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Characterization of the *Mycoplasma hominis ftsZ* gene and its sequence variability in mycoplasma clinical isolates

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Abstract

We cloned and sequenced *Mycoplasma hominis* chromosomal fragment containing *ftsZ* gene. The wild-type expression of the gene was shown at RNA level by reverse transcription followed by PCR amplification. We revealed that *M. hominis* FtsZ had a comparatively low similarity to proteins of *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. After full *ftsZ* gene sequencing for 14 clinical isolates of *M. hominis*, single-nucleotide substitutions were found in 21 positions, 6 of them being common for almost all isolates. This *ftsZ* gene polymorphism may be used for subtyping of *M. hominis* in clinical samples. Expression of the *M. hominis ftsZ* gene in different *Escherichia coli* strains was also demonstrated, and *M. hominis* FtsZ protein was purified from *E. coli* cells transformed with recombinant expression plasmid. Complementation between the *M. hominis* FtsZ and *E. coli* FtsZ could be shown. The comparison of FtsZ protein structures may also be used for investigation of bacterial phylogenetic relationships.

Keywords: ftsZ Gene; *Mycoplasma*; *M. hominis*; Gene expression; Bacterial evolution

Taxonomically, *Mycoplasma hominis* belongs to the class *Mollicutes*, the smallest cell-wall free prokaryotic organisms. *M. hominis* has been isolated as a part of the flora of bacterial vaginosis, from the lower genital tract of women [1]. Besides, *M. hominis* is a common spontaneous contaminant of eukaryotic cell cultures. Mycoplasmas used to be considered a *Clostridial* branch of Gram positive eubacteria and were supposed to lose a large part of genomic material and metabolic pathways during their evolution [2,3].

FtsZ protein plays a pivotal role in bacterial cell division process [4]. FtsZ is widely conservative, so the corresponding genes were found in all eubacterial groups, including *Mollicutes*, several archebacteria (*Archea*), and in chloroplasts of plants [5]. But three exceptions have been discovered recently: *ftsZ* genes were not revealed in the completely sequenced genomes of *Chlamidia trachomatis* [6], archebacteria *Aeropyrum pernix* [7], and *Ureaplasma urealyticum* [8]. So far it is not yet clear as to what other proteins perform the function of FtsZ in these microorganisms. About 15 genes and corresponding proteins are responsible for the division process in *Escherichia coli*, but only three genes (*ftsH, Y, Z*) of the division and cell-wall (dcw) group were found in the completely sequenced genomes of *Mycoplasma genitalium*, *Mycoplasma pneumoniae* [9,10], and *Mycoplasma pulmonis* [11]. Functions of *ftsH* and *ftsY* gene products in bacterial proliferation are unknown.

Polymerization of FtsZ into cytokinetic ring at the predivision site is considered the earliest event of the prokaryotic cell division process. The FtsZ protein has GTPase activity [12], and in vitro it forms filaments similar to those of tubulin [13]. Like eukaryotic tubulins, FtsZ proteins have a conservative N-terminal part, a GTP-binding motive, and a comparatively variable hydrophilic C-terminal region. The similarity was

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confirmed by crystal structure analyses of both proteins, and three-dimensional structure of FtsZ and tubulin was shown to be almost identical, which indicates considerable structural conservatism [14]. In bacterial cells, FtsZ forms a scaffold of contractile Z-ring and is located at the leading edge of the invaginating septum during the division process [15,16]. Other cell-wall bacterial proteins responsible for the site division formation and constriction of cytokinetic ring (Z-ring) in mycoplasmas are unknown. We assume that mycoplasmas have the simplest septation system among eubacteria.

Overproduction of FtsZ in *E. coli* cells results in transgression of cell division. High level expression of *Bacillus subtilis* or *M. pulmonis ftsZ* genes in *E. coli* inhibited its cell division to lead to filamentation [17,18]. This observation suggests that FtsZ also plays a basic role in the mycoplasma division process. Its characterization is necessary to understand the fundamental principles of the eubacterial cell proliferation.

The goal of the initial part of this work was to clone and to sequence the *M. hominis* H34 (laboratory strain) *ftsZ* gene. Comparative analysis of translated sequences of *M. hominis ftsZ* and *ftsZ* genes of other microorganisms was performed. We also studied the *ftsZ* gene expression in *M. hominis* itself and expression of recombinant plasmids carrying *M. hominis ftsZ* in *E. coli* cells. Then the *ftsZ* gene of *M. hominis* clinical isolates was amplified, sequenced, and compared to study *ftsZ* intraspecies polymorphism. *M. hominis* is a recognized human pathogen, so its subtyping might be of interest from the clinical aspect.

Materials and methods

Bacterial strains and plasmids. The *E. coli* strain DH5α was used as a routine cloning host. Strains B834(DE3), BL21(DE3), and DH5α were used as hosts for expressing the *M. hominis ftsZ* gene. RP61 (K-12, F-, *thrA*, *proA*, *lysA*, *dra*, *drm*, *ftsZ84*) and PAT84 (K12, F-, *thr1*, *leuB6*, *trp1*, *his1*, *thyA*, *argH1*, *thi1*, *lacY1*, *malY*, *malA1*, *mtl2*, *xyl7*, *tonA2*, *supE44*, *str9*, *mel*, *dapA*, *lysA*, *ftsZ84*) were used for complementation analysis. Bacteria were grown in LB medium supplemented with ampicillin (50 μg/ml) for the selection of recombinant plasmid clones. *Mycoplasma hominis* laboratory strain H34 (Cell Culture Collection of the Institute of Cytology of the Russian Academy of Sciences) was cultivated in a modified PPLO broth [19]. *M. hominis* clinical strains were kindly provided by Dr. A.Taraskina (D.O. Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, St. Petersburg). The strains were grown in a medium containing 39 g/l Brain Heart Infusion BHI (Difco, USA), 1% of arginine (Sigma, USA), fresh yeast extract (N.F. Gamalei Institute of Epidemiology and Microbiology, Moscow), 10% of horse serum (Flow Laboratories), and 0.5% phenol red. Plasmid vectors were pGEM-T-easy (Promega), PUC18, and pET15b (Novagen).

M. hominis ftsZ gene cloning and sequencing. DNA from *M. hominis* cells was extracted by a modified adsorption method on diatoms in the presence of a chaotropic agent, guanidinium thiocyanate [20]. Arbitrary primers (5′-AGAGGATCCGG(AT)GG(AT)GG (AT)AC(AT)GG(TAC)AC(ATC)GG and (5′-GATGAATTCAC (GA)TC(AT)GC(GA)AA(GA)TC(TA)A(GA)(GA)TT) which approximately corresponded to two highly conserved FtsZ regions of several bacteria, including mycoplasmas, GGGTGTG, and NLDFADV [18], were used for synthesis of the *M. hominis ftsZ* gene central fragment. The amplified fragment of expected length 310 bp was cloned into pGEM-T-easy vector (Promega) and sequenced in both directions by a standard method [21]. Further search for and amplification of unknown neighboring regions around the 310 bp fragment were made by targeted gene walking approach [22]. The fragment of 1636 bp length including the *M. hominis ftsZ* gene and small flanking regions was cloned into pGEM-T-easy vector and sequenced in both directions with synthetic primers and *fmol* DNA cycle sequencing system (Promega, USA). Nucleotide and deduced amino acid sequences were analyzed with DNAstar; the amplified fragments were identified basing on GenBank/EMBL searches (BLAST).

RT-PCR. RNA isolation from *M. hominis* cells was made with Trizol reagent according to Gibco BRL protocol. Synthesis of cDNA was performed with reagents including MMLV RT and random primers in accordance with manufacturer's recommendations (Promega, USA). *M. hominis* total cDNA was used as a template in PCR with the following primers: 5′-ATGGCAAAAGAATTAAATAATT TTAACCC-3′ (Pr47) and 5′-TCTGCAAGTTCATCGTTTGA-3′ (Pr23), corresponding to bases: from 1 to 29 and from 1130 to 1111, respectively, of the *M. hominis ftsZ* gene (GenBank Acc. No. AF468001). For the *ftsZ* fragment amplification, PCR started with denaturation at 93 °C for 2 min, then 30 cycles each of 93 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min. For the 16S rRNA gene fragment amplification, two primers were used: 5′-GGTTAGCAATAACCTAG CGGCGA-3′ (HF) and 5′-ACCATCTGTCACTCCGA TAACCTCC-3′ (HR), corresponding to bases 63–85 and 1016–993, respectively, of the 16s rRNA gene (GenBank Acc. No. M96660). In this case, PCR started with preheating at 93 °C for 2 min, then 35 cycles each at 93 °C for 10 s, at 60 °C for 10 s, and at 72 °C for 10 s, followed by incubation at 72 °C for 1 min.

Overproduction and isolation of FtsZ. The *M. hominis* H-34 *ftsZ* gene was cloned into PUC18 and pET15b expression vectors. The *ftsZ*-containing fragment was obtained by PCR, using the primers P1 (5′-CATATGGCAAAAGAATTAAATAATTTTAACCC-3′), and P2 (5′-GGATCCTCTTAGTGTTTTAATATATCTGCAAGTTCATCG-3′) containing the *Nde*I and *Bam*HI site, respectively, for cloning into pET15b plasmid, and primers P3 (5′-GAGCTCTATGGCAAAAGA ATTAAATAATTTTAACCC-3′) and P4 (5′-AAGCTTTTAGTGTT TTAATATATCTGCAAGTTCA TCG-3′) containing the *Sac*I and *Hin*dIII site for cloning into pUC18 plasmid. The resulting plasmids were called pUC18ftsZ and pET15bftsZ. FtsZ protein was isolated from extracts of B834(DE3)(pET15bftsZ) strain and purified according to manufacturer's protocol HisTrap (Pharmacia Biotech, GB).

Complementation analysis. After transformation with recombinant plasmids pUC18ftsZ, and pET15bftsZ, *E. coli* cells PAT84 ($ftsZ^{ts}$), RP61 ($ftsZ^{ts}$), and BL21(DE3) (control, $ftsZ^{+}$) were placed on LB-agar, supplemented with 100 μg/ml of ampicillin, and incubated both at 30 and 42 °C. Cells from the colonies grown up at both temperatures were cultivated on the liquid LB medium, supplemented with 100 μg/ml of ampicillin and 1 mM IPTG, and their phenotype was determined by light microscopy of the bacteria loaded on to slides.

Immunoblotting. Proteins were separated by 12% SDS–PAGE electrophoresis [22] and transferred to polyvinylidenedifluoride (PVDF) membrane (Immobilon, Millipore). Blot membranes were blocked with 1×PBS containing 3% of fat-free milk for 3 h. Then the filter was washed with 1×PBS, incubated with anti *E. coli* FtsZ Mab's for 3 h, and washed in PBS–Tw (1×PBS, 0.5% Tween20) three times, 10 min each. Proteins were detected with ECL-Western kit (Amersham). *E. coli* FtsZ Mab's were a gift from M. Vicente.

Results and discussion

Identification and cloning of the ftsZ gene from M. hominis

For the *ftsZ* gene identification, a PCR approach was used as the first step, in the same way as it was done earlier [18,23]. Degenerated primers were synthesized after FtsZ sequence comparison of eight eubacteria species and, in accordance with low GC% in the mycoplasma genome, A or T nucleotides were used in the third positions of the codons. PCR product of 310 bp length was synthesized with these primers and chromosomal DNA of *M. hominis* as a template. The amplified fragment was cloned and sequenced. Comparison of nucleotide sequence of this fragment revealed a significant similarity with other bacterial *ftsZ* genes. For the next step, we used targeted gene walking procedure according to Malo et al. [22]. As a result, two more fragments were amplified, cloned, and sequenced: one fragment, 490 bp long, which includes 5′-end region of the gene, and another one, 1200 bp long, containing 3′-end region of the gene. Finally, the full-size *ftsZ* gene copy was cloned into vector pGEM-T and sequenced completely in both directions.

M. hominis ftsZ gene structure

Analysis of the insert sequence (1589 bp long) revealed a large open reading frame corresponding to the full-size copy of *M. hominis ftsZ* (1145 bp long), with 65.9% G + C nucleotide composition. Recognizable RBS (AGGAG) presides the frame and situates four nucleotides upstream of the start codon. The translated FtsZ protein sequence consists of 381 amino acids (Fig. 1), and the predicted molecular weight of the polypeptide is 40.7 kDa. The protein has a high content of Ile (11.5%), Ala (8.9%), Gly (8.1%), Val (7.9%) and is much lower in Cys (1.0%), with the absence of Trp. Comparison of *M. hominis* FtsZ with other FtsZ proteins of different microorganisms demonstrates maximal homology level in the region of the first 315 amino acids of the N-terminus (Fig. 2). Thus, in this region of FtsZ of *M. hominis*, homology with FtsZ of *M. pulmonis*, *B. subtilis*, *E. coli*, and *Acholeplasma laidlawii* is 48%, 46%, 41%, and 39%, respectively, but maximal homology (41%) between FtsZ of *M. pneumoniae* and *M. genitalium* is only in the region of the first 223 amino acids of the protein. As a result of the FtsZ primary structure comparison (Fig. 2), a considerable divergence was found toward the C-terminus, including the first 70–80

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2     CGATTTGGACCTTCAAAGTCAAACGAAATGCTTTAAATAATGAAATTAGTAGGGAATTATTTACAATTGAAGTTTATAGTAATTCCAAAA

92    AGTACTACAATTTAATGCTAAACGATATAAATTAGGAGTTCTATGGCAAAAGAATTAAATAATTTTAACCCGGTGGCAAAAATAATTGTT
1                                          M  A  K  E  L  N  N  F  N  P  V  A  K  I  I  V

182   ATTGGAGTAGGTGGAGGCGGAAATAATAGTGTTGAAACTATGATAAATAGCCATTTAGATAGTTTTCAAATTATTGCTGCCAATACCGAT
 I  G  V  G  G  G  G  N  N  S  V  E  T  M  I  N  S  H  L  D  S  F  Q  I  I  A  A  N  T  D

272   AAACAAGTATTAGCAAAATTTCCTCAAGAATGCGTTTTACATTTAGGTGATGAACGTGGAATTGGCGCGGGTGCTAACCCTGAAATTGGC
     K  Q  V  L  A  K  F  P  Q  E  C  V  L  H  L  G  D  E  R  G  I  G  A  G  A  N  P  E  I  G

362   AAAACTGCTGCTGAATCTTCACGCGAAGAAATCAAAAGTAGATTGCAAGGAGCTGATCTAGTAATTATAACTGCAGGAATGGGTGGTGGA
76     K  T  A  A  E  S  S  R  E  E  I  K  S  R  L  Q  G  A  D  L  V  I  I  T  A  G  M  G  G  G

452   ACCGGCACAGGTGCAGCGCCCGTGATTGCACAAATAGCCAAGGAATGCAATGCTTTAGTAGTTGCAGTTGTAACTACTCCGTTTGACTTT
106    T  G  T  G  A  A  P  V  I  A  Q  I  A  K  E  C  N  A  L  V  V  A  V  V  T  T  P  F  D  F

542   GAAGGTCCAAAGAGAATGAGAATTGCAAAGCAAGGCTTACAAGAAATTAAAAAATGTGTTGATTCATATATTGTTATTTCAAACAATAAA
136    E  G  P  K  R  M  R  I  A  K  Q  G  L  Q  E  I  K  K  C  V  D  S  Y  I  V  I  S  N  N  K

632   CTATTACAACAATATGGAAATATTTCTTTTTCGGATGCATTTATTTGCGCAAATAATGTCTTAAAGCAAACTATTAGAACAATAGTTGAT
166    L  L  Q  Q  Y  G  N  I  S  F  S  D  A  F  I  C  A  N  N  V  L  K  Q  T  I  R  T  I  V  D

722   GTTATTGCCACCCCAAGCATTATTAATCTTGATTTTGCAGATTTATCAACCATTATTAAAAATAAAGGTGAAACAGTAATCGGCATTGGC
196    V  I  A  T  P  S  I  I  N  L  D  F  A  D  L  S  T  I  I  K  N  K  G  E  T  V  I  G  I  G

812   CAAGCAAATGGCCAAGATCGGGCTGTAAAAGCAATCACATCTGCAATAACAAGCCCAATTTTAGAATCAAGTGTTGTTGGGGCTAGTGAT
226    Q  A  N  G  Q  D  R  A  V  K  A  I  T  S  A  I  T  S  P  I  L  E  S  S  V  V  G  A  S  D

902   GCAATAGTAAATTTCAGTGCTTCTCAAAAAGTAACTTTAAATGAAATTCAAAGTGCTTTGGGAGCTATGAGGGAAATTGTTGGTAATGAA
256    A  I  V  N  F  S  A  S  Q  K  V  T  L  N  E  I  Q  S  A  L  G  A  M  R  E  I  V  G  N  E

992   ATTAACATTATTTTTGGTATAACAACTCTAGAATCTGAAGAAAGCAACAAATTAGGTGAGCTATTTGTCTCGGTAATCGCTACGGGATTA
286    I  N  I  I  F  G  I  T  T  L  E  S  E  E  S  N  K  L  G  E  L  F  V  S  V  I  A  T  G  L

1082  AGAAAAGACGCTCCTAAAGACATTGATCAAATTCAAGATGAAGTAATCAATGTAATAAAAAAAGACGATTTAAATTATGTTAACGATGAA
316    R  K  D  A  P  K  D  I  D  Q  I  Q  D  E  V  I  N  V  I  K  K  D  D  L  N  Y  V  N  D  E

1172  ACAAAGGAATTCTTTGTATCTGAAGGAACATTTAAAACACAATCATTTTTTTCAATGGATAACGATGAAGATTCAAACGATGAACTTGCA
346    T  K  E  F  F  V  S  E  G  T  F  K  T  Q  S  F  F  S  M  D  N  D  E  D  S  N  D  E  L  A

1262  GATATATTAAAACACTAAGATGATGTAAGAAATCTGTTTTAAAAAAATGCAATTAATTGTCTTTATTGCCATTTAAAAAATAAAAAAATT
376    D  I  L  K  H
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the *M. hominis ftsZ* gene. The nucleotide sequence of the *ftsZ* region from *M. hominis* and deduced amino acid sequence of the open reading frame are shown. Putative RBS is underlined.

amino acids. At present, *ftsZ* genes of many prokaryotic microorganisms are sequenced and the sizes of corresponding deduced FtsZ proteins vary from 320 to 550 amino acid residues. The N-terminal domains of FtsZ proteins are highly conservative, but only distal regions of carboxyl amino acid residues have a comparatively high homology level at the C-terminus.

Evolutionary conservative regions of FtsZ protein are located in the N-terminal domain in different microorganisms. The N-terminal region of FtsZ 200 amino acids long contains a loop enriched in Gly, and this region has maximal homology with α, β, and λ-tubulins [24,25]. The evolutionary conservative GTP-binding domain (GGGTGSG) of tubulins, which is involved into GTPase activity, differs from the FtsZ *M. hominis* corresponding motive only in one amino acid residue. The structure of this conservative GTP-binding *M. hominis* region (GGGTGTG) completely coincides with those of *M. pulmonis* and *A. laidlawii*, but differs in one position in *M. pneumoniae* and *M. genitalium* FtsZ.

Polymorphism of M. hominis ftsZ gene

The full *ftsZ* gene nucleotide sequences of 14 clinical isolates were determined and analyzed comparatively. Preliminary DNA preparations of 60 clinical isolates were screened by the SSCP method [26], 14 of them being chosen for further studies. The *ftsZ* sequence variants were discovered among the isolates (Table 1). Mutations (single-nucleotide substitutions) were revealed in 21 positions, six of them being common for almost all isolates (positions 249, 252, 531, 939, 963,

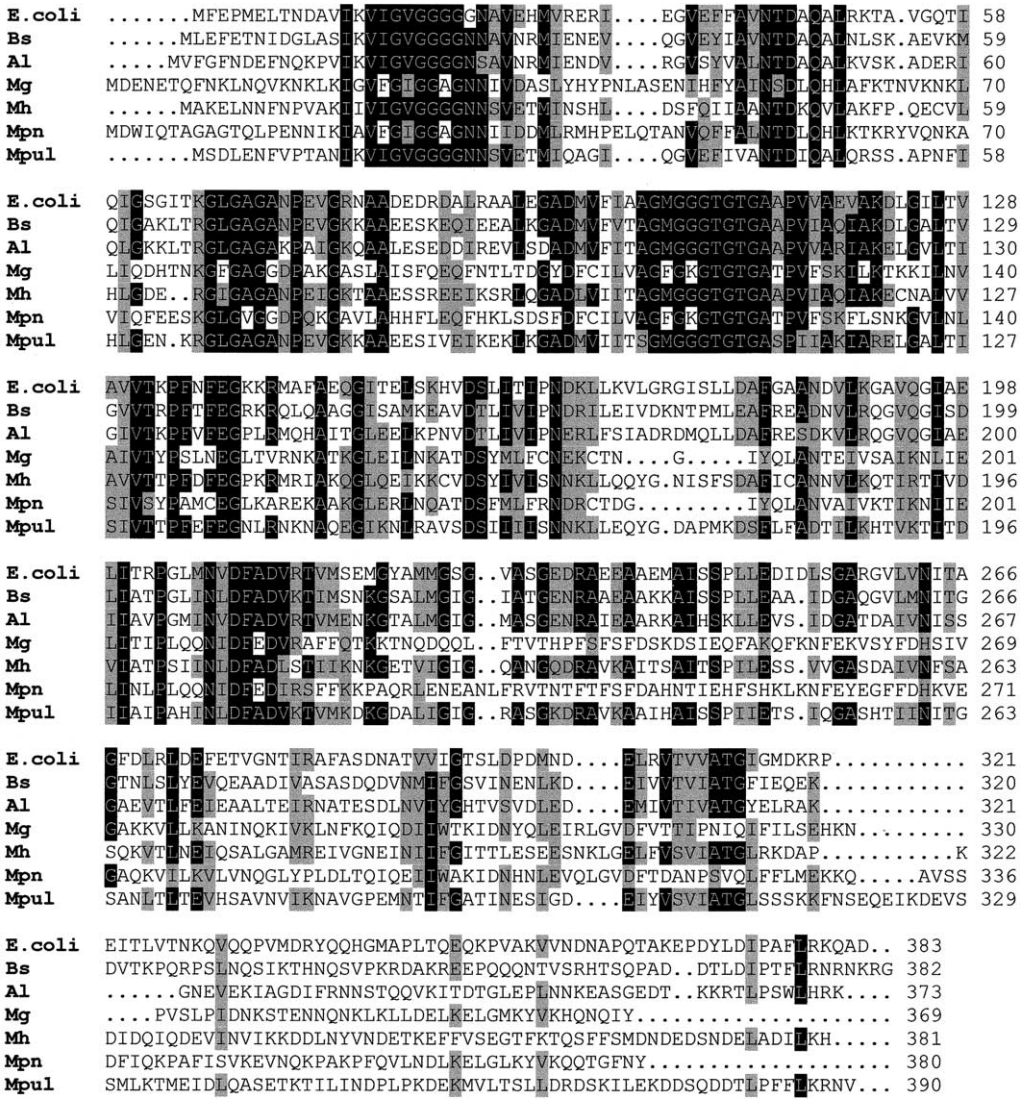

Fig. 2. Comparison of *M. hominis* FtsZ with other FtsZ proteins. The FtsZ protein sequences were aligned by using Megalign (DNAStar). The FtsZ proteins include *M. pulmonis* (Mpul), *M. pneumoniae* (Mpn), *M. genitalium* (Mg), *A. laidlawii* (Al), *B. subtilis* (Bs), *E. coli* (E. coli), and *M. hominis* (Mh). The sequences analyzed here have the following accession numbers: *A. laidlawii*, NP_389412 for *B. subtilis*, NP_414637 for *E. coli*, P47466 for *M. genitalium*, NP_110005 for *M. pneumoniae*, NP_326322 for *M. pulmonis*, and AF468001 for *M. hominis*.

1101). Almost in all cases, mutations take place at the third codon letter. This corresponds to the common principle that the single-nucleotide mutation leading to an amino acid change is a comparatively rare event. Only in one case, mutation at the first letter of the codon (GTT → CTT) was found, which corresponds to Val → Leu changing. Clinical strains (samples) S7, S9, S35, S39 (see Table 1) might be considered as a separate group, as 10 mutations in the isolates S9 and S39 completely coincide, S7 differs only in two, while S35, in three positions. Another group might be composed of isolates S11, S17, S38, and S45. Mutations in the isolates S17 and S38 completely coincide, whereas in S11 and S45 they differ in three positions. The third group includes isolates S36, S40, and S43. Mutations in the last two isolates coincide completely, while in S36, mutations differ in three positions. Each of the isolates S3, S33, and S48 has individual mutation sets.

Recently, *ftsZ* gene was used for clinical sample genotyping of *Bartonella henselae* [27]. The 701-bp region in the 3′-end of the *ftsZ* gene in 15 *B. henselae* isolates studied is particularly variable [28]. It is not yet clear whether there is any correlation between the FtsZ structure and pathogenicity of the *M. hominis* clinical strains. Anyway, the *M. hominis ftsZ* gene sequence variation demonstrated in this study might be useful for specifying the epidemiology of various *M. hominis* strains in clinical samples.

Expression of the M. hominis ftsZ

Overproduction of the *E. coli* FtsZ protein is known to give the minicell phenotype [15]; and expression of heterologous *ftsZ* genes at a high level causes transgression of the *E. coli* cell division and the appearance of filaments, multinucleoidal cell forms [17,18,29]. The latter phenomenon was observed with *M. pulmonis ftsZ* expression into *E. coli*, but was not found at *A. laidlawii ftsZ* expression into *E. coli* [23]. Natural expression of the *M. hominis ftsZ* gene and its expression in *E. coli* strains were checked by RT-PCR on the corresponding RNA preparations (Fig. 3) and by western blot (Fig. 4), respectively. Also, expression of the *M. hominis ftsZ* gene in *E. coli* cells was studied after transformation of PAT84 and RP61 (*E. coli ftsZ*^{ts} strains) and BL21(DE3) (*ftsZ*$^{+}$), as a control, with recombinant plasmids pUC18ftsZ and pET15bftsZ (see Materials and methods). No additional band was found in the Coomassie-stained gel of the transformed *E. coli* strain cell lysates, but a marked increase of intensity of a protein band at the region of 40 kDa was found (data not shown). Bacteria from the BL21(DE3)/pET15bftsZ strain despite a strong filamentation phenotype when growth under induction conditions, liquid LB medium with 1 mM IPTG (Fig. 5B). It has also been shown that *M. hominis* FtsZ protein is recognized with monoclonal antibodies raised against *E. coli* FtsZ (Fig. 4). These two results suggest that *M. hominis* FtsZ protein probably might be functional into *E. coli*, either as an analog mutant form of the *E. coli* FtsZ, or completely functional into *E. coli* cells, as the *E. coli* filamentation process can also take place in the case of disturbance of natural quantitative balance between FtsA and FtsZ proteins [30,31]. A possibility cannot be excluded that filamentation also occurs as a consequence of general disturbance due to overexpression of a heterologous protein.

Table 1
Sequence variations of *ftsZ* gene in *M. hominis* clinical samples

N°	Samples	Sequence variations in *M.hominis ftsZ* gene																					
		-38[a]	63	66	207	210	249	252	267	312	321	481	531	708	765	798	816	939	957	963	1022	1035	1101
		c-t[b]	a-t	c-t	g-a	t-c	a-g	c-t	t-c	t-c	c-t	g-c	g-a	a-g	t-c	a-g	t-c	t-c	c-t	t-c	a-g	t-c	c-t
1	3			■	■			■		■			■			■	■	■	■	■	■		■
2	7	■		■			■								■			■		■		■	■
3	9			■			■			■								■		■			■
4	11	■					■									■		■			■		■
5	17						■	■					■					■		■			
6	33												■		■			■		■			■
7	35	■		■			■	■		■		■	■	■	■				■		■		■
8	36	■		■			■	■							■					■			■
9	38						■	■								■		■		■			
10	39		■	■			■	■		■					■			■		■			■
11	40	■	■				■	■		■								■		■			■
12	43	■					■	■		■								■		■			■
13	45	■					■	■	■				■					■		■			■
14	48		■	■	■	■	■	■	■	■	■		■					■	■				■
15	H34																						

[a] position of the nucleotide in the sequence. [b] nucleotidechange. The corresponding mutation is indicated by a dark square.

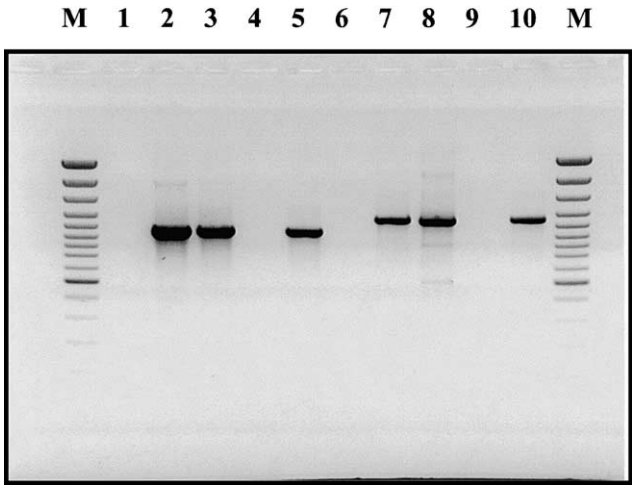

Fig. 3. Expression of *M. hominis ftsZ* detected by RT-PCR on total RNA samples. Lines 1–5, amplification of the 16S rRNA locus; lines 6–10, amplification of the *ftsZ* region. 1 and 6—negative control; 2 and 7—random-primed cDNA; 3 and 8—cDNA primed with HR for the 16S rRNA gene and PR23 for the *ftsZ* gene, respectively; 4 and 9—test for DNA contamination of cDNA; 5 and 10—*M. hominis* DNA (positive control). Lines M—size DNA markers.

Complementation analysis

Two *E. coli* strains defective in FtsZ, PAT84, and RP61 were used in transformation experiments in our attempts to understand whether filamentation results from the *ftsZ M. hominis* overexpression in *E. coli* cells or there is a complementation process between *E. coli* FtsZ and *M. hominis* FtsZ. In control experiments (without transformation), it was shown that normal septation process of PAT84 and RP61 took place at 30 °C, whereas at 42 °C, only long filaments were produced (Fig. 5C and D).

After transformation with recombinant plasmids pUC18ftsZ and pET15bftsZ, *E. coli* cells PAT84 ($ftsZ^{ts}$), RP61 ($ftsZ^{ts}$), and BL21(DE3) (control, $ftsZ^{+}$) were placed on LB-agar and incubated both at 30 and 42 °C. The grown cells from the colonies were cultivated on the liquid LB medium, supplemented with 100 mg/ml ampicillin and 1 mM IPTG, and their morphology was evaluated by light microscopy.

In the control experiment, 100% of the transformed colonies (strains BL21(DE3)/pET15bftsZ) tested at 37 °C under induction conditions was completely involved in filamentation (Fig. 5B). But in the case of BL21(DE3)/pUC18ftsZ, at 42 °C, where expression of the protein should be lower, only 5% of the cell material were filaments.

Two cell morphologies were revealed among transformed PAT84 and RP61 *E. coli* cells, both with pUC18ftsZ and pET15bftsZ: small colonies on plates, slow-growing cells in liquid medium, and large colonies, fast-growing cells on liquid medium. Thus, two effects of the *M. hominis ftsZ* gene expression in *E. coli* were

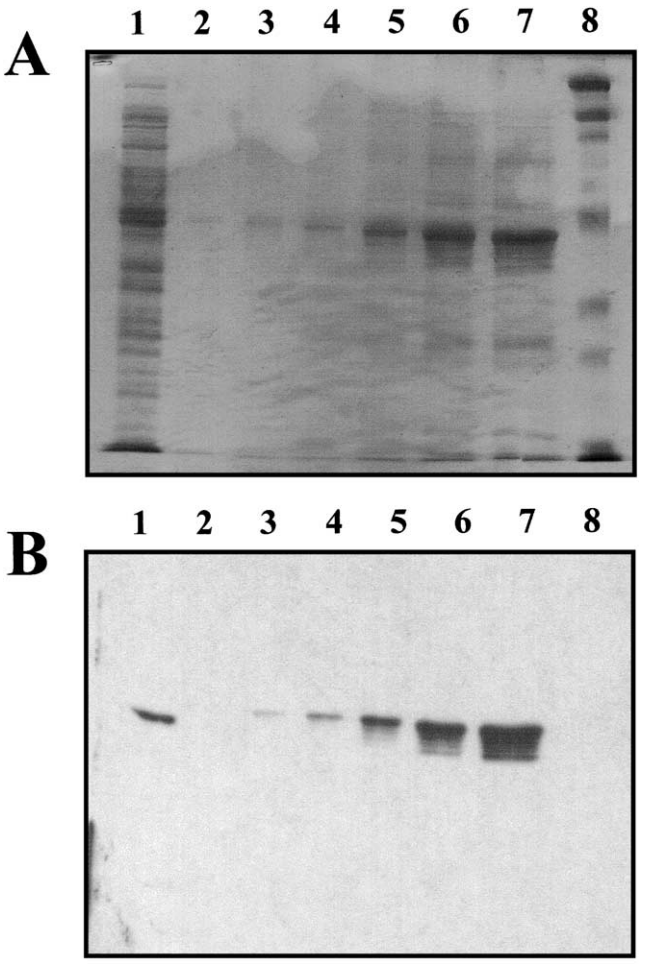

Fig. 4. Expression of the *M. hominis* FtsZ in *E. coli*. SDS–PAGE electrophoresis (A) and Western blot (B) of the *M. hominis* FtsZ. Line 1—(100 μg of protein) B834(DE3)/pET15bftsZ *E. coli* total cell lysate, lines 2–7—0.5, 1, 2, 5, 10, and 15 μg of the purified His-tagged FtsZ protein, line 8—molecular weight markers.

recorded (Fig. 5E–H). Fast-growing colonies of transformed *E. coli*, both PAT84 and RP61 strains, when at 30 °C, had normal phenotype, whereas at 42 °C, some filamentation takes place, specifically, in the case of RP61/pUC18ftsZ, up to 100% of cell material. However, the RP61/pUC18ftsZ cell at 42 °C consists of short filaments (Fig. 5F), two to three cell-length units, comparatively with the BL21(DE3)/pET15bftsZ long filaments at 42 °C (Fig. 5B) (also up to 100% of the material). Analysis of the slow-growing colonies revealed complementation between FtsZ proteins of *M. hominis* and *E. coli*. Cellular phenotype in the cases of RP61/pET15bftsZ and PAT84/pET15bftsZ was practically the same at 42 °C and at 30 °C: only 5–10% of the material was involved in filamentation, these filaments being of the small type (Fig. 5G and H).

These results suggest that formation of small filaments indicates the complementation process between FtsZ proteins of *E. coli* and *M. hominis*, while the

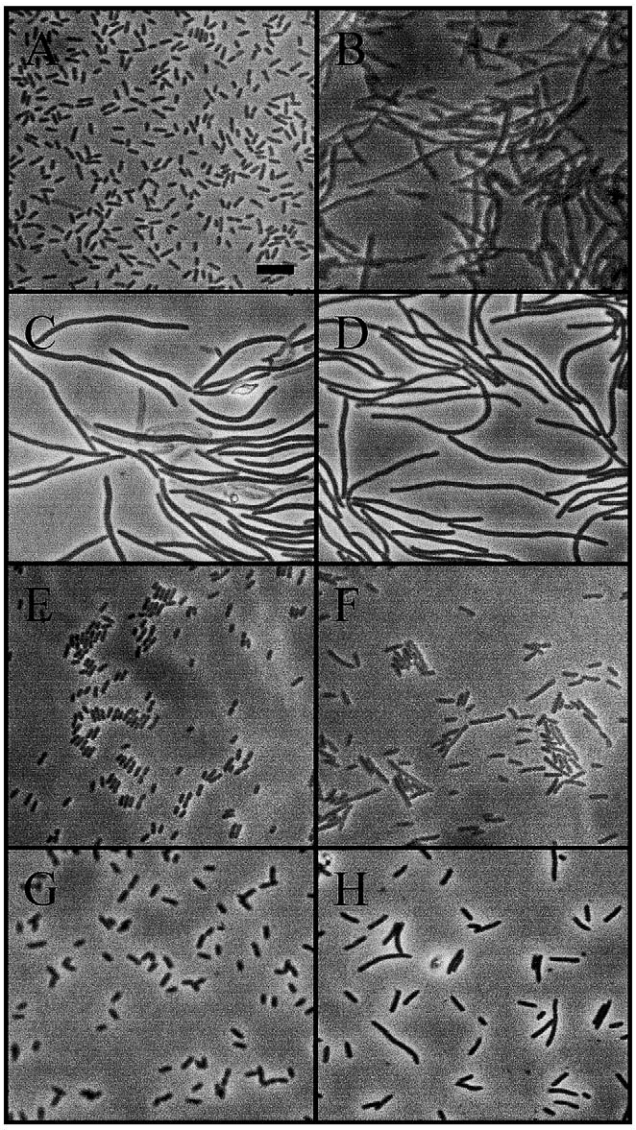

Fig. 5. Effects of overproduction of the *M. hominis* FtsZ in *E. coli* cells and complementation analysis between the *M. hominis* FtsZ and *E. coli* FtsZ. Cells from a culture of *E. coli* BL21(DE3)/pET15bftsZ growing exponentially at 37 °C in LB medium supplemented with 100 μg/ml of ampicillin, without IPTG (A) or with 1 mM IPTG (B), and from a culture of PAT84 (C) and RP61(D) *E. coli* growing in LB medium at 42 °C were placed on an agarose-covered slide and photographed. *E. coli* RP61 (*ftsZ*^{ts}) (E and F) and PAT84 (*ftsZ*^{ts}) (G and H) strains were transformed with the plasmids carrying the *M. hominis ftsZ* gene, pUC18ftsZ (E and F) or pET15bftsZ (G and H). Selected clones of the transformant strains were grown at 30 °C (E and G) and at 42 °C (F and H) in LB medium supplemented with 100 μg/ml of ampicillin and with 1 mM IPTG, placed on agarose-covered slides and photographed. Scale bar corresponds to 5 μm.

appearance of long type filaments indicates the effect of FtsZ protein overproduction.

Gene ftsZ structure and fine-scale phylogenetic analysis

The important role of 16S rDNA data for phylogenetic analysis [2] does not exclude usefulness of other nucleotide sequences for the same purpose. These are particularly 16S–23S rRNA intergenic spacer sequences, genes of ribosomal proteins [32], *tuf* gene of EF-Tu elongation factor [33], *gltA* gene of citrate synthase [34], and *ftsZ* [28]. The inter-strain differences in *ftsZ* gene structures were found in comparing two strains of *M. fermentans* [35]. Bacterial cell-cycle *ftsZ* is considered to be a rapidly evolving gene. Studies of bacterium *Wolbachia* sp. (*Rickettsia*, reproductive parasite of *Arthropodes*) also based on the *ftsZ* sequence revealed 38 new strains of this bacterium, and a new, more detailed phylogenetic tree of the *Wolbachia* was constructed [36]. We have found that in the structure of *M. hominis* FtsZ, like in the structure of *A. laidlawii* FtsZ, there are both some conservative and some evolutionary variable regions. Mutations in a variable region probably do not affect polypeptide conformation and function of the protein. Gene *ftsZ* is widely spread, functionally important, and sufficiently extended: this gene was found in all bacterial groups, and it encodes the indispensable protein. Therefore, it fits the basic criteria for the sequences useful for phylogenetic purposes [2]. The comparison of deduced FtsZ protein structures can be used as a sensitive approach for investigation of short-range bacterial evolution relationships.

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