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ORIGINAL PAPER

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Gene cloning, expression and partial characterization of cell division protein FtsZ1 from extremely halophilic archaeon *Haloarcula japonica* strain TR-1

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Abstract The gene encoding a cell division protein FtsZ1 was cloned from an extremely halophilic archaeon, *Haloarcula japonica* strain TR-1. Nucleotide sequencing analysis of the *ftsZ1* gene revealed that the structural gene consisted of an open reading frame of 1,158 nucleotides encoding 386 amino acids. Transcription of the *ftsZ1* gene in *Ha. japonica* was confirmed by RT-PCR. A modified *ftsZ1* gene was inserted into the shuttle vector pWL102 and used to transform *Ha. japonica*. The recombinant FtsZ1 was produced as a fusion with hexahistidine-tag in *Ha. japonica* host cells and purified. Purified recombinant FtsZ1 exhibited GTP-dependent polymerization activity and GTP-hydrolyzing activity in the presence of high concentrations of KCl.

Keywords FtsZ1 · Extremely halophilic archaeon · *Haloarcula japonica* · Gene cloning · Gene expression · Polymerization · GTPase

Introduction

FtsZ is a key protein in the cell division processes of *Escherichia coli* and other bacteria (Erickson 1997; Lutkenhaus and Addinall 1997; Margolin 2000; Addinall and Holland 2002). It self-assembles to form a circumferential ring at the inner face of cytoplasmic membrane at the division site. During cell cycle, the ring designated as FtsZ ring directs a process of septation. As cell division proceeds, the FtsZ ring decreases in diameter at the leading edge of the invanigating septum that eventually separates the two daughter cells (Bi and Lutkenhaus 1991). FtsZ is thought to be the prokaryotic homolog of eukaryotic tubulins based on its biochemical properties. Like tubulins, the *E. coli* FtsZ is a GTP/GDP-binding protein with GTPase activity and polymerization activity which forms filaments in the presence of GTP (de Boer et al. 1992; RayChaudhuri and Park 1992; Mukherjee et al. 1993; Bramhill and Thompson 1994; Mukherjee and Lutkenhaus 1994).

The genes encoding FtsZs, *ftsZ* genes, are found not only in bacteria but also in archaea. Several *ftsZ* genes from archaea *Halobacterium salinarum* (Margolin et al. 1996), *Haloferax mediterranei* (Poplawski et al. 2000), *Haloferax volcanii* (Wang and Lutkenhaus 1996), *Pyrococcus woe sei* (Baumann and Jackson 1996), *Thermococcus kodakaraensis* (Nagahisa et al. 2000) and *Thermoplasma acidophilum* (Yaoi et al. 2000) have been cloned. Recent genome analyses indicated that most euryarchaea had multiple *ftsZ* paralogs and they almost commonly possessed *ftsZ1*. In extremely halophilic archaea, *Halobacterium* sp. strain NRC-1 and *Hb. salinarum* have been shown to have five *ftsZ* paralogs (Ng et al. 2000; http://www.halolex.mpg.de/). Currently, complete genome sequence of *Haloarcula marismortui* was reported, and *Ha. marismortui* had also five *ftsZ* paralogs (Baliga et al. 2004). The *ftsZ1* genes of *Hf. volcanii* (Wang and Lutkenhaus 1996), *Methanococcus jannashii* (Díaz et al. 2001; Andreu et al. 2002; Oliva et al. 2003; Huecas and Andreu 2003; Huecas and Andreu 2004) and *P. woesei* (Baumann and Jackson 1996), as well as the *ftsZ* gene of *T. kodakaraensis* (Nagahisa et al. 2000) were expressed in *E. coli*. Recombinant proteins were purified and some properties, such as GTP-binding, GTPase and/or polymerization activities, were discussed. The three-dimensional structure of *M. jannaschii* FtsZ1 (Löwe and Amos 1998), as well as that of a bacterial FtsZ from *Mycobacterium tuberculosis* (Leung et al. 2004), is highly similar to those of eukaryotic α- and β-tubulins (Nogales et al. 1998). However, archaeal cell division mechanisms, and phys-

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iological roles of archaeal FtsZ1s and other FtsZ paralogs remain unsolved.

Haloarcula japonica strain TR-1 is a predominantly triangular disk-shaped halophilic archaeon (Horikoshi et al. 1993). The course of *Ha. japonica* cell division was analyzed by time-lapse microscopic cinematography. The *Ha. japonica* cells have been shown to divide asymmetrically (Hamamoto et al. 1988), however, the cell division process remains unclear.

In this paper, the *Ha. japonica ftsZ1* gene which encoding FtsZ1 was cloned and sequenced. Transcriptional analysis of the *Ha. japonica* genomic *ftsZ1* gene was also investigated. Furthermore, the *C*-terminally hexahistidine-tagged FtsZ1 was produced in *Ha. japonica* and characterized.

Materials and methods

Plasmids, strains and media

Plasmids pUC119 and pBluescript II KS(+) were obtained from Takara Shuzo (Kyoto, Japan) and Stratagene (La Jolla, CA, USA), respectively. Plasmid pWL102 is an *E. coli*-haloarchaea shuttle vector (Lam and Doolittle 1989; a gift from W. F. Doolittle of Dalhousie University, Halifax, Canada). *Halobacterium salinarum* strain R1 (a gift from W. F. Doolittle) and *Ha. japonica* strain TR-1 (ATCC 49778 and JCM 7785) were grown at 37°C in liquid media as described previously (Nishiyama et al. 1995). *Escherichia coli* strains DH5α and JM109 were used as the hosts for gene cloning. *E. coli* strain JM110 (Yanisch-Perron et al. 1985) was a *dam* and *dcm* strain and used for preparation of unmethylated plasmids. All *E. coli* strains were cultured at 37°C in L broth containing 50 μg/ml Ampicillin (Sambrook et al. 1989).

Isolation of genomic DNA and total RNA from haloarchaea

Halobacterium salinarum and *Ha. japonica* genomic DNA were isolated according to the method described Ng et al. (1995) with minor modifications. Total RNA of *Ha. japonica* was prepared using Sepasol RNA I (Nacalai Tesque, Kyoto, Japan) according to the manufacture's instructions. The resulting total RNA was further treated with DNase I (Amersham, Uppsala, Sweden) to remove trace amounts of contaminating genomic DNA.

Polymerase chain reaction and reverse transcription-PCR

Oligonucleotide primers for PCR and RT-PCR were purchased from Bex (Tokyo, Japan). The oligonucleotide primers used in this study are shown in Table 1. PCR was carried out using KOD –Plus– (Toyobo, Osaka, Japan) according to the manufacture's instructions. One-step RT-PCR was performed under the following conditions. Total RNA (0.1 μg) was reverse transcribed at 60°C for 30 min in 50 μl of the reaction buffer containing 20 pmol of each primer, 0.3 mM of each deoxynucleotide triphosphate, 2.5 mM manganese (II) acetate, 10% dimethyl sulfoxide (DMSO), 20 U RNase inhibitor (Toyobo), and 5 U rTth DNA Polymerase (Toyobo). The generated cDNA was then amplified by 40 cycles of PCR. The temperature profile for amplification was as follows: denaturation for 1 min at 94°C and annealing and extension for 1.5 min at 65°C.

Table 1 Oligonucleotide primers used in this study

Name	Sequence
K5	5′-ATTACCGTGGTTGGGTGTGGC-3′
K6	5′-CTCCTCGATGCTCATGCCC-3′
HZ10	5′-ATCCAGCAGTCCATCGACGGCTCG-3′
HZ11	5′-CTTCTCCTGTTCGGCCTCGCTCTG-3′
HZ12	5′-GTCGATGCCGCTGCAGGACGCGTTCAAG-3′
HZ13	5′-GACGACGAGGCGGACTAGTGGTGGTGGTG-GTGGTGGTCGACGTAGTC-3′
HZ14	5′-GACTACGTCGACCACCACCACCACCACCAC-TAGTCCGCCTCGTCGTC-3′
HZ15	5′-GGTACCGCCGATACCACCGCCTGCGTTG-3′

Recombinant DNA techniques

Restriction enzymes, DNA ligation kit, bacterial alkaline phosphatase and T4 polynucleotide kinase were purchased from Toyobo. Southern hybridization and colony hybridization were performed according to the standard protocols (Sambrook et al. 1989) using a DIG DNA Labeling and Detection Kit (Roche, Mannheim, Germany). DNA sequencing was carried out by the dideoxy chain termination method of Sanger et al. (1977) with a Shimadzu (Kyoto, Japan) model DSQ-2000L sequencer and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Transformation of *E. coli* and *Ha. japonica*

Transformation of *E. coli* was performed by electroporation (MicroPulser; Bio-Rad, Hercules, CA, USA). An expression plasmid was passaged through *E. coli* strain JM110 to avoid a haloarchaeal restriction barrier (Holmes et al. 1991). Transformation of *Ha. japonica* was performed using the polyethylene glycol method (Cline et al. 1989; Dyall-Smith 2004) with some modifications. Transformants were selected on agar plates containing 8 μg/ml Plavastatin (a gift from Sankyo Co., Ltd., Tokyo, Japan) instead of Mevinolin.

DNA sequence accession number

The DNA sequence data reported in this article will appear in the DNA Data Bank of Japan (DDBJ),

European Molecular Biology Laboratory (EMBL), and GeneBank nucleotide sequence databases under the accession number AB196145.

SDS-PAGE and Western blotting analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done following the method of Laemmli (1970) using 10% gels. SDS-PAGE Standards (Low; Bio-Rad) was used as molecular mass markers. After electrophoresis, proteins in the gel were stained with Coomassie brilliant blue (CBB) R-250.

Western blotting analysis was carried out according to the standard protocols (Sambrook et al. 1989) using a nitrocellulose filter (Trans-Blot Transfer Medium; Bio-Rad). Recombinant FtsZ1 blotted on the filter was reacted with a mouse monoclonal antibody raised against hexahistidine (Genzyme Tecne, Minneapolis, MI, USA) as the primary antibody and anti-mouse immunoglobulin G (IgG) horse radish peroxidase (HRP) conjugate (Bio-Rad) as the secondary antibody. Protein A-HRP conjugate (Bio-Rad) was also used to enhance intensity of the assay. Immuncomplexes were detected using an ECL Western Blotting Detection System (Amersham) with an ECL Mini-Camera (Amersham).

Purification of the *C*-terminally hexahistidine-tagged recombinant FtsZ1

Haloarcula japonica transformant containing an expression plasmid was grown to a late exponential phase. Cells were collected and disrupted by sonication in 20 mM Tris–HCl buffer (pH 7.5) containing 2.6 M NaCl, 2 mM EDTA and 2 mM phenylmethylsulfonyl fluoride (PMSF). The extracts were centrifugated at 5,000×*g* for 10 min to remove unbroken cells, and then centrifugated at 100,000×*g* for 1.5 h to remove cell envelope components. The obtained supernatant (cytoplasmic fraction) was dialyzed against 20 mM K_2HPO_4–KH_2PO_4 buffer (pH 7.0) containing 2.6 M NaCl and 10 mM imidazole, and loaded onto a Ni^{2+}-immobilized HiTrap Chelating HP (Amersham) column equilibrated with the same buffer. The absorbed proteins were eluted using a linear gradient from 10 mM to 500 mM imidazole in the same buffer. The recombinant FtsZ1-containing fractions were collected and dialyzed against 20 mM Tris–HCl buffer (pH 7.5) containing 2.6 M NaCl, and stored at 4°C. Protein concentrations were estimated by the Lowry method (DC Protein Assay; Bio-Rad) with bovine serum albumin as a standard.

N-terminal amino acid sequencing

The purified recombinant FtsZ1 was separated by SDS-PAGE and subsequently transferred onto a polyvinylidene difluoride (PVDF) membrane (Sequi-Blot PVDF Membrane; Bio-Rad). The recombinant FtsZ1 blotted on the membrane was stained with CBB G-250. A protein band of the recombinant FtsZ1 was cut off, and then subjected to an automated Edman-type protein sequencer Procise 494HT (Applied Biosystems).

Analytical gel filtration

The purified recombinant FtsZ1, 100 μg of protein in 100 μl, was applied to a Superose 12 HR 10/30 (Amersham) column equilibrated with 20 mM Tris–HCl buffer (pH 7.5) containing 2.6 M NaCl. The molecular mass of recombinant FtsZ1 was estimated by comparison with protein size standards.

Sedimentation assay for polymerization

The purified recombinant FtsZ1 (20 μg) was incubated at 37°C for 20 min in 100 mM MES-NaOH buffer (pH 6.5) containing 5 mM GTP, 3 M KCl and 2 mM $MgCl_2$. After centrifugation at 100,000× *g* and 37°C for 20 min, the supernatant and pellet were applied to SDS-PAGE followed by CBB staining to estimate the amount of insoluble FtsZ1 generated by polymerization.

GTPase assay

The purified recombinant FtsZ1 (100 μg) was incubated at 37°C for 10 min in 100 mM MES-NaOH buffer (pH 6.5) containing 2 mM GTP, 3 M KCl and 2 mM $MgCl_2$. Inorganic phosphate in the reaction mixture was assayed by the Malachite green method (Kodama et al. 1986) to estimate the GTPase activity.

Results

Cloning of the *Ha. japonica ftsZ1* gene

In order to clone the *Ha. japonica ftsZ1* gene, we used the previously reported *Hb. salinarum ftsZ1* gene (http://www.halolex.mpg.de/) as a probe. A part of *Hb. salinarum ftsZ1* gene was amplified by PCR using the K5 and K6 primers. Genomic DNA of *Ha. japonica* was digested with various combinations of restriction enzymes, and analyzed by Southern hybridization using the PCR product as a probe. Genomic DNA digested with *Sac*I- *Sma*I showed a single hybridization band at about 4.4 kb. DNA fragments around 4.4 kb were isolated from *Sac*I- *Sma*I digested genomic DNA, ligated to the *Sac*I- *Sma*I site of pUC119, and then introduced into *E. coli* strain DH5α. Transformants were screened by colony hybridization with the same probe. Several positive clones were obtained and found to contain recombinant plasmids with an identical 4.4 kb *Sac*I- *Sma*I genomic insert. One such plasmid was designated pJFZ9.

DNA sequence of the *Ha. japonica ftsZ1* gene

Nucleotide sequencing analysis of the 4.4 kb *Sac*I-*Sma*I fragment revealed that the *Ha. japonica ftsZ1* gene was found to be included in this fragment. The putative *Ha. japonica ftsZ1* gene contained an open reading frame (ORF) of 1,158 nucleotides encoding 386 amino acids, although there were two possible in-frame initiation codons downstream of the predicted one. Molecular mass of the *Ha. japonica* FtsZ1 was calculated to be 40,484. Deduced amino acid sequence of the *Ha. japonica* FtsZ1 was compared to other archaeal FtsZ1 orthologs and *E. coli* FtsZ (Fig. 1). The *Ha. japonica* FtsZ1 indicated 68%, 68%, 54% and 33% identities to the *Hb. salinarum*, strain NRC-1, *M. jannaschii* FtsZ1 and the *E. coli* FtsZ, respectively. Furthermore, the *Ha. japonica* FtsZ1 had the same amino acid sequence as that deduced from the genome sequence of the *Ha. marismortui ftsZ1* gene except only one amino acid. A comparison with amino acid sequences between the *Ha. japonica* FtsZ1 and FtsZ paralogs of other archaea showed that these proteins had only limited sequence similarity to each other (data not shown). The glycine-rich sequence motif GGGTGTG (in single letter codes) implicated in GTP-binding (GTP-binding motif) was also conserved in the *Ha. japonica* FtsZ1. Like other haloarchaeal proteins, the *Ha. japonica* FtsZ1 was an acidic one and contained 19% of negatively charged amino acid residues. The multiple alignment also showed that the *Ha. japonica* FtsZ1 and other haloarchaeal FtsZ1s had an additional *N*-terminal domain of about 40 amino acids rich in negatively charged residues.

Transcription of the *Ha. japonica ftsZ1* gene

To determine whether the cloned *ftsZ1* gene is transcribed in *Ha. japonica*, RT-PCR was performed for the detection of the *ftsZ1* mRNA. The specific primers for RT-PCR, HZ10 and HZ11, were designed on the determined DNA sequence of the *Ha. japonica ftsZ1* gene. An expected RT-PCR product of about 700 bp was obtained on agarose gel electrophoresis (Fig. 2). It was confirmed to correspond to a part of the *ftsZ1* gene by DNA sequencing. No RT-PCR products were observed when the reverse transcription step was omitted from the temperature profile of RT-PCR, indicating that the observed product was not due to possible genomic DNA contamination in the total RNA preparation. This result confirmed that the *ftsZ1* gene was transcribed in *Ha. japonica*.

Fig. 1 Alignment of amino acid sequences of the *Ha. japonica* FtsZ1 and other FtsZs. Amino acid sequences are given in *single-letter codes*. *Asterisks* indicate positions of perfectly conserved residues. The GTP-binding motif is *boxed*

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Ha. japonica FtsZ1        1  MDSIIDDAIDEAEQDGEDAAGGTVDETTSTPSQDMSTSGTMSDEELASVVKDLETKITVVGCGGAGGNTVTRMMEEGIHGAKLVAANTDA   90
Ha. marismortui FtsZ1     1  MDSIIDDAIDEAEQDGEDAAGGTVDETTSTPSQDMSTSGTMSDEELASVVKDLETKITVVGCGGAGGNTVTRMMEEGIHGAKLVAANTDA   90
NRC-1 FtsZ1               1  MDSIVQDAIDEAE-ESEDSASEPAD--VAGGGGDTVPTGTMTDNELEDVLQELQTNITVVGCGGAGSNTVDRMATEGIHGADLVAANTDV   87
Hb. salinarum FtsZ1       1  MDSIVQDAIDEAE-ESEDSASEPAD--VAGGGGDTVPTGTMTDNELEDVLQELQTNITVVGCGGAGSNTVDRMATEGIHGADLVAANTDV   87
M. jannaschii FtsZ1       1                  MKFLKNVLEEGSKLEEFNELELSPEDKELLEYLQQTKAKITVVGCGGAGNNTITRLKMEGIEGAKTVAINTDA   73
E. coli FtsZ              1                                                MFEPMELTNDAVIKVIGVGGGGGNAVEHMVRERIEGVEFFAVNTDA   46
                                                                                  * * * ** * *       * * *    * ***

Ha. japonica FtsZ1       91  QHLADEVAADTKILIGRKRTGGRGAGSVPKIGEEAAQEDIEDIQQSIDGSDMVFVTAGLGGGTGTGAAPVVAQAAQEAGALTISIVTIPF  180
Ha. marismortui FtsZ1    91  QHLADEVAANTKILIGRKRTGGRGAGSVPKIGEEAAQEDIEDIQQSIDGSDMVFVTAGLGGGTGTGAAPVVAQAAQEAGALTISIVTIPF  180
NRC-1 FtsZ1              88  QHLVD-IEADTKILMGQQKTKGRGAGSLPQVGEEAAIESQGEIRDSIAGSDMVFVTAGLGGGTGTGSAPVVAKAAREQGALTIAIVTTPF  176
Hb. salinarum FtsZ1      88  QHLVD-IEADTKILMGQQKTKGRGAGSLPQVGEEAAIESQGEIRDSIAGSDMVFVTAGLGGGTGTGSAPVVAKAAREQGALTIAIVTTPF  176
M. jannaschii FtsZ1      74  QQLIR-TKADKKILIGKKLTRGLGAGGNPKIGEEAAKESAEEIKAAIQDSDMVFITCGLGGGTGTGSAPVVAEISKKIGALTVAVVTLPF  162
E. coli FtsZ             47  QALRK-TAVGQTIQIGSGITKGLGAGANPEVGRNAADEDRDALRAALEGADMVFIAAGMGGGTGTGAAPVVAEVAKDLGILTVAVVTKPF  135
                             * *         *  *   * * ***  *  *  ** *           ****   * ******* *****      * **   ** **

Ha. japonica FtsZ1      181  TAEGERRRANADAGLERLRSVSDTVIVVPNDRLLDYAPS-MPLQDAFKICDRVLMRSVKGMTELITKPGLVNVDFADVRTIMENGGVAMI  269
Ha. marismortui FtsZ1   181  TAEGERRRANADAGLERLRSVSDTVIVVPNDRLLDYAPS-MPLQDAFKICDRVLMRSVKGMTELITKPGLVNVDFADVRTIMENGGVAMI  269
NRC-1 FtsZ1             177  TAEGEVRRTNAEAGLERLRDVADTVIVVPNDRLLDSVGK-LPVREAFKVSDEVLMRSVKGITELITKPGLVNLDFADVRTVMEKGGVAMI  265
Hb. salinarum FtsZ1     177  TAEGEVRRTNAEAGLERLRDVADTVIVVPNDRLLDSVGK-LPVREAFKVSDEVLMRSVKGITELITKPGLVNLDFADVRTVMEKGGVAMI  265
M. jannaschii FtsZ1     163  VMEGKVRMKNAMEGLERLKQHTDTLVVIPNEKLFEIVPN-MPLKLAFKVADEVLINAVKGLVELITKDGLINVDFADVKAVMNNGGLAMI  251
E. coli FtsZ            136  NFEGKKRMAFAEQGITELSKHVDSLITIPNDKLLKVLGRGISLLDAFGAANDVLKGAVQGIAELITRPGLMNVDFADVRTVMSEMGYAMM  225
                               **  *   *  *   *    *     **  *            **     **   * *  ****  ** * *****   *   * **

Ha. japonica FtsZ1       270  GLGESDSENKAQDSIRSALRSPLL-DVEFDGANSALVNVVGGPDMSIEEAEGVVEEIYDRIDPDARIIWGASVNNEFEGKMETMIVVTGV  358
Ha. marismortui FtsZ1    270  GLGESDSENKAQDSIRSALRSPLL-DVEFDGANSALVNVVGGPDMSIEEAEGVVEEIYDRIDPDARIIWGASVNNEFEGKMETMIVVTGV  358
NRC-1 FtsZ1              266  GLGEADSDAKAADSVQSALRSPLL-DVDISSANSALVNVTGGPGMSIEEAEGVVEQLYDRIDPDARIIWGTSIDEQIQEEMRTMVVVTGV  354
Hb. salinarum FtsZ1      266  GLGEADSDAKAADSVQSALRSPLL-DVDISSANSALVNVTGGPGMSIEEAEGVVEQLYDRIDPDARIIWGTSIDEQIQEEMRTMVVVTGV  354
M. jannaschii FtsZ1      252  GIGESDSEKRAKEAVSMALNSPLL-DVDIDGATGALIHVMGPEDLTLEEAREVVATVSSRLDPNATIIWGATIDENLENTVRVLLVITGV  340
E. coli FtsZ             226  GSGVASGEDRAEEAAEMAISSPLLEDIDLSGARGVLVNITAGFDLRLDEFETVGNTIRAFASDNATVVIGTSLDPDMNDELRVTVVATGI  315
                              * *       *      *  **** *     *   *           *   *          *    *              * **

Ha. japonica FtsZ1       359  ESPQIYGQSEAEQEKAAQQLGEDIDYVD                                                        386
Ha. marismortui FtsZ1    359  ESPQIYGQSEAEQEKAAQQLGEDIDYVD                                                        386
NRC-1 FtsZ1              355  DSPQIYGRNEAAEGDGPAQESTPEPEPEPQAGSEIEDIDYVE                                          396
Hb. salinarum FtsZ1      355  DSPQIYGRNEAAEGDGPAQESTPEPEPEPQAGSEIEDIDYVE                                          396
M. jannaschii FtsZ1      341  QSRIEFTDTGLKRKKLELTGIPKI                                                            364
E. coli FtsZ             316  GMDKRPEITLVTNKQVQQPVMDRYQQHGMAPLTQEQKPVAKVVNDNAPQTAKEPDYLDIPAFLRKQAD                383
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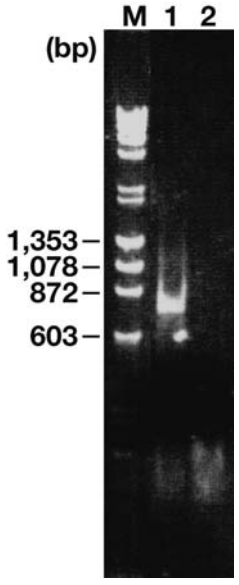

Fig. 2 Agarose gel electrophoresis of the RT-PCR product. *Lane 1* the RT-PCR product, *lane 2* the control reaction product with total RNA not subjected to reverse transcription. *M* indicates the *Hin*dIII digest of λDNA- *Hae*III digest of Φ X174 DNA markers

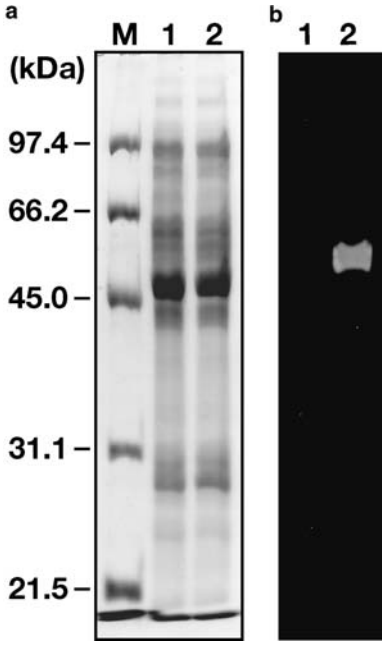

Fig. 3 SDS-PAGE of cell extracts of *Ha. japonica* transformants. After electrophoresis, proteins in the gel were stained with CBB R-250 (**a**), or subjected to Western blotting analysis using anti-hexahistidine antibody (**b**). *Lanes 1* the cell extract of *Ha. japonica* carrying pWL102, *lanes 2* the cell extract of *Ha. japonica* carrying pJFZ14. *M* indicates the molecular mass markers

Production of the *C*-terminally hexahistidine-tagged FtsZ1 in *Ha. japonica*

A modified *Ha. japonica ftsZ1* gene encoding the *C*-terminally hexahistidine-tagged FtsZ1 was generated by PCR using two sets of primers. A part of the *Ha. japonica ftsZ1* gene (0.5 kb) was amplified by PCR using the HZ12 and HZ13 primers, which contained *Pst*I and *Spe*I sites, respectively. The 3′ non-coding region of the *Ha. japonica ftsZ1* gene (0.4 kb) was amplified by PCR using the HZ14 and HZ15 primers, which contained *Spe*I and *Kpn*I sites, respectively. The two PCR fragments were subcloned into pBluescript II KS(+) digested with *Sma*I. A 0.9-kb *Pst*I- *Hin*dIII fragment, obtained by recombining the 0.5-kb *Pst*I- *Spe*I and the 0.4-kb *Spe*I- *Hin*dIII fragment [*Hin*dIII site was derived from the multiple cloning sites of pBluescript II KS(+)] at a common *Spe*I site, was replaced by the *Pst*I- *Hin*dIII fragment of pJFZ9 to construct pJFZ13. In this construct, six histidine codons [$(CAC)_6$] were inserted just upstream of the stop codon of the *Ha. japonica ftsZ1* structural gene. Finally, a 2.8-kb *Eco*RI- *Eco*RI fragment of pJFZ13 was ligated into the *Eco*RI site of shuttle vector pWL102 to obtain a novel haloarchaeal expression, plasmid pJFZ14.

The plasmid pJFZ14 was introduced into *Ha. japonica*. Cell extract of the successful transformant was separated by SDS-PAGE and then applied to Western blotting analysis using anti-hexahistidine tag antibody. By immunological detection of the hexahistidine-tagged recombinant FtsZ1, a positive band at about 55 kDa was revealed (Fig. 3). This result showed that the recombinant FtsZ1 was produced by *Ha. japonica* carrying pJFZ14.

Purification of the recombinant FtsZ1

The recombinant FtsZ1 was purified from the cytoplasmic fraction of *Ha. japonica* carrying pJFZ14 using a Ni^{2+}-chelating column. The purified recombinant FtsZ1 showed a single protein band at about 55 kDa on SDS-PAGE (Fig. 4). About 10 mg of the purified recombinant FtsZ1 was obtained from 16 g (wet-weight) of transformant cells. The purified recombinant FtsZ1 was separated by SDS-PAGE, transferred to PVDF membrane, and then subjected to the protein sequencer. The partial *N*-terminal amino acid sequence of recombinant FtsZ1 was determined to be: H_2N–MDSIIDDAIDEA–. The methionine residue was detected at the *N*-terminus, and the *N*-terminal sequence was identical to that anticipated from the DNA sequence of *Ha. japonica ftsZ1* gene. The molecular mass of recombinant FtsZ1 was determined to be 50 kDa by gel filtration (data not shown).

Polymerization activity and GTPase activity of the recombinant FtsZ1

The sedimentation assay for polymerization activity was carried out as described in the materials and methods. The recombinant FtsZ1 exhibited polymerization activity only in the presence of GTP (Fig. 5). It has no activity in the absence of GTP or in the presence of GDP and GMP instead of GTP. Polymerization activity of the

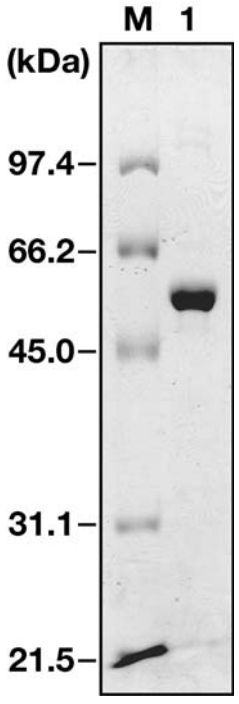

Fig. 4 SDS-PAGE of the purified recombinant FtsZ1. After electrophoresis, proteins in the gel were stained with CBB R-250. *Lane 1* the purified recombinant FtsZ1 (5 μg). *M* indicates the molecular mass markers

recombinant FtsZ1 was also assessed at various concentrations of KCl or NaCl (Fig. 6). Polymerization of the recombinant FtsZ1 was observed at KCl concentrations of 2.25–3.00 M. However, the recombinant FtsZ1 showed no polymerization activity at KCl concentrations below 2.00 M. Furthermore, the FtsZ1 polymer was not detected using the reaction mixtures containing several concentrations of NaCl instead of KCl. These results indicated that polymerization activity of the recombinant FtsZ1 required high concentrations of KCl.

GTPase activity for the recombinant FtsZ1 was determined by the Malachite green method. The effects of KCl and NaCl concentrations on GTPase activity of the recombinant FtsZ1 was shown in Fig. 7. The GTPase activity was detected at high KCl concentrations. The maximum activity was found in the presence of 3.00 M KCl, whereas only 20% of the maximum

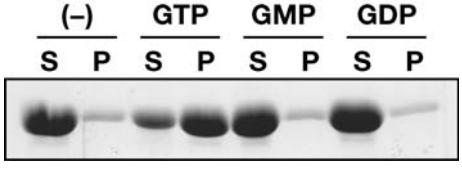

Fig. 5 Effect of nucleotides on polymerization of the recombinant FtsZ1. Sedimentation assay was performed in the presence of GTP, GDP and GMP. (–) indicates no addition of nucleotides. *S* supernatant, *P* pellet

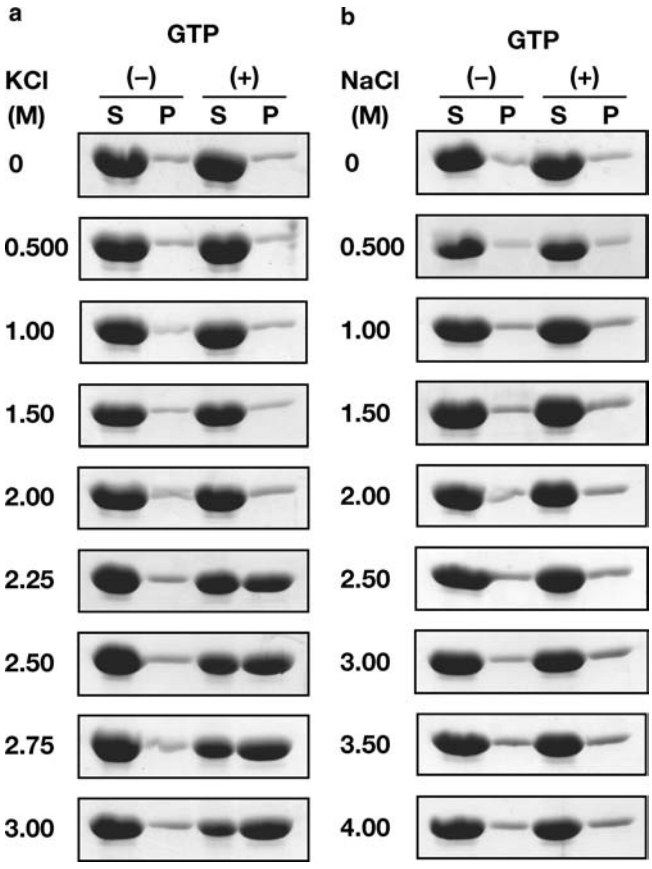

Fig. 6 Effects of KCl (**a**) and NaCl (**b**) concentrations on polymerization of the recombinant FtsZ1. Sedimentation assay was performed in the presence (+) or absence (-) of GTP. *S* supernatant, *P* pellet

activity was observed at lower KCl concentrations. In the presence of 0.5–4.0 M NaCl, little GTPase activity (less than 20% of the activity detected in the presence of

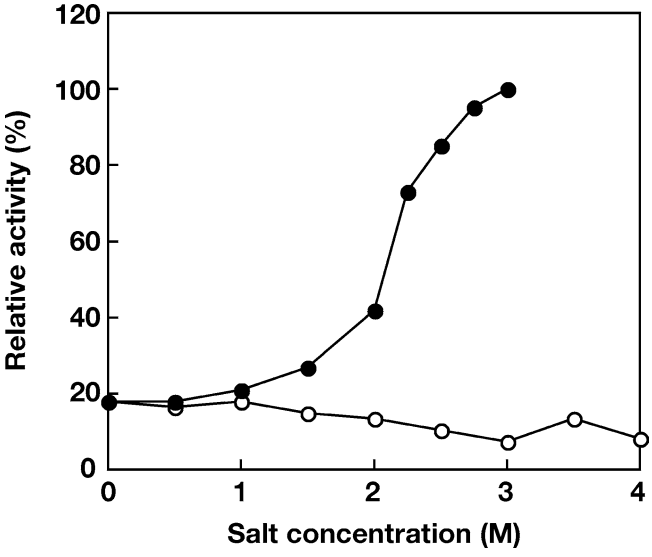

Fig. 7 Effects of KCl (*filled circle*) and NaCl (*open circle*) concentrations on GTPase activity of the recombinant FtsZ1

3.0 M KCl) was observed. Similar to polymerization activity, GTPase activity of the recombinant FtsZ1 also required high concentrations of KCl.

Discussion

Recent complete genome analyses revealed that most euryarchaea had multiple *ftsZ* paralogs, except *Picrophilus torridus* which possessed no *ftsZ* gene (Fütterer et al. 2004). In extremely halophilic archaea, *Ha. marismortui*, strain NRC-1 and *Hb. salinarum* had five *ftsZ* paralogs (Baliga et al. 2004; Ng et al. 2000; http://www.halolex.mpg.de/). However, biochemical properties and physiological roles of archaeal FtsZs remain unclear (Bernander 2003).

We cloned the *ftsZ1* gene from *Ha. japonica* using the *Hb. salinarum ftsZ1* gene as a probe. As shown in Fig. 1, deduced amino acid sequence of the *Ha. japonica* FtsZ1 was closely identical to those of other archaeal FtsZ1s. The glycine-rich GTP-binding motif was completely conserved. The *Ha. japonica ftsZ1* gene was not a pseudo gene and transcription of the cloned *ftsZ1* gene in *Ha. japonica* was confirmed by RT-PCR.

To characterize the *Ha. japonica* FtsZ1 in vitro, production of the *C*-terminally hexahistidine-tagged *Ha. japonica* FtsZ1 was examined. The modified *Ha. japonica ftsZ1* gene was successfully expressed in the *Ha. japonica* host to produce recombinant FtsZ1 as a fusion with hexahistidine-tag in the cytoplasm. The recombinant FtsZ1 could be purified using a Ni^{2+}-chelating column under the physiological hypersaline conditions. The *N*-terminal amino acid sequence of the purified recombinant FtsZ1 coincided with the deduced amino acid sequence, suggesting that the initiation codon of *Ha. japonica ftsZ1* gene should to be the predicted one. The *Ha. japonica* FtsZ1 and other haloarchaeal FtsZ1s had an additional *N*-terminal domain rich in acidic amino acids, which was not present in *M. jannaschii* FtsZ1 and *E. coli* FtsZ. To maintain the biological function under hypersaline conditions, haloarchaeal proteins have to compete with the excess inorganic ions for water binding. The *N*-terminal domain conserved in the haloarchaeal FtsZ1s may provide the means for larger solvent-accessible surface areas, and thus enable efficient water binding in their hypersaline biological niches. In support of this notion, additional domains rich in acidic amino acids have been observed in other haloarchaeal proteins (Frolow et al. 1996; Matsuo et al. 2001). It has been found that aspartic and glutamic acids are good water binders (Frolow et al. 1996). The molecular mass of purified recombinant FtsZ1, as determined by gel filtration, agreed closely with the estimated value by SDS-PAGE. These results indicated that the recombinant FtsZ1 was purified as a monomeric form.

The *Ha. japonica* FtsZ1 exhibited in vitro polymerization and GTPase activities. Both activities required high concentrations of KCl. The dependence of KCl concentration on GTPase activity of the *Ha. japonica* FtsZ1 was comparable to that of the *Hf. volcanii* FtsZ (Wang and Lutkenhaus 1996), although in vitro polymerization activity of the *Hf. volcanii* FtsZ had never been described. Extremely halophilic archaea grow under high NaCl conditions. They are known to contain high concentrations of K^+ (about 3 M), instead of Na^+, in their cytoplasm (Lanyi 1974). Therefore, haloarchaeal proteins located in the cytoplasm are adapted to the high concentrations of intracellular KCl. Thus, the *Ha. japonica* FtsZ1, one of haloarchaeal cytoplasmic proteins, was also stable and active under high KCl conditions. It is possible that the *Ha. japonica* FtsZ1 is a component of the FtsZ ring during the *Ha. japonica* cell division.

In conclusion, we have cloned and expressed the *Ha. japonica ftsZ1* gene and characterized some properties of the *Ha. japonica* FtsZ1. This is the first report about in vitro polymerization of a haloarchaeal FtsZ. We are now in the process of disrupting the *Ha. japonica ftsZ1* gene to clarify the physiological role of FtsZ1. Furthermore, we are trying to characterize other *ftsZ* paralogs from *Ha. japonica*. To date, the *ftsZ2* paralog has been cloned from *Ha. japonica* using the *Hb. salinarum ftsZ2* gene as a probe. Biochemical and genetic approaches of multiple *ftsZ* paralogs will lead to the understanding of the physiological role and the evolution of archaeal cell division apparatus.

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