Marine Genomics 30 (2016) 87–91

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

Genomics/technical resources

A transcriptome resource for the deep-sea bacterium *Shewanella piezotolerans* WP3 under cold and high hydrostatic pressure shock stress

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ARTICLE INFO

Article history:
Received 11 September 2016
Received in revised form 27 September 2016
Accepted 27 September 2016
Available online 4 October 2016

Keywords:
Deep-sea bacterium
Cold shock
High hydrostatic pressure
Microarray

ABSTRACT

Low temperature and high hydrostatic pressure (HHP) are two of the most remarkable environmental factors influencing deep-sea ecosystem. The adaptive mechanisms of microorganisms which live in these extreme environments to low temperature and high pressure warrant investigation. In this study, the global gene expression patterns of the deep-sea bacterium *Shewanella piezotolerans* WP3 in response to cold (0 °C) and HHP (50 MPa) shock were evaluated through DNA microarray analysis. Results revealed that 22, 66, and 106 genes were differentially expressed after WP3 was respectively exposed to cold shock for 30, 60, and 90 min. Of these genes, 16 genes were identified as common differentially expressed genes (DEGs). After 30 min and 120 min of HHP shock, 5 and 10 genes were respectively identified as DEGs. The hierarchical clustering analysis of the DEG pattern indicated that WP3 may employ different adaptive strategies to cope with cold and HHP shock stress. Taken together, our study provided a transcriptome resource for deep-sea bacterial responses to cold and HHP stress. This study also established a basis for further investigations on environmental adaptive mechanisms utilized by benthic bacteria.

1. Introduction

The deep-sea environments are generally characterized by permanently low temperature (2 °C to 4 °C) with high hydrostatic pressure (HHP) (10 MPa to 110 MPa) (Lauro and Bartlett, 2008; Arístegui et al., 2009; Orcutt et al., 2011). As such, microorganisms inhabiting benthic environments should adapt to these extreme conditions. In mesophilic bacteria, such as *Escherichia coli* (Phadtare et al., 1999; Yamanaka, 1999; Phadtare, 2004), cold shock response, a specific pattern of gene expression in response to abrupt shifts to low temperatures (Jones and Inouye, 1994), has been quite extensively studied. However, the response mechanisms of deep-sea bacteria to extreme cold conditions are largely unknown. The effects of HHP on transcription have been respectively demonstrated in *E. coli* and *Pyrococcus yayanosii*, an obligate piezophilic archaea, by microarray and RNA sequencing (Ishii et al., 2005; Michoud and Jebbar, 2016). Moreover, the global transcriptomic analysis of benthic bacterium *Photobacterium profundum* SS9 has also been performed (Campanaro et al., 2005; Vezzi et al., 2005; Campanaro et al., 2012).

In previous studies, the piezotolerant and psychrotolerant bacterium *Shewanella piezotolerans* WP3 (hereafter referred to as WP3) was isolated from deep-sea sediments at a depth of 1914 m in the west Pacific and extensively analyzed in terms of its adaptive mechanisms to the extreme benthic environment (Wang et al., 2004; Xiao et al., 2007; Wang et al., 2008; Jian et al., 2015; Jian et al., 2016). WP3 grows at a temperature range of 0 °C-28 °C (optimum growth at 20°C) and a pressure range of 0.1–50 MPa (optimum growth at 20 MPa) (Xiao et al., 2007). In our study, the whole genome DNA microarray of WP3 was used to investigate dynamics of global gene expression profiles in response to cold and HHP conditions. It is well-known that low temperature and HHP are two distinguishing features of the deep-sea environment. Therefore, our study helped enhance our understanding of the environmental adaptive strategies utilized by benthic bacteria.

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http://dx.doi.org/10.1016/j.margen.2016.09.004
1874-7787/

2. Data description

2.1. Bacterial culture conditions, cold and HHP shock treatment

The WP3 wild-type stain (WP3) was cultured in modified 2216E marine medium (2216E) (5 g/l tryptone, 1 g/l yeast extract, 0.1 g/l $FePO_4$, and 34 g/l NaCl). The single clone of the WP3 strain was inoculated into a 5 ml test tube, and the culture was used to inoculate to 100 ml of the same medium with shaking (220 rpm) at 20 °C (optimal growth temperature). The growth of the WP3 strain was determined using turbidity measurements at 600 nm with a spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). Samples (zero time) were obtained from the 100 ml culture when the cells reached the early exponential phase ($OD_{600} \approx 0.2$) under aerobic conditions. For cold shock treatment, an 80 ml aliquot was transferred to a 500 ml flask precooled to 0 °C and then incubated in a 0 °C water bath shaker. Subsequently, 20 ml culture aliquots were collected at 30, 60, and 90 min. For HHP shock treatment, the cultures were transferred into sterile syringes (50 ml), which were sealed with no air space and placed inside pressure vessels. Pin closure pressure vessels (Yayanos and Dietz, 1982) were used in this study (Feiyu Petrochemical Instrument Equipment Inc., Nantong, China). Pressure was applied using a hand-operated pump with a quick-fit connector to the pressure vessel. The cells were then incubated at a hydrostatic pressure of 50 MPa (200 atm) at 20 °C. Subsequently, 50 ml culture aliquots were collected at 30, and 120 min. For each treatment, 3 separate cultures were used. The samples were centrifuged for 30 s at the maximal speed (16,000 × g). The cells were immediately frozen in liquid nitrogen for subsequent RNA extraction.

2.2. RNA isolation

Total RNA was isolated from the WP3 cultures with TRI reagent-RNA/DNA/protein isolation kit (Molecular Research Center, Cincinnati, OH, USA) following the manufacturer's instructions as described previously (Wang et al., 2009; Jian et al., 2012). The quality of RNA samples was determined by running 1.0% Tris-acetate-EDTA agarose gel. Total RNA was treated with DNase I at 37 °C for 1 h to remove DNA contamination and purity was assessed by PCR amplification with RNA as template. The quantity and integrity of RNA were evaluated with a UV spectrophotometer (Nanodrop 2000c, Thermo Scientific). In general, the ratio of 260 nm/280 nm > 2 and 260 nm/280 nm ≈ 2.2 indicated that RNA was pure and could be used for follow-up microarray analysis.

2.3. Microarray analysis

The WP3 custom microarray design has been previously described (Table 1) (Jian et al., 2013). The total RNAs were reverse transcribed with Superscipt II (Invitrogen, Carlsbad, USA), and the cDNAs were labeled with Cy3 and Cy5 by using a Klenow enzyme (Takara Bio Inc, Shiga, Japan) based on the manufacturers' instructions. Labeled cDNA was purified with a PCR purification kit (Macherey-Nagel, Düren, Germany) and resuspended in elution buffer. The labeling efficiency was evaluated with a UV spectrophotometer (Nanodrop 2000c, Thermo Scientific), and the florescence should be >150 pmol. Labeled controls and test samples were quantitatively adjusted based on the efficiency of Cy-dye incorporation and mixed with 30 μl of hybridization solution (50% formamide, 1× hybridization buffer; Amersham Biosciences). The DNA in hybridization solution was denatured at 95 °C for 3 min prior to loading onto a microarray. The arrays were hybridized overnight at 42 °C and washed with two consecutive solutions (0.2% SDS, 2× SSC for 5 min at 42 °C, and 0.2× SSC for 5 min at RT). The microarray slides were hybridized with cDNA prepared from three biological replicate samples. As a measure of technical replication, the dye-swap experiment was performed on each sample so that a total of six data points were available for every ORF on the microarrays.

Table 1
Characteristics of the transcriptome analysis project, compliant with the MIGS standard.

Item	Description
Investigation_type	Prokaryote
Project_name	Transcriptome analysis of the deep-sea bacterium *Shewanella piezotolerans* WP3 under cold and high pressure shock stress
Collected_by	Shengkang Li
Collection_date	2005-08
Sample source location	China (Xiamen, Fujian)
Organism	*Shewanella piezotolerans* WP3
Isolation environment	West Pacific sediment at a depth of 1914 m (Wang et al., 2004)
Biome	ENVO:01000320 (marine environment)
Feature	ENVO:01000007 (microbial feature)
Material	ENVO:00002113 (marine sediment)
Genome information	GenBank accession no. CP000472 (Wang et al., 2008)
Sequencing method or array type	CapitalBio custom designed *S. piezotolerans* WP3 genome array
Microarray Platform	GEO accession no. GPL16568
Microarray Series	GEO accession no. GSE82259 and GSE82267
Sample material	*S. piezotolerans* WP3 pure culture in 2216E medium
Data format	Raw data: GPR files, normalized data: EXCEL files
Experimental factors	Cold shock at 0 °C for 30, 60, and 90 min; high hydrostatic pressure shock at 50 MPa for 30 and 120 min
Experimental features	Whole genome analysis to identify genes response to cold and high pressure shock stress

A LuxScan 10K scanner and microarray scanner 2.3 software (CapitalBio, Beijing, China) were used for array image acquisition. We quantified the signal intensities of individual spots from the 24-bit TIFF images using SpotData Pro 2.2 (CapitalBio, Beijing, China). The linear normalization method was used for data analysis, and it was based on the expression levels of the WP3 housekeeping genes in combination with the yeast external controls. The normalized data were log-transformed and loaded into MAANOVA under R environment for multiple testing, by fitting a mixed-effects ANOVA model (Wu et al., 2003). Microarray spots with P values <0.01 in Student's *t*-test and the average fold-change ratio >2 or <0.5 were considered as differentially expressed genes (DEGs).

2.4. Identification of differentially expressed genes (DEGs) in cold shock stress

Overall, 22 genes, including 11 up-regulated and 11 down-regulated genes, were differentially expressed after 30 min of cold shock (Supplementary Table S1). A total of 66 (34 and 32 genes with increased and decreased transcription levels, respectively) and 106 (45 and 61 genes with increased and decreased transcription levels, respectively) DEGs were detected in WP3 after 60 and 90 min of cold shock, respectively (Supplementary Tables S2 and S3). The differentially transcribed genes were then subjected to functional classification with the Clusters of Orthologous Groups of Proteins database (Fig. 1A). Furthermore, the 16 genes that respectively accounted for 72.7%, 24.2%, and 15.1% of the total number of DEGs in the 30, 60, and 90 min cold shock microarray dataset were identified as common DEGs (Fig. 1B and Supplementary Table S4). These DEGs may comprise the core genes responsible for the response of WP3 to cold shock stress.

2.5. Identification of DEGs in HHP shock stress

A total of 5 (4 and 1 genes with up- and down-regulated transcription levels, respectively) and 10 (5 and 5 genes with up- and down-regulated transcription levels, respectively) DEGs were detected in WP3 after 30 and 120 min of HHP shock, respectively (Supplementary Tables S5 and S6). Hierarchical clustering analysis was performed to identify the common DEGs involved in the responses of WP3 to cold and HHP shock (Fig. 2). Surprisingly, no common DEGs were identified