN32, containing the \( L. \ lactis \) ftsH gene, and the mutation \( \Delta \)ftsH::kan. Colonies of transformants with pLN2 and pLN39, containing only part of \( L. \ lactis \) ftsH, were all ApR, CmR and KanR, showing no loss of pAR171 was obtained. Thus pAR171, and hereby the \( E. \ coli \) wild-type ftsH gene, can be lost only from strains containing the complete \( L. \ lactis \) ftsH gene. One strain, AR3120 \( \Delta \)ftsH::kan (pLN32 \( L. \ lactis \) ftsH), was saved for further studies.

Western blots with anti-\( E. \ coli \) FtsH serum were performed using extracts of AR423, AR423 containing the plasmids in Fig. 5(a) and AR3120 to detect the gene products of \( E. \ coli \) ftsH and/or \( L. \ lactis \) ftsH (Fig. 5b). The \( E. \ coli \) FtsH protein could be detected in the AR423 derivatives but not in AR3120, whereas the \( L. \ lactis \) FtsH protein was detected in AR423(pLN32) and AR3120. No \( L. \ lactis \) ftsH protein was detected in AR423, AR423(pLN39), AR423(pLN2) or AR423(pKS+). This

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Fig. 1. Nucleotide sequence of the \( L. \ lactis \) ftsH gene. The numbers to the right indicate nucleotide positions ('htp, nucleotides 1–72; ftsH, nucleotides 278–2365, 696 codons). The deduced amino acid sequences of 'htp and ftsH (FtsH) are shown in one-letter code below, with the translation stop codons indicated by asterisks. The numbers to the left indicate the deduced amino acid residue position of FtsH. The putative ribosome binding sites (RBS) and promoter regions (−35, −16, −10) are indicated. Two putative stem-loop structures are underlined. The \( Tn5phoA1 \) insertions in pLN22–27 are indicated above the sequence. The EcoRV sites used to construct the integration plasmid pLN43 are shown above the sequence.
**Fig. 2.** Amino acid sequence alignment of *L. lactis* (L.) FtsH, *E. coli* (Ec) FtsH, Yme1p and the conserved domains found in *L. lactis* FtsH (residues 191–410); TBP-1 (Nelbock et al., 1991); Paslp (Erdmann et al., 1992; Shibuya et al., 1990), and Secl8p resembling NSF (Eakle et al., 1989). 54 of the 265 protease, MSSl, and SUGl resembling TBP-1 (Dubiel et al., 1992; Shibuya et al., 1992; Swaffield et al., 1992), VCP and p97 resembling CDC48p (Koller & Brownstein, 1987; Peters et al., 1988) also contain the

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**Table 2.** The amino acid sequence alignment of *L. lactis* (L.) FtsH, *E. coli* (Ec) FtsH, Yme1p and the conserved domains found in *L. lactis* FtsH (residues 191–410); TBP-1 (Nelbock et al., 1991); Paslp (Erdmann et al., 1992; Shibuya et al., 1990), and Secl8p resembling NSF (Eakle et al., 1989). 54 of the 265 protease, MSSl, and SUGl resembling TBP-1 (Dubiel et al., 1992; Shibuya et al., 1992; Swaffield et al., 1992), VCP and p97 resembling CDC48p (Koller & Brownstein, 1987; Peters et al., 1988) also contain the conserved domain (not shown). Identical amino acid residues found in these sequences are indicated in boldface type above the sequences. Identical amino acid residues of *L. lactis* FtsH, *E. coli* FtsH and Yme1p are indicated by asterisks. The two putative membrane-spanning sequences of *L. lactis* FtsH and Yme1p are underlined.
Fig. 3. Localization of *L. lactis* FtsH in cell fractions. Fractionation of extracts from MG1363 and DN4302, and treatment of membrane fractions with 1 M NaCl were done as described in Methods. Electrophoresis of the cell fractions on an SDS-polyacrylamide gel (a and b) was done, blotted to nitrocellulose and gold-stained (lanes 1–3) or subjected to Western blotting with anti-*E. coli* FtsH serum (lanes 4–6). Positions of molecular mass reference markers, FtsH and a truncated form of FtsH (FtsH') are indicated. All other bands are a result of unspecific binding of the secondary anti-serum used (control not shown). (a) Lanes: 1 and 4, cytoplasmic fraction of MG1363; 2 and 5, supernatant derived from membrane fraction of MG1363 washed with 1 M NaCl; 3 and 6, pellet derived from membrane fraction of MG1363 washed with 1 M NaCl. (b) Lanes: same as (a) except extract from DN4302 was used.

Fig. 4. Deduced topology of the *L. lactis* FtsH protein. Numbers indicate amino acid residue positions. Shaded boxes illustrate putative membrane-spanning regions. The encoded FtsH–PhoA fusions of pLN22–27 that results in AP activity are fused at positions indicated by triangles. The region with similarity to the conserved region of the AAA-protein family is indicated by an open box. This region contains the ATP binding motifs.

demonstrates that AR3120 was able to grow without the essential *E. coli ftsH* gene product when containing the *L. lactis ftsH* gene product. The plasmid pLN2 encodes a truncated *L. lactis* FtsH protein containing the epitope for the anti-*E. coli* FtsH serum, when transcribed and translated in *vitro* (Nilsson & Lauridsen, 1992; data not shown). This truncated FtsH was not detected by the anti-*E. coli* FtsH serum in the Western blot, possibly due to rapid degradation in vivo.

*E. coli* AR3120 showed temperature-sensitive growth in that it could grow at 30 °C and 37 °C but not at 42 °C. A *B. subtilis* mutant with a salt-sensitive phenotype has been shown to be impaired in a putative homologue of ftsH (Geisler & Schumann, 1993). AR423, AR423(pLN32) and AR3120 were tested for growth on LB agar plates containing 4% (w/v) NaCl. AR423 and AR423(pLN32), but not AR3120, were able to grow on this medium.

**Construction of a *L. lactis* ftsH mutant**

A 1·3 kb *EcoRV* fragment of pLN32, containing an internal part of *L. lactis ftsH* (Fig. 1), was cloned into the *L. lactis* integration vector pV2 giving pLN43 (Table 1).
Transformation of pLN43 into L. lactis strain MG1363, selecting for erythromycin resistance, resulted in strain DN4302ftsH::pLN43. The integration of pLN43 into ftsH of DN4302 was verified by Southern hybridization analysis (not shown). The construction of DN4302 indicates that the ftsH gene is not essential in L. lactis or that the truncated form of FtsH encoded by the disrupted ftsH gene (Fig. 3b, lane 6) is enough to retain any essential function. DN4302 and MG1363 were tested for growth on M17-agar plates containing 4% or 1% NaCl. MG1363 could grow at both 4% and 1% NaCl, whereas DN4302 grew slowly at 1% and not at all at 4% NaCl. Fig. 6 shows the growth of DN4302 and MG1363 at various salt concentrations in M17 broth. MG1363 grew at 4% NaCl after a lag phase of about 2 h, whereas DN4302 at 4% NaCl showed almost no growth. No growth of either strains occurred with 7% NaCl. DN4302 and MG1363 were also tested for growth on M17-agar plates containing 0.5 M sucrose. Both strains appeared to grow equally well on this medium.

Growth at 38 °C, 30 °C and 16 °C on M17-agar plates was also tested. DN4302 only grew at 30 °C, whereas MG1363 grew at all temperatures. However, incubating DN4302 anaerobically restored growth at 38 °C.

**Homologous genes in other bacteria**

A 1.0 kb HindIII–KpnI fragment (nucleotides 667–1600, Fig. 1) was used as probe in Southern hybridization analysis with ScaI-digested chromosomal DNA from various bacterial strains (Fig. 7). Single chromosomal bands can be detected from the Gram-positive bacteria L. lactis, B. subtilis, Leuconostoc sp. and Lactobacillus sp. (Fig. 7, lanes 1, 3–8). With E. coli DNA only a very faint band was detected (Fig. 7, lane 2). Under the same experimental conditions, homology to hpt, using a hpt probe, was only detected in L. lactis (results not shown).

**DISCUSSION**

The ftsH gene of L. lactis was cloned and shown to encode a transmembrane protein with putative ATPase activity. The deduced amino acid sequence indicates that FtsH belongs to the AAA-protein family of putative ATPases containing several members of eukytotic proteins (Erdmann et al., 1991; Kunau et al., 1993) and also includes the membrane protein FtsH of E. coli (Tomoyasu et al., 1993a, b). FtsH from L. lactis and E. coli seem to be homologous proteins based on the high similarity of the amino acid sequences and that the L. lactis ftsH gene can complement certain defects of an E. coli ΔftsH::kan strain. Southern hybridization analysis of various Gram-positive bacteria using L. lactis ftsH as probe suggests that homologous genes exist. In B. subtilis a putative ftsH gene has been located on the chromosome of B. subtilis adjacent to the hpt gene (Geisler & Schumann, 1993; Ogasawara et al., 1994), indicating a similar arrangement of hpt and ftsH as in L. lactis. It seems from these results that ftsH genes are widely conserved in bacteria.

The function of FtsH is unknown. Our L. lactis ftsH mutant and the B. subtilis ftsH mutant (Geisler & Schumann, 1993) were constructed by a Campbell-type integration with an internal gene fragment. This should inactivate the genes. In E. coli, ftsH is essential for growth. Since the resulting mutants are viable, it is possible that the ftsH genes are not essential in L. lactis and B. subtilis. However, in at least L. lactis, the truncated form of FtsH produced by the mutant DN4302 may still retain essential functions.

The L. lactis ftsH mutant DN4302 is impaired in salt tolerance and other stress responses, and shows a different pattern of membrane-associated proteins compared to the wild-type strain (Fig. 3, lanes 2). These phenotypes could be explained by an improper assembly of membrane proteins, some necessary for the salt tolerance, caused by the ftsH mutations in L. lactis. In E. coli the maturation of penicillin binding protein 3 (PBP3) and β-lactamase is dependent on FtsH function, in that post-translational processing at the C-terminal part of PBP3 seems to be defective and accumulation of the plasmid-encoded precursor of β-lactamase in the cytoplasm was observed in the thermosensitive ftsH1 mutant of E. coli (Begg et al., 1992; Tomoyasu et al., 1993a). Analysis of several newly constructed E. coli ftsH mutants encoding FtsH variants, including C-terminally truncated forms with dominant phenotypes, suggests that FtsH is involved in assembly/folding of proteins into and through the membrane and that FtsH is needed to assure efficient stop-transfer of some membrane proteins (Akiyama et al., 1994a, b).
phenotypes is not known. Recently it was shown that a mutation in E. coli causing increased lysogenization frequencies of bacteriophage λ, and other direct biochemical evidence for these functions has been reported so far. Comparative biochemical and genetic analysis of FtsH from L. lactis and E. coli will provide better understanding of the biological significance of FtsH, if it is a chaperone or a protease, and what are its substrates.

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development controlled by the same essential Escherichia coli gene, fsiH/fbF. Proc Natl Acad Sci USA 90, 10861–10865.


