Cloning and sequencing of the *ftsZ* gene from a deep-sea piezophilic bacterium, (*Shewanella violacea* strain DSS12)

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We have cloned and sequenced the *ftsZ* gene, encoding a protein essential for cell division, from a λ phage library of the chromosome of the deep-sea bacterium *Shewanella violacea* strain DSS12. This gene, 1,179 bp in length, was found to encode a protein consisting of 392 amino acid residues with a molecular mass of 40,727 Da. Significant homology was evident comparing the *ftsZ* gene of *S. violacea* with that of *Escherichia coli* (63% identity), *Pseudomonas putida* (51% identity), *Bacillus subtilis* (48% identity), and *Streptomyces coelicolor* (43% identity). Comparison of the amino acid sequences of the FtsZ proteins of *S. violacea* and *E. coli* revealed the presence of a GTPase domain in N-terminal portion and a specific conserved region in the C-terminal portion (extreme C-terminus) which is associated with other cell division proteins. A variable region between the GTPase domain and the C-terminal region was also observed. Phylogenetic analyses of 16S rDNAs and FtsZ proteins of several bacteria showed that *S. violacea* is closely related to *E. coli*.

Key Words : cell division, high pressure, *ftsZ*, *Shewanella violacea*, *Escherichia coli*

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1. Introduction

The moderately piezophilic and psychrophilic bacterium *Shewanella violacea* strain DSS12 from the Ryukyu Trench (depth 5,110 m)[1] grows optimally at 30 MPa and 8ºC, but also grows at atmospheric pressure (0.1 MPa) and 8ºC[2]. These growth properties are useful for comparative study of cell physiology under high and low pressure conditions. *S. violacea* shows good growth under high pressure conditions[1, 2], whereas *E. coli*, which is closely related to *S. violacea*, shows poor growth under the same conditions[3] . We are interested to further elucidate the differences in cell growth properties of these two species under high pressure conditions. When *E. coli* is cultured under high pressure conditions, it grows as a long filamentous form[4]. This phenomenon seems to resemble the observed morphological features of certain *E. coli* mutants, called *fts* mutants (the meaning of *fts* is filament forming temperature sensitive), which are defective in cell division at the non-permissive temperature. The defective gene in these mutants, designated the *fts* gene[5], is known to be important for cell division. The Fts proteins are assembled into the cell division apparatus at the cell division site[6, 7] and the assembly process is initiated by the FtsZ protein, which is a tubulin-like protein with GTPase activity, and it provides the cytoskeletal framework of a cytokinetic ring for membrane constriction in bacteria[8)–13]. Our recent observation of filamentous *E. coli* under high pressure conditions, by immunofluorescence microscopy using anti-FtsZ antibody, revealed that the FtsZ protein is not functional (data not shown). This suggests that the *fts*-like mutant phenotype is caused by inhibition of the function of FtsZ in *E. coli* under high pressure conditions. Thus, to understand cell division under high pressure conditions, characterization of the FtsZ protein both *in vivo* and *in vitro* is definitely required.

In this study, we isolated and cloned the *ftsZ* gene from a deep-sea piezophilic bacterium as the first step in studying the FtsZ protein. In this report, we describe the sequence of the FtsZ protein and the results of phylogenetic analyses based on comparison of the FtsZ protein and 16S rDNA of *S. violacea* with those of other bacteria.

2. Materials and Methods

2.1. Cloning of the *ftsZ* gene from *S. violacea*

Two degenerate oligonucleotides were designed and synthesized, based on highly conserved sequences, TKGLGAGAN and NVDFADVR, in FtsZ proteins. The nucleotide sequences of the oligonucleotides were 5'-CTIGGIGCIGGIGCIAAYCC-3' (primer 1) and 5'-CKIACRTCIGCRAARTCIAC-3' (primer 2). The PCR product obtained using *S. violacea* chromosomal DNA as the template and the above primers, was cloned into the pCR2.1 vector (Invitrogen Co., Carlsbad, CA) and the nucleotide sequence was determined by the dye terminator method using a DNA sequencer model 377 (Perkin-Elmer/Applied Biosystems Co., Foster, CA). Based on the determined nucleotide sequence, two other PCR primers were designed for use in amplification of the digoxigenin (DIG)-labeled *ftsZ* fragment. The DIG-labeled fragment was employed as the probe for plaque hybridization in screening by means of the DIG detection system (Boehringer Mannheim Co., Mannheim, Germany).

2.2. Construction of a λ phage library of the chromosome of *S. violacea* and screening of the library for the *ftsZ* gene region.

Chromosomal DNA isolated from *S. violacea* was partially digested with S*au*3AI. These fragments were inserted into the B*am*HI site of lambda DASH II (Stratagene Co., La Jolla, CA). Then, *in vitro* packaging of the ligated DNA was performed using GIGAPACK III XL packaging extracts (Stratagene Co.) according to the manufacturer's instructions. The λ phage library was screened for plaque hybridization with the *ftsZ* probe and several positive clones were obtained. The positive clones containing the *ftsZ* gene were each purified by several single plaque isolation steps. Each of the inserts in λ phage were amplified by long and accurate PCR and were subcloned into the pCR-Blunt vector (Invitrogen Co.). For sequencing of these cloned fragments, the random shotgun sequencing method was used with a DNA sequencer model 377 (Perkin-Elmer/Applied Biosystems Co.). Assembling and editing of the determined DNA sequences were performed with AutoAssembler Version 2.0 (Perkin-Elmer/Applied Biosystems Co.). GENETYX-MAC version 10.1 from Software Development (Tokyo, Japan) was used for sequence analysis.

2.3. Phylogenetic analysis of the *ftsZ* genes and 16S rDNAs

To determine the phylogenetic position of the *S.*

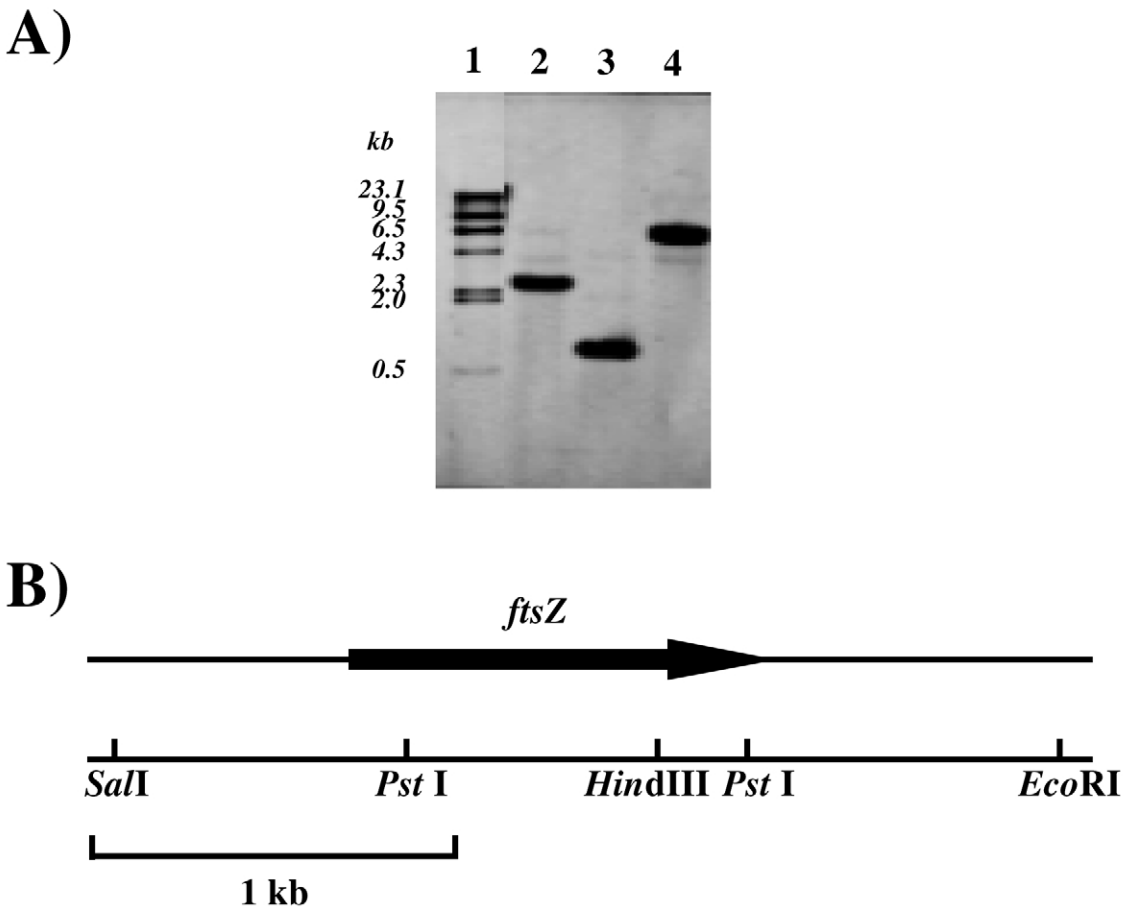

Fig.1 Southern blot analysis and restriction map of the *ftsZ* gene of *S. violacea*.
A) *S. violacea* chromosomal DNA was digested with *Hind* III, *Pst* I, and *Sal* I. A single major band was detected in each lane using the *S. violacea ftsZ*-specific probe. Lane 1 : λ *Hin*dII marker. Lane 2: *Hin*d III-digested DNA with a fragment approx. 2.5 kb in size showing hybridization with the probe. Lane 3 : *Pst* I-digested DNA with a fragment approx. 0.9 kb in size showing hybridization. Lane 4 : *Sal*I-digested DNA with a fragment approx. 6 kb in size showing hybridization. The sizes of the nucleotides are indicated in kb.
B) Restriction map of the *ftsZ* gene region. The *ftsZ* ORF is shown by an arrow.

violacea ftsZ gene, evolutionary distance was calculated and a phylogenetic tree was constructed. The evolutionary distance was based on comparison of 12 bacteria. The 16S rDNA sequences or the FtsZ amino acid sequences were aligned and the tree was constructed by the neighbor-joining method. The alignment and calculation were performed using CLASTALX and the PHYLP package (ver. 3.573; obtained from J. Gelsenstein, University of Washington, Seattle).

3. Results and Discussion

3.1. Isolation of the *ftsZ* gene

To clone a part of the *ftsZ* gene from *S. violacea*, the amino acid sequences of the FtsZ proteins from several bacteria were aligned and a highly conserved domain was found in the N-terminal portion (Fig. 3). These conserved sequences were used to design and synthesize degenerate oligonucleotides (primer 1 and primer 2) in order to amplify a part of the *ftsZ* gene from *S. violacea* (Fig. 2). A fragment containing part of the *ftsZ* gene, approximately 450 bp, amplified by PCR with the primers, was cloned into the pCR2.1 vector and its nucleotide sequence was determined. The deduced amino acid sequence of the cloned gene was significantly similar to the FtsZ of other bacteria. To clone the complete *ftsZ* gene, the partial *ftsZ* gene fragment was labeled with digoxigenin by PCR for use as a hybridization probe. Southern hybridization was carried out using this probe to confirm the restriction map of the *ftsZ* region. The *ftsZ* probe hybridized with chromosomal DNA digested with HindIII, *Pst*I

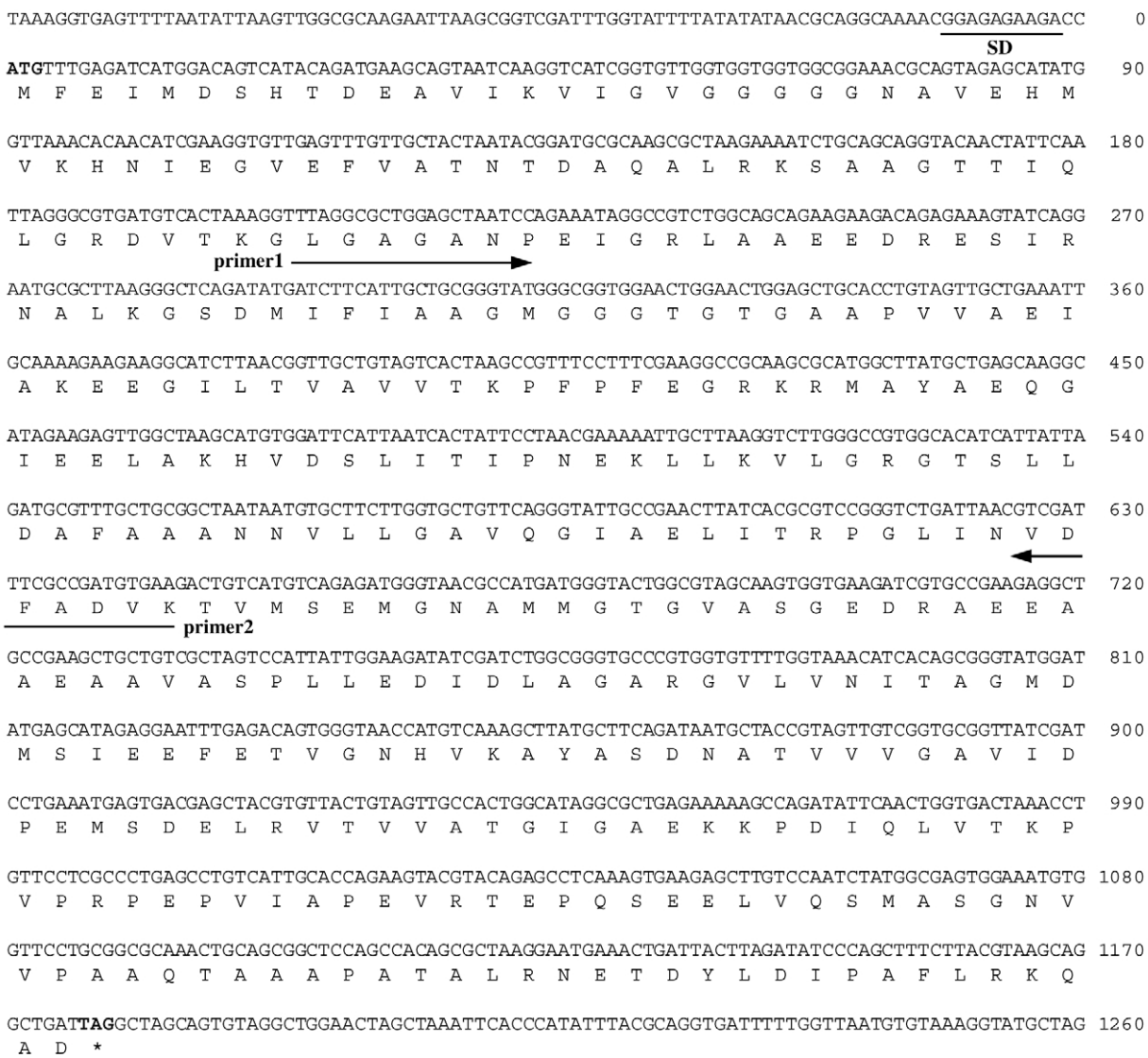

Fig.2 Nucleotide and amino acid sequences of the *S. violacea ftsZ* gene.
The deduced amino acid sequence is shown in single-letter code below the nucleotide sequence and the stop codon is shown with a asterisk. Primers 1 and 2 used for degenerate PCR amplification are indicated by arrows. A putative ribosome binding site (SD) is underlined.

and *Sal*I as shown in Fig. 1A, and a single major band was detected in each lane. Based on these results, several restriction sites in the *ftsZ* gene region were mapped (Fig. 1B).

3.2. Structural analysis of the complete *ftsZ* gene of *S. violacea*

A λ phage library of *S. violacea* chromosomal DNA was constructed and screened using the *ftsZ* probe. Several positive clones were isolated from among 10^4 plaques and purified by repeated single plaque isolation. These clones were confirmed to contain the *ftsZ* gene by PCR at each isolation step. As a result of the screening, we obtained five positive clones and the nucleotide sequence of one of these clones containing a DNA insert approximately 18 kb in size was determined by the random shotgun sequencing method. The open reading frame of the *ftsZ* gene, 1,179 bp in length, was found to encode a polypeptide consisting of 392 amino acid residues with a molecular mass of 40,727 Da. A possible ribosome binding site (SD), 5'-GGAGAGAAAGA-3', was found upstream of the start codon (Fig. 2). Restriction analysis showed that the *ftsZ* gene region was present within a *Pst*I fragment approximately 900 bp in size which hybridized with the *ftsZ* probe (Fig. 2B). Analysis of the deduced amino acid sequence confirmed that the cloned

Fig.3 Multi-alignment of FtsZ proteins from *Shewanella violacea* (*Sv*), *Escherichia coli* (*Ec*), *Pseudomonas putida* (*Pp*), *Bacillus subtilis* (*Bs*), and *Streptomyces coelicolor* (*Stc*). Identical residues are indicated by asterisks. A putative GTP binding region and a GTP-hydrolyzing region (underlined) are highly conserved.

gene contained the complete sequence of the FtsZ protein. It was found to be significantly similar to the FtsZ protein of *E. coli* (63 % identity), *P. putida* (51% identity), *B. subtilis* (48% identity), and *S. coelicolor* (43% identity) (Fig. 3). Comparison of the FtsZ of *S. violacea* with that of *E. coli* showed that the GTPase domain in the N-terminus was highly conserved; the GTP binding and GTP hydrolyzing motifs (GMGGGTGTGAAP[10)–12)] and INVFADV[14)], as shown in Fig. 3), were completely identical. The sequence at the C-terminus (DYLDIPAFLRKQAD as shown in Fig. 3), which is associated with other cell division proteins such as FtsA and ZipA[15), 16)], was also highly conserved (Fig. 3). These observations indicate that the FtsZ proteins of *S. violacea* and *E. coli* have the same functions, that is, GTPase activity and protein-protein interaction.

Under high pressure conditions, *S. violacea* grows without filamentation, but *E. coli* cells form filaments. It seems possible that the difference in morphology between these species may depend on the functions of the FtsZ protein under high pressure conditions. Comparing the FtsZ protein of *S. violacea* and that of *E. coli* it is evident that the amino acid sequence is highly conserved and thus the function of FtsZ in these species must be very similar. This suggests that the difference in FtsZ function under high pressure conditions is likely to be due to the variable region of the sequence. To understand the effect of pressure on cell division, characterization of the FtsZ proteins in vitro under high pressure conditions is definitely required. Characterization of the polymerization of FtsZ *in vitro* will help us to understand the effect of pressure on cell division.

3.3. Phylogenetic analyses of the evolutionary distance of *S. violacea* FtsZ.

The *ftsZ* gene is conserved in a wide range of eubacteria, archaea and eukaryotes[9)]. Because the sequence of the 16S rDNA of *S. violacea* is very similar to that of *E. coli*, it is evident that these species are closely related[1), 2)] . To confirm the phylogenetic position of the *S. violacea ftsZ*, the evolutionary distance was calculated based on comparison of 12 bacteria and a phylogenetic tree was constructed (Fig. 4). The 16S rDNA sequences or the FtsZ amino acid sequences were aligned and the tree was con-

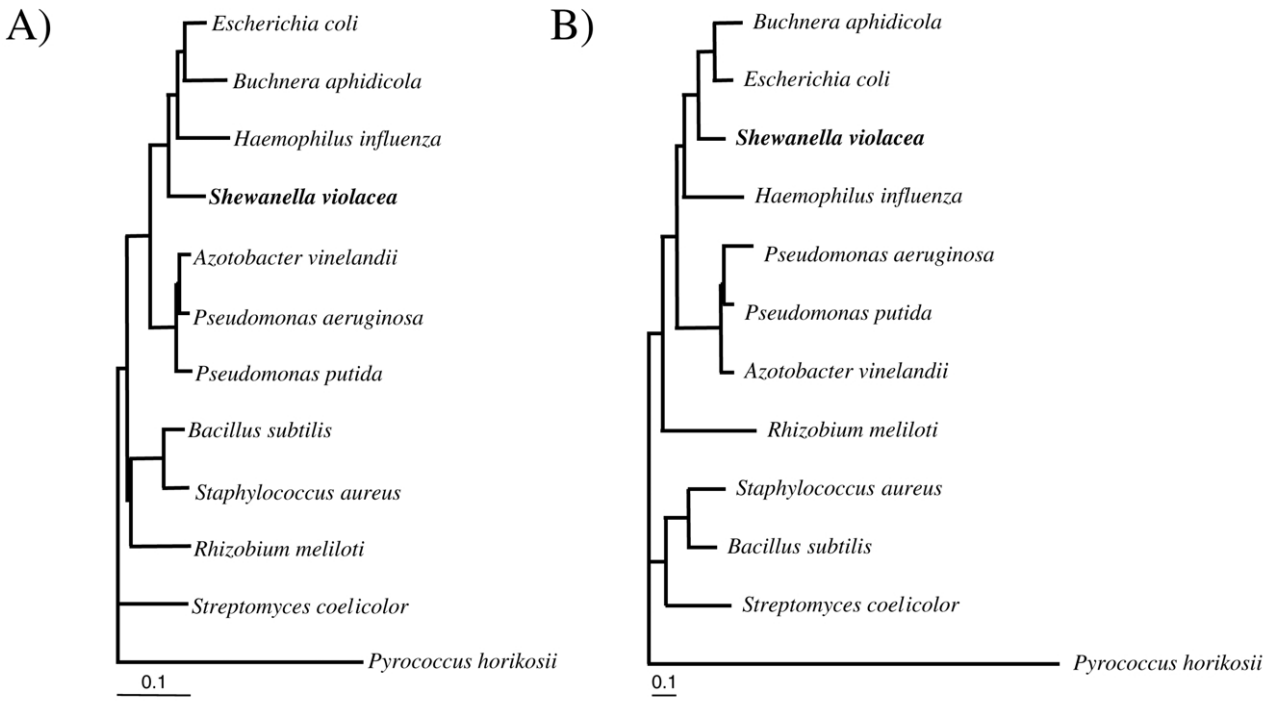

Fig.4 Phylogenetic tree inferred by neighbor joining analysis of 16S rDNA and FtsZ protein sequences.
A tree showing the positions of A) 16S rDNA sequences, B) FtsZ amino acid sequences of 12 bacteria. The *S. violacea ftsZ* gene is most closely related to that of *E. coli*.

structed by the neighbor-joining method. The results obtained in calculation of the evolutionary distance comparing the FtsZ amino acid sequences of 12 bacteria showed that the FtsZ protein of *S. violacea* is closely related that of *E. coli*. This finding is consistent with the results obtained comparing the 16S rDNA sequences.

The results of sequence and phylogenetic analyses demonstrate that the FtsZ proteins of *S. violacea* and *E. coli* have highly conserved sequences and are closely related, but the difference in cell morphology under high pressure conditions indicates that these FtsZ proteins differ in terms of function under high pressure conditions.

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