近畿大学工学部研究報告 No.38, 2004年, pp.23-26
Research Reports of the School of Engineering,
Kinki University No.38, 2004, pp.23-26

# Expression analysis *dnaJ* and *dnaK* gene under high pressure conditions from deep-sea piezophilic *Shewanella violacea*

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The *dnaK* and *dnaJ* genes of the deep-sea piezophilic and psychrophilic bacterium *Shewanella violacea*, was cloned and sequenced by screening lambda phage library. The molecular masses of DnaK and DnaJ proteins were calculated to be 68,861 and 40,762 Da, respectively. Expression studies by Northern blot analysis under high pressure conditions showed that the amount of the mRNA was increased by elevated pressure conditions. Primer extension analysis showed that tree transcription initiation sites were existed.

**Key words** *Shewanella violacea*, heat shock protein, *dnaK*, *dnaJ*, molecular chaperone, piezoresponse

## Introduction

The deep-sea environment is a unique habitat characterized by extremely high pressure and low temperature and microorganisms living there have adapted to this extreme environment. We have identified several piezophilic bacteria isolated from deep-sea mud samples [1]. *Shewanella violacea* strain DSS12 is one of the piezophilic organisms isolated from the Ryukyu Trench at a depth of 5110 m, and it grows optimally at 30 MPa and 8°C [1, 2]. We are interested in examining the molecular mechanisms of gene regulation in piezophilic bacteria at high pressure. In our previous studies, a pressure-regulated operon, controlled by elevated pressure at the level of transcription, was cloned from this strain [3]. A variety of environmental stresses induce the synthesis of a set of highly conserved proteins called heat shock proteins (HSPs) [4]. These proteins play important roles in assisting the folding, assembly and degradation of proteins, not only under stress conditions, but also under normal growth conditions. Although many reports have described the HSPs of mesophilic and thermophilic bacteria, little is known about the mechanisms of the response of piezophilic bacteria to not only high temperature conditions, but also high pressure conditions [5]. One of HSPs, the DnaK and DnaJ proteins are thought to be evolutionarily conserved with those of mesophilic and thermophilic bacteria. Thus, in order to understand piezoresponse of these stress proteins in the deep-sea bacterium, it is important to clone *dnaK* and *dnaJ* genes from the strain. In this paper, we describe the cloning of *dnaK* and *dnaJ* genes from this strain, and analysis of its sequence, and it is demonstrated that expression of *dnaK* and *dnaJ* is positively controlled at the transcriptional level by elevated pressure. Our results suggest that $\sigma^{32}$ may contribute to pressure-regulation in this piezophilic bacterium.

## Materials and Methods

Bacterial strains and culture conditions

*S. violacea* strain DSS12 [1, 2] and *E. coli* strain JM109 were used in this study. *S. violacea* cells were grown at atmospheric pressure and 8°C with vigorous shaking in Marine Broth 2216 medium (Difco, Sparks, MD, USA). Cells of *E. coli* strain JM109 were grown at 37°C with vigorous shaking in Luria-Bertani medium.

Isolation of the *dnaK* and *dnaJ* genes from *S. violacea*

First in order to isolate *dnaK* and *dnaJ* genes we prepared a hybridization probe for the gene. Based on the amino acid sequences of the gene from several gram-negative bacteria (*E. coli*, *Pseudomonas putida*, *Vibrio cholerae*, and *Yersinia pestis*) were aligned, conserved sequences, two synthetic

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degenerate oligonucleotide primers (data not shown), were designed and synthesized to amplify part of the *dnaKJ* region from *S. violacea*. A fragment of approximately 300 bp amplified by PCR and expected to contain part of the *dnaK* gene was cloned into the pCR2.1 vector and its nucleotide sequence was determined. To clone the complete *dnaKJ* region, the partial *dnaK* gene fragment was labeled with digoxygenin (DIG) in PCR as a hybridization probe for plaque hybridization. Chromosomal DNA isolated from *S. violacea* was partially digested with *Sau*3AI. These fragments were inserted into the *Bam*HI site of lambda DASH II (Stratagene Co., La Jolla, CA, USA). 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 DSS12 λ phage library was screened for plaque hybridization with the *dnaK* probe and a positive clone was obtained. The positive clone containing the *dnaK* gene was purified by several single-plaque isolation steps. The insert in the λ phage was amplified by long PCR and was subcloned into the pCR-Blunt vector (Invitrogen Co., Carlsbad, CA, USA). For sequencing of these cloned fragments, the random shotgun sequencing method was used with a DNA sequencer model 377 (Perkin-Elmer/Applied Biosystems Co., Foster City, CA, USA).

Northern hybridization analysis

Total cellular RNA was prepared from *S. violacea* cells cultivated at 0.1, 10, 30, and 50 MPa, using Sepasol RNA I (Nakalai Tesque, Japan) as described previously [6]. The RNA pellet was dissolve in diethyl pyrocarbonate-treated water, quantified by spectrophotometry, and stored at -80°C. Northern blot analysis was performed by the method reported previously [6]. Probes for Northern blot analysis were constructed by the PCR DIG Probe Synthesis Kit(Boehringer Mannheim Co.).

Primer extension analysis

Total cellular RNA was prepared from *S. violacea* cells cultivated at 0.1, 10, 30, and 50 MPa, using Sepasol RNA I (Nakalai Tesque, Japan) as described previously [6]. Primer extension analysis was carried out according to the procedure previously reported [7], using the following synthesized oligonucleotides primers, ES-1, 5'-GAA TCT CCC TTG TCC-3' and ES-2, 5'-TAG AAG GGG TCG TAC GAT CGC CTT CGG C -3'. Transcripts were analyzed on a 5% polyacrylamide gel under denaturing conditions. The sequencing ladder was generated using the same primers as those used in the dideoxy sequencing reactions.

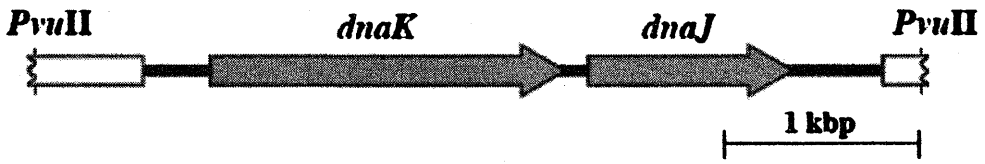


**Fig. 1 Genetic map of the region containing the *dnaK* and *dnaJ* genes in *Shewanella violacea*.**

## Results and discussion

Structural analysis of the *dnaK* and *dnaJ* genes of *S. violacea*

By screening the λ phage library, a fragment containing *dnaK* followed by *dnaJ* was obtained (Fig. 1). Sequence analysis showed that the gene organization and orientation of the fragment containing *dnaKJ* region is identical with those in *E. coli*. Therefore, *dnaKJ* region is likely to be transcribed as an operon, as reported for *E. coli*. The open reading frames of the *dnaK* and *dnaJ* genes consist of 1,923 and 1,311 bp, respectively. The *dnaK* gene encodes a protein consisting of 640 amino acid residues with a molecular mass of 69,034 Da, whereas the *dnaJ* gene encodes a protein consisting of 376 amino acid residues with a molecular mass of 40,883 Da (Table 1). Significant homology was found when comparing the DnaK and DnaJ proteins of *S. violacea* with those of *S. oneidensis*, *E. coli*, *P. putida* and *Bacillus subtilis*, respectively (Tabel 1). Evolutionarily conserved regions were also found to exist over the predicted amino acid sequences.

Expression studies of the *dna K* and *dnaJ* genes of *S. violacea*

In order to understand pressure response (piezoresponse) at molecular level of the genes for *dnaK* and *dnaJ*, Northern blot analysis of RNAs from the moderately piezophilic bacterium, *Shewanella violacea* grown under varied pressure conditions, was carried out. As shown in Fig. 2, small quantity of mRNA for both *dnaK* and *dnaJ* genes was observed under atmospheric pressure

condition (0.1 MPa) and relatively large quantity of mRNA for both genes was observed under both 30 MPa and 50 MPa (Fig. 2).

**Table 1　Gene analysis of *dnaK* and *dnaJ* genes**

| | nt | aa | M.W. | pI | SD/initiation codon | stop codon | characteristics | identity |
|---|---|---|---|---|---|---|---|---|
| *dnaK* | 1,923 | 640 | 69,034 | 4.48 | tCaggagCACCCCATG | TAA | similar to chaperone protein DnaK in *Shewanella oneidensis* MR-1 | 86% |
| *dnaJ* | 1,311 | 376 | 40,883 | 8.24 | aaggAgAgCGTGAGAATATG | TAA | similar to chaperone protein DnaJ in *Shewanella oneidensis* MR-1 | 75% |

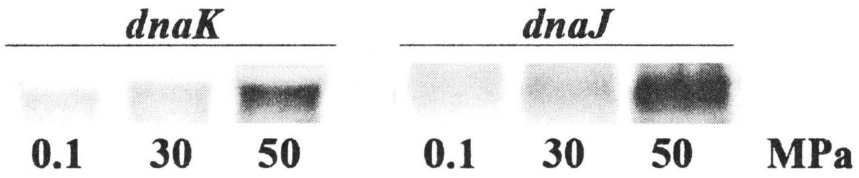


**Fig. 2. Northern blot analysis of mRNA for *dnaK* and *dnaJ* genes in *S. violacea* under the pressure conditions.**
Total RNA was isolated from the cell cultured at 0.1 MPa, 30 MPa, 50 MPa, respectively.

The result shows that the expression of the genes, *dnaK* and *dnaJ*, were positively controlled at transcriptional level by elevated pressure. The molecular basis for the mechanism in transcription under high pressure conditions may depends upon the natures upstream region (promoter sequence) of the gene for *groESL* operon. It is, therefore, necessary to determine transcription initiation site(s) of the operon and analyze sequence features of the *cis*-elements [8, 9].

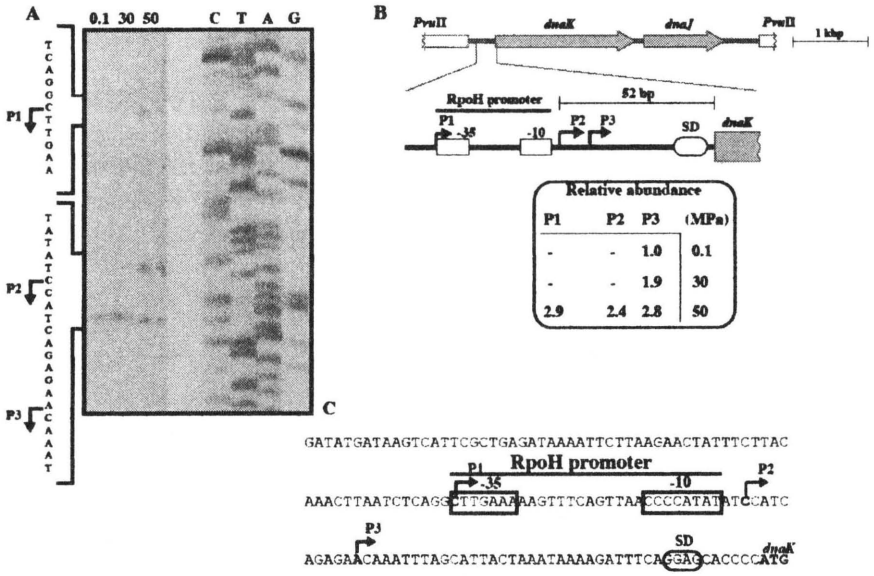


**Fig. 3.　Primer extension analysis of transcription of the *dnaKJ* region in *S. violacea* under different pressure conditions.**
Total cellular RNA was prepared from cells grown at 0.1, 10, 30 and 50 MPa. The sequencing ladder was generated using the same primers as those used in the dideoxy sequencing reactions. The transcripts are shown by arrows.

Primer extension and promoter analysis of the region upstream of the *dnaKJ* region.

To identify the promoter regions of the *dnaKJ* region, we determined the transcription initiation sites by primer extension analysis. As shown in Fig. 3A and B, tree transcription sites (P1, P2 and P3) for the *dnaKJ* region were detected. They were located from 42 to 82 nucleotides upstream of the translational initiation site of *dnaK*. The levels of these extension products were increased by

elevated pressures. The sequences of the -35 and -10 regions of the putative promoter (P2) for *groES* were CTTGAAA and CCCCATAT, respectively. These are similar to the consensus sequence of the $\sigma^{32}$-dependent promoter in *E. coli* [10]. No consensus sequences were not found in the promoter for the transcription initiation site for P1 and P3 (Fig. 3C). Similar results were reported in psychrophilic bacterium, Colwellia maris for *dnaK* and *dnaJ* genes [11]. For future study, detailed sequence analysis of the promoter elements is definitely required to understand molecular mechanisms for transcription under high pressure conditions.

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