J. Biochem. 132, 183–188 (2002)

Isolation and Characterization of the *dcw* Cluster from the Piezophilic Deep-Sea Bacterium *Shewanella violacea*[1]

Akihiro Ishii,*,† Kaoru Nakasone,‡ Takako Sato,† Masaaki Wachi,§ Motoyuki Sugai,¶ Kazuo Nagai,§ and Chiaki Kato*,†,[2]

Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa 226-8503; †Japan Marine Science and Technology Center, 2-15 Natsushima-cho, Yokosuka, Kanagawa 237-0061; ‡Department of Chemistry and Environmental Technology, Kinki University School of Engineering, 1 Umenobe Takaya, Higashi-Hiroshima 739-2116; §Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa; and ¶Department of Microbiology, Hiroshima University School of Dentistry, 1-2-3 Kasumi, Minami-ku, Hiroshima, Hiroshima

Received March 4, 2002; accepted May 12, 2002

The *dcw* cluster of genes involved in cell division and cell wall synthesis from the piezophilic deep-sea bacterium *Shewanella violacea* was isolated and characterized. It comprises 15 open reading frames, of which the organization is *mraZ-mraW-ftsL-ftsI-murE-murF-mraY-murD-ftsW-murG-murC-ftsQ-ftsA-ftsZ-envA*, in that order. To analyze transcription upstream from the *ftsZ* gene, Northern blot and primer extension analyses were performed. The results showed that gene expression is not pressure dependent. Western blot analysis showed that the FtsZ protein is equally expressed under several pressure conditions in the range of atmospheric (0.1 MPa) to high (50 MPa) pressures. Using immunofluorescence microscopy, the FtsZ ring was observed in the center of cells at pressure conditions of 0.1 to 50 MPa. These results imply that the FtsZ protein function is not affected by elevated pressure in this piezophilic bacterium.

Key words: cell division, *dcw* cluster, *ftsZ*, high pressure, *Shewanella violacea*.

The moderately piezophilic bacterium *Shewanella violacea* strain DSS12 isolated from the Ryukyu Trench (depth 5,110 m) grows optimally at 30 MPa and 8°C, but also grows under atmospheric pressure conditions (0.1 MPa) at 8°C (*1, 2*). These growth properties are useful for comparative studies of cell physiology under high- and low-pressure conditions. This bacterium grows well under high-pressure conditions (*1, 2*), but *Escherichia coli* cells which is closely related to *S. violacea*, grows poorly under high-pressure conditions (*3, 4*). When *E. coli* are cultured at high pressure, they grow as a long filamentous form (*5*), the phenomenon that appears to resemble the observed morphological features of certain *E. coli* mutants, called filament-forming temperature sensitive (*fts*) mutants, which are defective in cell division at non-permissive temperatures. The defective genes in these mutants, designated the *fts* genes (*6*), are known to be important for cell division. In many bacterial species, some of the genes involved in cell division and cell wall synthesis are organized as a conserved gene cluster, called *dcw* cluster (*7*). The *dcw* cluster contains several *fts* genes, such as the *ftsZ* gene, which is widely conserved in bacteria. This gene codes for a tubulin-like protein with GTPase activity and provides the cytoskeletal framework of a cytokinetic ring for membrane constriction in bacteria (*8–13*).

Under high hydrostatic pressure conditions, the volume of the system (for example any physical, biochemical processes and included molecules) is decreased obeying the principle of Le Chathelier (*14, 15*). Previous studies showed that in the assembly process of microtubules, myosin and actin increase in total volume and their filaments become destabilized and the processes disassemble to monomeric proteins under high-pressure conditions (*16–18*). But assembled filaments from deep-sea life are more stable than those from species living at normal pressures because the assembly processes for proteins in deep-sea life forms are altered so that their volumes are much less than those of proteins from normal pressure species under high-pressure conditions (*16–18*).

From these studies, we are interested in the relationship between the physical effect of hydrostatic pressure on the FtsZ protein, a tubulin homologue, and its function and cell division. Thus, as a first step toward understanding the cell division mechanisms under high-pressure conditions, the characteristics of the FtsZ protein from a deep-sea bacterium were studied *in vivo*. In the present study, we cloned and sequenced the *dcw* cluster containing the *ftsZ* gene in *S. violacea* (*SvftsZ*), and the gene expression of *ftsZ* was analyzed at the transcriptional and translational levels. FtsZ protein expression and function were investigated using immunofluorescence microscopy.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—*S. violacea* was cul-

[1] This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas: Single-Cell Molecular Technology (area number 736).

[2] To whom correspondence should be addressed at: Tel: +81-468-67-9520, Fax: +81-468-67-9575, E-mail: katoc@jamstec.go.jp

tured at 8°C and at pressures of 0.1, 30, and 50 MPa in Marine Broth 2216 (Becton Dickinson, Sparks, MD, USA) with the addition of oxygenated Fluorinert FC-72 (Sumitomo 3M, Tokyo) according to the method described previously (*1*). *E. coli* strain TOP10 (Invitrogen, Carlsbad, CA, USA) was used as the host strain for gene cloning. *E. coli* cells were grown at 37°C in LB medium with ampicillin 50 $\mu g\ ml^{-1}$ or kanamycin 20 $\mu g\ ml^{-1}$.

Isolation and Sequencing of the dcw Cluster—To isolate the *dcw* cluster, a λ phage library of *S. violacea* was screened using the DIG detection system (Roche Diagnostics, Mannheim, Germany) using part of the *ftsZ* gene of *S. violacea* as a probe (*19*). Based on a comparison of the amino acid sequences of the FtsZ proteins from several Gram-negative bacteria, two degenerate oligonucleotide primers, 5′-CTIGGIGCIGGIGCIAAYCC-3′ and 5′-CKIACRTCIGCRAARTCIAC-3′, were designed and synthesized. A target DNA fragment containing part of the *ftsZ* gene was amplified by polymerase chain reaction (PCR) using these primers, and the products (approximately 300 bp) were cloned into the TA cloning vector pCR2.1 (Invitrogen) and sequenced. This fragment was labeled with digoxigenin to prepare a hybridization probe. Plaque hybridization was carried out with the probe and a positive clone was isolated from the λ phage library. The nucleotide sequence of the DNA insert of this clone was determined using an ABI prism 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA).

The nucleotide sequence data in this study will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases under the accession number AB052554.

Gene Analysis—Assembly and editing of the determined DNA sequences were performed with AutoAssembler Version 2.0 (Applied Biosystems), and GENETYX-MAC version 10.1 (Software Development, Tokyo) was used for sequence analysis. The ribosome binding site of each ORF was predicted by the GeneMark and GeneMark.hmm programs (http://opal.biology.gatech.edu/GeneMark/). The sequence of the predicted open reading frames (ORFs) was compared with other bacterial sequences in a homology search by the FASTA program (http://spiral.genes.nig.ac.jp/homology/fasta.shtml).

Northern Blot and Primer Extension Analyses—For transcription analysis, total cellular RNA was prepared from log-phase cells of *S. violacea* grown under 0.1, 30, or 50 MPa conditions for 42 h with Sepasole-RNA I (Nacalai Tesque, Kyoto) as described previously (*20*).

For Northern blot analysis, 30 mg of total RNA was used per sample. Amplification of the Sv*ftsZ*-specific DIG-labeled DNA probe was performed by PCR with two primers (5′-GTGATGTCACTAAAGGTTTAGGCGCTGGAG-3′ and 5′-TCTAATAAGATGTGCCACGGCCCAAGACC-3′) and *ftsZ* mRNA was detected using the DIG detection system (Roche Diagnostics) according to the manufacturer's instructions.

Reverse-transcription (RT)-PCR and the sequence reaction for primer extension analysis were performed according to the method previously described (*20*). Fifty micrograms of total RNA was employed as a template and two biotinylated primers (5′-biotin-CTCTACTGCGTTTCCGCCACCACC-3′ and 5′-biotin-GATACCAGCAGCGACTTGGTCCTC-3′) were used for RT. For electrophoresis of the RT products, the GENOQUENSER (ATTO, Tokyo) system was used with detection by an Imaging High-color device (TOYOBO, Kyoto).

In Vitro and In Vivo Immuno-Detection of SvFtsZ—*S. violacea* was grown at 0.1, 30, or 50 MPa and the soluble fraction was obtained for analysis by sonication. The soluble fraction was separated in a 10% acrylamide gel and immuno-detected with anti–*S. violacea* FtsZ polyclonal antibody.

Immunofluorescence microscopy was performed as previously described (*21*). SvFtsZ was immunostained with the same antibody as for Western blot analysis with Alexa™ 488 goat anti-rabbit IgG conjugate (Molecular Probes, Eugene, OR, USA) used as the second antibody. The immunofluorescence micrographs were modified with Photoshop 5.5 software (Adobe Systems, San Jose, CA, USA).

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of the S. violacea dcw Cluster—To isolate the *dcw* cluster surrounding the *ftsZ* gene from *S. violacea*, a partial DNA fragment of the Sv*ftsZ* gene was amplified by PCR with degenerate oligonucleotides designed based on the highly conserved region of several Gram-negative bacterial FtsZ proteins. The nucleotide sequence of the obtained fragment was determined, and a database search confirmed that the fragment encoded part of the *ftsZ* gene. Two specific primers were designed to amplify the DIG-labeled Sv*ftsZ* probe (approximately 300 bp), and the probe was employed as the hybridization probe. Distribution of the Sv*ftsZ* gene on the chromosome was determined by Southern blot analysis using the same probe (Fig. 1A). As shown in Fig. 1A, analysis showed that the Sv*ftsZ* gene exists as a single copy in the *S. violacea* genome. Plaque hybridization of the *S. violacea* λ phage library was carried out and few positive clones were obtained. One positive clone containing the *ftsZ* gene was 21 kb in size, and its nucleotide sequence was determined by the random shotgun sequencing method. Sequence analysis of the fragment revealed that it comprises 15 open reading frames (ORFs) thought to be involved in cell division and cell envelope biosynthesis. As shown in Fig. 1B, the gene organization is *mraZ-mraW-ftsL-ftsI-murE-murF-mraY-murD-ftsW-murG-murC-ftsQ-ftsA-ftsZ-envA*, in that order. Upstream and downstream from the *mraZ* and *envA* genes, respectively, no cell division or cell wall synthesis gene was found. The *dcw* cluster was organized surrounding the *ftsZ* gene in *S. violacea* as in other Gram-negative bacteria (*22*).

The ORFs in the *S. violacea dcw* cluster are listed and their positions, lengths, putative SD sequences and descriptions are shown (Table I). The deduced proteins in the *S. violacea dcw* cluster are similar to those in *E. coli*, *Haemophilus influenzae*, and *Vibrio cholerae* (Table I). The similarity of the MraW proteins between *S. violacea* and *E. coli* was the highest (75.8% identity), with the lowest similarity found for *ftsQ* between *S. violacea* and *E. coli* (36.5% identity). The others show similarities of 40–60%. The FtsZ protein showed the next highest degree of similarity between *S. violacea* and *E. coli* (72.2% identity). A comparison of the FtsZ amino acid sequences among *S. violacea* and other bacteria living under normal atmospheric pressure condition is shown in Fig. 2. The FtsZ protein was analyzed according to its functional domains as the GTPase and its

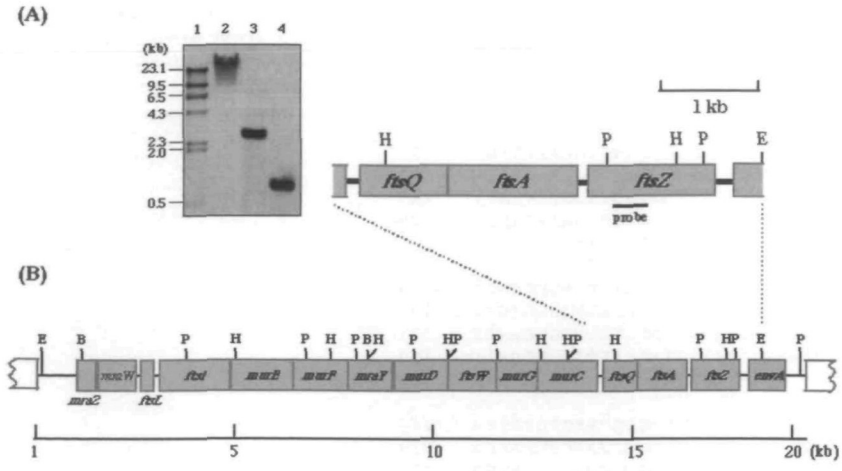

Fig. 1. **Southern blot analysis and gene organization of the *dcw* cluster in *S. violacea*.** (A) Southern blot analysis with the Sv*ftsZ* probe. The DNA molecular size markers are shown in lane 1. *S. violacea* chromosomal DNA was digested with *Eco*RI (lane 2), *Hin*dIII (lane 3), and *Pst*I (lane 4). (B) Restriction sites (B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I) in the *S. violacea dcw* cluster.

TABLE I. **ORF analysis of the *dcw* cluster of *S. violacea*.**

Gene	Position	(n t)	(a.a)	(kDa)	SD/initiation codon	Characteristics
mraZ	1203-1661	458	152	17 5	AAGGACAAGCTAACGTG	Similar to *H influenzae* Rd conserved hypothetical protein (56 6% identity)
mraW	1693-2634	941	313	35.0	GAAGATACTAATG	Similar to *E coli* YabC protein (62.2% identity)
ftsL	2705-3019	314	104	12 6	AGGGAGTTCTGATATG	Similar to *V cholerae* FtsL protein (41.5% identity)
ftsI	3088-4830	1,742	580	64.3	AACGAGAGCATCATG	Similar to *V cholerae* penicillin-binding protein 3 protein (51.0% identity)
murE	4830-6308	1,478	492	53 0	CAAGAGGACAGAATAATG	Similar to *E. coli* UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase (EC 6.3.2.13) (45 1% identity)
murF	6305-7672	1,367	455	48.9	CCGGAGAACAGATATG	Similar to *E coli* UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D- alanyl ligase (EC 6.3.2 15) (60 0% identity)
mraY	7672-8754	1,082	360	39 8	AGGGAGTTTGTTTAATG	Similar to *E coli* phospho-N-acetylmuramoyl-pentapeptide-transferase (EC2.7 8 13) (75 8% identity)
murD	8761-10110	1,349	449	47 8	AAGGTAATTGACGATG	Similar to *E coli* UDP-N-acetylmuramoylalanine-D-glutamate ligase (EC 6.3.2 9) (47 1% identity)
ftsW	10103-11317	1,214	404	44 9	GAGGCTAGTGCTGATG	Similar to *E coli* FtsW protein (57 1% identity)
murG	11317-12414	1,097	365	39 0	AGGGAAAAATAGTTAATG	Similar to *E coli* UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol n-acetylglucosamine transferase (EC 2 4 1 -) (52.5% identity)
murC	12383-13867	1,484	494	54.1	CCGAAAAAGTGGCTGATG	Similar to *V cholerae* UDP-N-acetylmuramate-alanine ligase (EC6.3 2.8) (65.8% identity)
ftsQ	13971-14783	812	270	30.8	GTGGGGTACCTATG	Similar to *E coli* FtsQ protein (36.5% identity)
ftsA	14783-16018	1,235	411	41.6	GAAGAGAGCCAGTAAATAATG	Similar to *E coli* FtsA protein (59.5% identity)
ftsZ	16093-17271	1,178	392	40 7	ACGGAGAGAAGACCATG	Similar to *E. coli* FtsZ protein (72 2% identity)
envA	17436-18356	920	306	33 9	CAGGTAAAAATATG	Similar to *V cholerae* UDP-3-O-[3-hydroxymyristoyl]-N-acetylglucosamine deacetylase (EC 3.5 1 -) (64.1% identity)

The position and length of each ORF in *S. violacea dcw* cluster, the putative protein size and molecular mass are listed. The predicted SD sequence (underlined) and initiation codon (bold) are also shown. Proteins homologous to the encoded protein in each ORF and their identities (%) are given.

self-interaction (*23, 24*). The GTPase domain in the N-terminus is highly conserved in *S. violacea* (Fig. 2, broken underline). The sequence at the C-terminus from amino acid 320 to the end, which is especially for the self-interaction of FtsZ, however, shows little conservation among bacteria (Fig. 2, solid underline). This indicates that the FtsZ proteins of *S. violacea* and other ground bacteria have similar GTPase activities and a specific interaction property in each FtsZ protein.

Northern Blot Analysis of the SvftsZ Gene—Northern blot analysis was performed to analyze the expression of Sv*ftsZ* mRNA under several pressure conditions. The RNAs were hybridized and visualized with the DIG-labeled Sv*ftsZ*-specific probe and the DIG detection system (Fig. 3). Two RNA bands, about 2.6 kb and 1.5 kb in size, were detected under these pressure conditions. The effects of high pressure on *S. violacea* gene expression have been reported (*19, 25–28*), but the expression of *ftsZ* was almost the same under all pressure conditions.

In *E. coli*, *ftsZ* gene expression has been well studied. *E. coli ftsZ* promoters were found inside the *ddlB* (*ftsQ*2p), *ftsQ* (*ftsA*p), and *ftsA* (*ftsZ*1p-4p) genes in several studies (*29–31*), and the contribution of the promoter of the *mraZ* gene (*mraZ*p), about 18 kb upstream from *ftsZ*, has been suggested (*32*). After transcription, *ftsZ* mRNA is cleaved by RNase E behind the *ftsZ*p in *E. coli* (*33*). The multiple bands in the present Northern blot analysis under different pressure conditions indicate that the regulation of *ftsZ* transcription in *S. violacea* is similar to that in *E. coli*.

Primer Extension Analysis of the ftsZ Upstream Region

```
(Sv)   1 MFEIMDSHTDEAVIKVIGVGGGGGNAVEHMVKHNIEGVEFVATNTDAQALRKSAAGTTIQ  60
(Ec)   1 MFEPMELTNDA-VIKVIGVGGGGGNAVEHMVRERIEGVEFFAVNTDAQALRKTAVGQTIQ  59
(Hi)   1 MFDFNDSMVSNAIIKVVGVGGGGGNAVQHMIEEVSD-VEFFALNTDGQALSKSKVQNILQ  59
(Pp)   1 MFELVDNVPQSPVIKVIGVGGGGGNAVNHMVKSSIEGVEFICANTDAQALKNIGARTILQ  60
         **.  .      .***.********** **.   ...*** . ***.*** .       *
                                                  .....

(Sv)  61 LGRDVTKGLGAGANPEIGRLAAEEIRESIRNALKGSDMIFIAAGMGGGTGTGAAPVVAEI  120
(Ec)  60 IGSGITKGLGAGANPEVGRNAADEIRDALRAALEGADMVFIAAGMGGGTGTGAAPVVAEV  119
(Hi)  60 IGTNLTKGLGAGANPEIGKRAAAEIRAKIEQLLEGADMVFITAGMGGGTGTGGAPVVAEV  119
(Pp)  61 LGTGVTKGLGAGANPEVGRQAALEIRERIAEVLQGTNMVFITTGMGGGTGTGAAPIIAEV  120
          *   *********** *. ** ***  .   * * .*.** .**********.**..**.
                                                  ..........

(Sv) 121 AKEEGILTVAVVTKPFPFEGRKRMAYAEQGIEELAKHVDSLITIPNEKLLKVLGRGTSLL  180
(Ec) 120 AKDLGILTVAVVTKPFNFEGKKRMAFAEQGITELSKHVDSLITIPNDKLLKVLGRGISLL  179
(Hi) 120 AKEMGILTVAVVTKPFPFEGPRRMKAAEQGIDELTKHVDSIITVPNEKLLSVLGKGASLI  179
(Pp) 121 AKEMGILTVAVVTRPFPFEGRKRMQIADEGIRMLAESVDSLITIPNEKLLTILGKDASLL  180
         **. *********.**.*** .**  *..** .* ..***.**.**.*** .** . **.
                                                  ............

(Sv) 181 DAFAAANNVLLGAVQGIAELITRPGLINVDFADVKTVMSEMGNAMMGTGVASGEDRAEEA  240
(Ec) 180 DAFGAANDVLKGAVQGIAELITRPGLMNVDFADVRTVMSEMGYAMMGSGVASGEDRAEEA  239
(Hi) 180 DAFNAANDVLGNAVKGVSELITKPGLINVDFADVRAVMTNMGLAMMGMGEASGENRAREA  239
(Pp) 181 SAFAKADDVLAGAVRGISDIIKRPGMINVDFADVRTVMGEMGMAMMGTGCASRPNRAREA  240
         .** .*..** .** *. ..*..**..*******..** .** **** * **.. ** **
                                                  ..........

(Sv) 241 AEAAVASPLLEDIDLAGARGVLVNITAGMDMSIEEFETVGNMVKAYASDNATVVVGAVID  300
(Ec) 240 AEMAISSPLLEDIDLSGARGVLVNITAGFDLRLDEFETVGNTIRAFASDNATVVIGTSLD  299
(Hi) 240 AEAAISSPLLEDINLDGAKGVIVNITAGMDMSIGEFEEVGEVIRSFISDEAIVIAGTVID  299
(Pp) 241 TEAAIRNPLLEDVNLQGARGILVNITAGPDLSLGEYSDVGSIIEAFASDHAMVKVGTVID  300
         .*.*. .*****. * **.*..****** * .  *.. **  . ...** * *  *...*

(Sv) 301 PEMSDELRVTVVATGIGAEKKPDIQLVTKP-VPRPEPVIAPEVRTEPQSEELV-QSMASG  358
(Ec) 300 PDMNDELRVTVVATGIGMDKRPEITLVTNKQVQQ---PVMD---RYQQHGMAPLTQEQKPV  354
(Hi) 300 PDMSDSMKVTVVVTGI----EKVA------MKR---GFGVEKTSSLQ--QSAS--SFSNK  342
(Pp) 301 PDMRDELHVTVVATGLGARIEKPVKVVDNTLQTAQQAYEASNPQSCAGAQEQPAVNYRDL  360
         *.* *.. ****.**..          .

(Sv) 359 NV-VPAAQTAAAPATALRNET----DYL---DIPAFLRKQAD                     392
(Ec) 355 AKVVND-NA---PQTAKEP------DYL---DIPAFLRKQAD                     383
(Hi) 343 TSAPFLRKETEVVTGASNAPKTDSIDV-NKSDIPSFLRRR--                     381
(Pp) 361 ERPTVMRNQAKA--GAAAAAKLNPQDDLDYLDIPAFLRRQAD                     400
                         *          * .   ***.*** ...
```

Fig. 2. **Multi-alignment of FtsZ proteins from *Shewanella violacea* (*Sv*), *Escherichia coli* (*Ec*), *Haemophilus influenzae* (*Hi*), and *Pseudomonas putida* (*Pp*).** Identical residues are indicated by asterisks. The conserved GTPase domain (broken) and variable self-interaction domain (solid) are underlined.

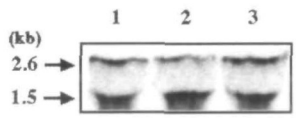

Fig. 3. **Northern blot analysis of Sv*ftsZ* gene expression under different pressure conditions (lane 1, 0.1 MPa; lane 2, 30 MPa; lane 3, 50 MPa).** Two RNA bands were detected in each lane and estimated to be approximately 2.6 and 1.5 kb in size. RNA sizes are indicated on the left.

in S. violacea under High-Pressure Conditions—To measure *ftsZ* expression levels in *S. violacea* under several pressure conditions and to determine the transcription initiation site, primer extension analysis was performed. The same transcription initiation sites were identified (Fig. 4) at pressures of 0.1, 30, and 50 MPa. In the region upstream from these sites, a putative σ^{70}-type promoter consensus sequence and a putative ribosome binding site were seen (Fig. 4).

In Vivo and In Vitro Immuno-Detection of SvFtsZ Protein—Western blot analysis was carried out to study the pressure effect on SvFtsZ translation (Fig. 5). Specific bands were detected in all lanes, and the relative amounts of SvFtsZ protein in a cell were equal under each pressure condition.

To study the function of the SvFtsZ protein *in vivo*, the localization of the SvFtsZ protein in cells under atmospheric and high-pressure conditions was observed using immunofluorescence microscopy. *S. violacea* cells grown at 0.1, 30, and 50 MPa were observed by differential interference contrast microscopy. In *S. violacea*, rod-shaped morphology and membrane constriction in the cell division site were observed in the range of 0.1 to 50 MPa (Fig. 6, upper panel). A single band exhibiting FtsZ ring formation was visualized at the center of a *S. violacea* cell, which corresponds to the constriction site (Fig. 6, lower panel). This observation confirms that SvFtsZ is present *in vivo* at a cell division site under atmospheric and high-pressure conditions.

CONCLUSIONS

We cloned and characterized the *dcw* cluster in the piezophilic bacterium *S. violacea* to study the pressure effect on the cell division mechanism, especially on the FtsZ protein, under high-pressure conditions. Northern blot analysis and primer extension analysis showed that *ftsZ* gene expression was not affected by elevated pressure in *S. violacea*. Furthermore, immunodetection analysis *in vitro* and *in vivo* revealed that the SvFtsZ protein was translated equally and was functional at pressures from atmospheric to high-pressure conditions. These results show that FtsZ is not altered quantitatively and the cell division function is not inhibited

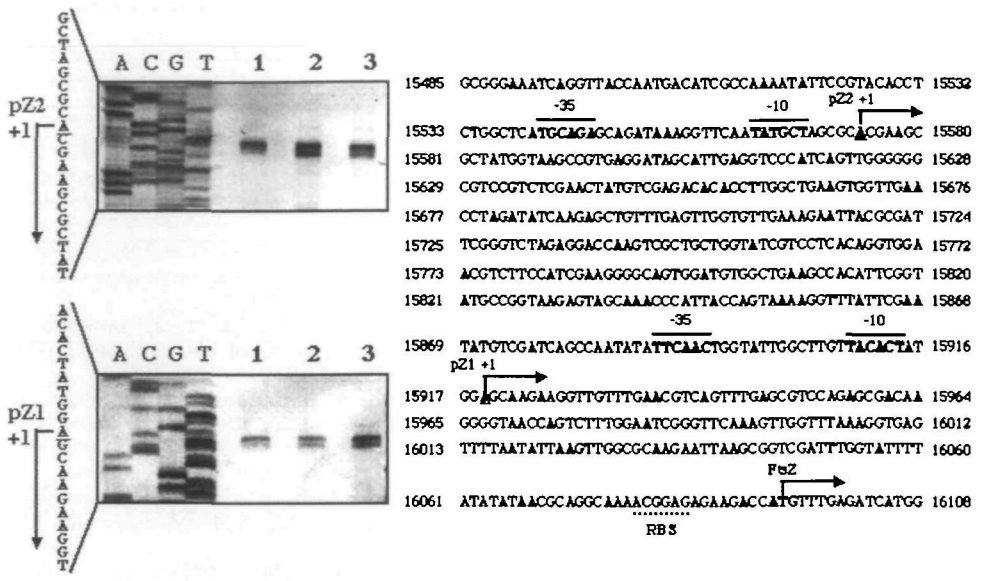

Fig. 4. **Determination of the transcription initiation site by primer extension analysis.** Transcription upstream from the *S. violacea ftsZ* gene at 0.1 MPa (lane 1), 30 MPa (lane 2), and 50 MPa (lane 3) is shown. The transcription initiation site is indicated by an arrow and underlining. The nucleotide sequence in this region is shown on the right. The predicted σ^{70}-type promoter consensus sequences and ribosome binding site (RBS, dotted underlining) are indicated. The DNA sequence ladders of the gene are shown on the left

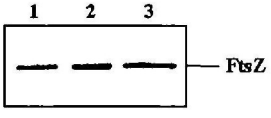

Fig. 5. **Western blot analysis of the SvFtsZ protein.** *S. violacea* proteins from cells grown at 0.1 MPa (lane 1), 30 MPa (lane 2), and 50 MPa (lane 3) were immunodetected with anti–*S. violacea* FtsZ antibody.

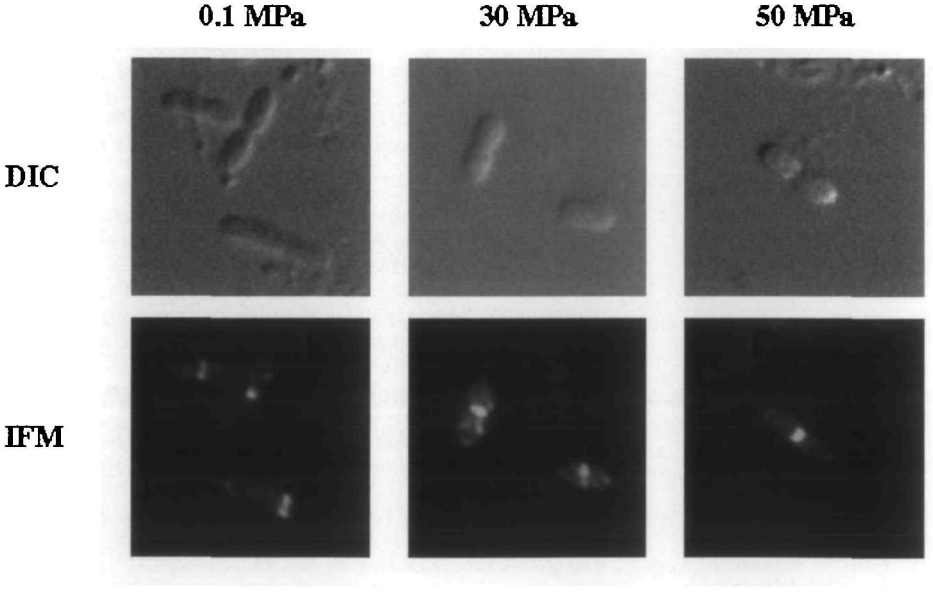

Fig. 6. **Observation of FtsZ ring formation in *S. violacea*.** Upper panel: Observation of *S. violacea* cells grown at 0.1, 30, and 50 MPa using differential interference contrast (DIC) microscopy. Lower panel: Observation of FtsZ ring formation using immunofluorescence microscopy (IFM).

by high-pressure in *S. violacea*. The cell morphology of *E. coli* under high-pressure conditions is, however, quite different from that of *S. violacea*. The filamentous cells indicate a stop in the cell division steps and we expect that a hydrostatic pressure effect on FtsZ protein function causes the inhibition of cell division. In fact, the C-termini of FtsZ from the four bacteria compared in this study are not conserved and the region is essential for polymerization activity. The characteristic property under high-pressure is considered from the variety.

We attempted to grow *E. coli* under high-pressure by means of transduction of the *SvftsZ* gene. However, the expression of SvFtsZ caused filamentation and the *E. coli* were not able to grow under any pressure conditions (data not shown). Therefore, we expect that the characterization of the biochemical features and polymerization activities of SvFtsZ and terrestrial bacteria (*eg. E. coli*) *in vitro* will help us to understand the effect of pressure on cell division steps *in vivo*.

We thank Ms. C. Yenches for assistance in editing the manuscript.

REFERENCES

1. Kato, C., Sato, T., and Horikoshi, K. (1995) Isolation and properties of barophilic and barotolerant bacteria from deep-sea

mud samples. *Biodiv. Conserv.* **4**, 1–9
2. Nogi, Y., Kato, C., and Horikoshi, K. (1998) Taxonomic studies of deep-sea barophilic *Shewanella* strains and description of *Shewanella violacea* sp. nov. *Arch. Microbiol.* **170**, 331–338
3. Kato, C., Sato, T., Smorawinska, M., and Horikoshi, K. (1994) High-pressure conditions stimulate expression of chloramphenicol acetyltransferase regulated by the *lac* promoter in *Escherichia coli*. *FEMS Microbiol. Lett.* **122**, 91–96
4. Zobell, C.E. and Cobet, A.B. (1962) Growth, reproduction, and death rates of *Escherichia coli* at increased hydrostatic pressures. *J. Bacteriol.* **84**, 1228–1236
5. Zobell, C.E. and Cobet, A.B. (1963) Filament formation by *Escherichia coli* at increased hydrostatic pressures. *J. Bacteriol.* **87**, 710–719
6. Hirota, Y., Ryter, A., and Jacob, F. (1968) Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division. *Cold Spring Harbor Symp. Quant. Biol.* **33**, 677–693
7. Ayala, J.A., Garrido, T., de Pedro, M.A., and Vicente, M. (1994) Molecular biology of bacterial endospore formation in *New Comprehensive Biochemistry—Bacterial Cell Wall* (Hakenbeck, R. and Ghuysen, J.M., eds.) Vol. 27, pp. 73–101, Elsevier, Amsterdam
8. Addinall, S.G. and Lutkenhaus, J. (1996) FtsZ-spirals and -arcs determine the shape of the invaginating septa in some mutants of *Escherichia coli*. *Mol. Microbiol.* **22**, 231–237
9. Bi, E.F. and Lutkenhaus, J. (1991) FtsZ ring structure associated with division in *Escherichia coli*. *Nature* **354**, 161–164
10. de Boer, P., Crossley, R., and Rothfield, L. (1992) The essential bacterial cell-division protein FtsZ is a GTPase. *Nature* **359**, 254–256
11. Lutkenhaus, J. (1993) FtsZ ring in bacterial cytokinesis. *Mol. Microbiol.* **9**, 403–409
12. Mukherjee, A., Dai, K., and Lutkenhaus, J. (1993) *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. *Proc. Natl. Acad. Sci. USA* **90**, 1053–1057
13. RayChaudhuri, D. and Park, J.T. (1992) *Escherichia coli* cell-division gene *ftsZ* encodes a novel GTP-binding protein. *Nature* **359**, 251–254
14. Gross, M. and Jaenicke, R. (1994) Proteins under pressure. The influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes (Review). *Eur. J. Biochem.* **221**, 617–630
15. Heremans, K. and Smeller, L. (1998) Protein structure and dynamics at high pressure (Review). *Biochim. Biophys. Acta* **1386**, 353–370
16. Josephs, R. and Harrington, W.F. (1966) Studies on the formation and physical chemical properties of synthetic myosin filaments. *Biochemistry* **5**, 3474–3487
17. Josephs, R. and Harrington, W.F. (1967) An unusual pressure dependence for a reversibly associating protein system; sedimentation studies on myosin. *Proc. Natl. Acad. Sci. USA* **58**, 1587–1594
18. Salmon, E.D. (1975) Pressure-induced depolymerization of brain microtubules *in vitro*. *Science* **189**, 884–886
19. Yamada, M., Nakasone, K., Tamegai, H., Kato, C., Usami, R., and Horikoshi, K. (2000) Pressure regulation of soluble cytochrome *c* in a deep-sea piezophilic bacterium, *Shewanella violacea*. *J. Bacteriol.* **182**, 2945–2952
20. Fujii, S., Nakasone, K., and Horikoshi, K. (1999) Cloning of two cold shock genes, *cspA* and *cspG*, from the deep-sea psychrophilic bacterium *Shewanella violacea* strain DSS12. *FEMS Microbiol. Lett.* **178**, 123–128
21. Hiraga, S., Ichinose, C., Niki, H., and Yamazoe, M. (1998) Cell cycle-dependent duplication and bidirectional migration of SeqA-associated DNA-protein complexes in *E. coli*. *Mol. Cell.* **1**, 381–387
22. Vicente, M., Gomez, M.J., and Ayala, A. (1998) Regulation of transcription of cell division genes in the *Escherichia coli dcw* cluster. *Cell. Mol. Life Sci.* **54**, 317–324
23. Wang, X., Huang, J., Mukherjee, A., Cao, C., and Lutkenhaus, J. (1997) Analysis of the interaction of FtsZ with itself, GTP, and FtsA. *J. Bacteriol.* **179**, 5551–5559
24. Di Lallo, G., Anderluzzi, D., Ghelardini, P., and Paolozzi, L. (1999) FtsZ dimerization in vivo. *Mol. Microbiol.* **32**, 265–274
25. Ikegami, A., Nakasone, K., Kato, C., Nakamura, Y., Yoshikawa, I., Usami, R., and Horikoshi, K. (2000) Glutamine synthetase gene expression at elevated hydrostatic pressure in a deep-sea piezophilic *Shewanella violacea*. *FEMS Microbiol. Lett.* **192**, 91–95
26. Kato, C., Nakasone, K., Quereshi, M., and Horikoshi, K. (2000) How do deep-sea microorganisms respond to changes in environmental pressure? in *Environmental Stressors and Gene Responses* (Storey, K.B. and Storey, J., eds.) pp. 277–291, Elsevier, Amsterdam
27. Nakasone, K., Ikegami, A., Kato, C., Usami, R., and Horikoshi, K. (2000) Molecular mechanisms of transcriptional regulation in a deep-sea piezophilic bacterium. *Rec. Res. Dev. Microbiol.* **4**, 255–269
28. Nakasone, K., Ikegami, A., Fujii, S., Kato, C., and Horikoshi, K. (2000) Isolation and piezoresponse of the *rpoA* gene encoding the RNA polymerase α subunit from the deep-sea piezophilic bacterium *Shewanella violacea*. *FEMS Microbiol. Lett.* **193**, 261–268
29. Aldea, M., Garrido, T., Pla, J., and Vicente, M. (1990) Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters. *EMBO J.* **9**, 3787–3794
30. Dewar, S.J. and Dnachie, W.D. (1990) Regulation of expression of the *ftsA* cell division gene by sequences in upstream genes. *J. Bacteriol.* **172**, 6145–6151
31. Garcia-Lara, J., Shang, L.H., and Rothfield, L.I. (1996) An extracellular factor regulates gene expression of *sdiA*, a transcriptional activator of cell division genes in *Escherichia coli*. *J. Bacteriol.* **178**, 2742–2748
32. Mengi-Lecreulx, D., Ayala, J., Bouhss, A., Heijenoort, J.V., Parquet, C., and Hara, H. (1998) Contribution of the P_{mra} promoter to expression of genes in the *Escherichia coli mra* cluster of cell envelope biosynthesis and cell division genes. *J. Bacteriol.* **180**, 4406–4412
33. Cam, K., Rome, G., Krisch, H.M., and Bouche, J.P. (1996) RNase E processing of essential cell division gene mRNA in *Escherichia coli*. *Nucleic Acids Res.* **24**, 3065–3070