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Data in Brief

Microarray analysis of *lexA* gene deletion mutant of deep-sea bacterium *Shewanella piezotolerans* WP3 at low-temperature and high-pressure

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ABSTRACT

Addressing DNA lesion, the SOS response is conserved in bacterial domain and governed by DNA binding protein LexA, which have been well characterized in model microorganism such as *Escherichia coli*. However, our understanding of the roles of SOS pathway in deep-sea bacteria is limited. To indentify the composition of SOS regulon and function of LexA, we performed whole genome transcriptional profiling using a custom designed microarray which contains 95% open reading frames of *Shewanella piezotolerans* WP3. Here we describe the experimental procedures and methods in detail to reproduce the results (available at Gene Expression Omnibus database under GSE66790) and provide resource to be employed for comparative analyses of SOS response in microorganisms which inhabited in different environments, and thus broaden our understanding of life strategy of bacteria against various environment stresses.

Specifications	
Organism/cell line/tissue	*Shewanella piezotolerans* WP3
Sex	N/A
Sequencer or array type	CapitalBio custom designed *S. piezotolerans* WP3 genome array
Data format	Raw data: LSR files, normalized data: EXCEL files
Experimental factors	*lexA* gene mutant vs. wild-type strain
Experimental features	Whole genome analysis to identify genes response to *lexA* gene deletion at 20 MPa and 4 °C
Consent	N/A
Sample source location	Shanghai, China

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66790

Experimental design, materials and methods

Bacterial culture conditions and RNA isolation

The *Shewanella piezotolerans* WP3 (hereafter referred to as WP3) strains were cultured in modified 2216E marine medium (2216E) (5 g/l tryptone, 1 g/l yeast extract, 0.1 g/l $FePO_4$, 34 g/l NaCl). The single clone of WP3 strains were inoculated into a 5 ml test tube with shaking (220 rpm) at 0.1 MPa (1 atm) and 20 °C. The culture was diluted 1000-fold in the same medium and was grown to late-exponential phase ($OD_{600} \approx 2.0$), and then 100 μl cultures were transferred into 5 syringes (50 ml), which were sealed with no air space and placed inside pressure vessels. Pin closure pressure vessels [1] 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 20 MPa (200 atm) at 4 °C. The growth of the WP3 strains was determined using turbidity measurements at 600 nm with a spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). The culture of WP3 wild-type stain (WP3-WT) and *lexA* gene mutant (WP3Δ*lexA*) was collected immediately when the cells reached late-exponential phase ($OD_{600} \approx 0.15$). The samples were centrifuged for 30 s at the maxim speed (16,000 × *g*). The cells were immediately frozen in liquid nitrogen for subsequent RNA extraction.

Total RNA was isolated from the WP3 culture growing at different temperatures with TRI reagent-RNA/DNA/protein isolation kit (Molecular Research Center, Cincinnati, USA) according to the manufacturer's instructions as described previously [2,3]. The quality of RNA samples were determined by visualizing the nearly 2:1 ratio of 23S:16S ribosomal RNA by running a 1.0% TAE (Tris-Acitate-EDTA) agarose gel (Fig. 1A). The total RNA was treated with DNase I at 37 °C for 1 h to remove DNA contamination and the purity was checked by PCR amplification with RNA as template. The quantity and integrity of RNA was evaluated with a UV spectrophotometer (Nanodrop 2000c, Thermo Scientific).

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In general, the ratio of 260 nm/280 nm > 2 and 260 nm/280 nm ≈ 2.2 indicate the RNA is pure and could be used for the follow-up microarray analysis.

PCR primers for 4744 of the 4945 predicted ORFs in the WP3 genome (excluding 200 CDSs shorter than 150 bp) were designed using Primer version 5.0 and then synthesized (BioAsia Biotech, Shanghai, China). The following criteria were used to identify the optimal forward and reverse primers to generate PCR products specific for each selected ORF: (1) the entire ORF was used as a probe if it was <75% similar to all other genes in the genome; (2) for homologous genes, the maximal portion of the genes showing <75% similarity was selected as specific probes; (3) for homologous genes where no specific fragments could be identified, one of the genes was selected as a probe to represent the entire gene group; (4) each oligonucleotide primer contained 20 to 25 bases. To simplify the PCR amplification, most of the primer sets were designed to have annealing temperatures of ~60 °C. ORF-specific fragments were amplified by Taq DNA polymerase with the following cycling conditions: denaturation for 30 s at 95 °C, annealing for 1 min at 60 °C and extension for 1.5 min at 72 °C, along with an initial 5 min denaturation at 95 °C and a final extension of 10 min at 72 °C. All PCR products were purified using ethanol precipitation. The quality of the amplified products was checked by 1.5% agarose gel electrophoresis and ethidium bromide staining. Amplified DNA fragments were considered correct if the PCR results contained a single product of the expected size. The PCR for 94 genes consistently failed to yield satisfactory products (e.g., no product, product of the wrong size, multiple or faint bands). Of the 4744 genes with designed primers, 4650 ORFs were correctly amplified. We used specific 70-mer oligonucleotides to represent the 39 ORFs which were not successfully amplified. In total, the PCR amplicons and oligonucleotide probes represented 95% of the total predicted gene content of WP3. The PCR products and microarray reagents were arrayed from 384-well microtiter probes printed in triplicate onto Telechem Superamine slides (Telechem, Sunnyvale, USA). The printed slides were dried and subjected to UV cross-linking (Scientz, Ningbo, China).

Preparation of fluorescent dye-labeled DNA and hybridizations

The total RNAs were reverse transcribed with Superscript II (Invitrogen, Carlsbad, USA) and the cDNAs were labeled with Cy3 and Cy5 by using a Klenow enzyme (Takara Bio Inc., Shiga, Japan) according to the manufacturer's 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 value should be >150 pmol. Labeled controls and test samples were quantitatively adjusted based on the efficiency of the 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 2 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 3 biological replicate samples. As a measure of technical replication, the dye-swap experiment was performed on each sample so that a total of 6 data points were available for every ORF on the microarrays.

Image acquisition, data processing and validation

A LuxScan 10 K scanner and microarray scanner 2.3 software (CapitalBio, Beijing, China) were used for the 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, based on the expression levels of 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 testings, by fitting a mixed-effects ANOVA model [4]. Microarray spots with P values < 0.001 in the F-test were regarded as differentially expressed genes (DEGs) (Fig. 1B). In addition, all of the DEGs were confirmed with Significance Analysis of Microarrays (SAM) software [5].

To validate the microarray data, 7 genes, including those that were up-regulated, down-regulated or unchanged, were selected for quantitative real-time PCR (qPCR) (ABI, Foster City, CA, USA). It is a need to be pointed out that the same samples were used for the microarray and qPCR. The relative mRNA levels for each gene were calculated and log-transformed. The correlation coefficient (R^2) between the data obtained by microarray and qPCR was 0.9599 (Fig. S1), demonstrating that the microarray data is reliable.

Discussion

Here we describe the data of differentially expressed genes in *lexA* deletion mutant by compared to wild-type strain using our

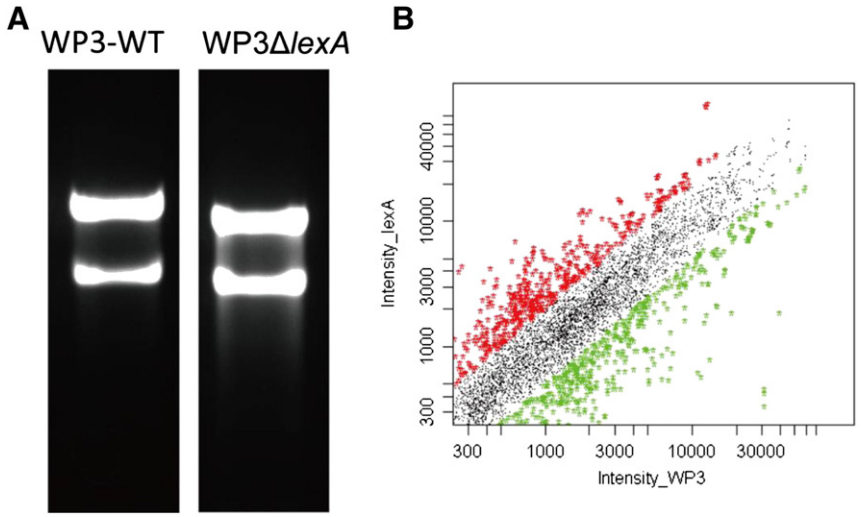

Fig. 1. A. Electrophoresis of total RNA of WP3 wild-type strain and *lexA* gene mutant. B. Overview of gene expression by compares WP3-WT with WP3Δ*lexA* in the present experiment. X and Y axes present the intensity of gene transcription in WP3-WT and WP3Δ*lexA*, respectively. The black dots indicate genes with no significant change of transcriptional level, while red and green dots indicate up-regulated and down-regulated genes, respectively.

custom designed genome-wide *S. piezotolerans* array. The microarray data is of high quality as evidenced by the SAM analysis and qPCR validation. The *Shewanella* species are well-known for their versatile respiration ability and widely distributed in aquatic environment including deep-sea [6,7]. Meanwhile, the LexA protein is the key transcriptional regulator of SOS pathway [8]. The present data thus would significantly assist investigation of the function of SOS response and adaptation mechanism of bacterium in the extreme deep-sea environment.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.03.013.

Conflict of interest

The authors declare no conflict of interest.

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