Genomics Data 5 (2015) 51–53

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Data in Brief

Transcriptional profiling of CRP-regulated genes in deep-sea bacterium *Shewanella piezotolerans* WP3

Huahua Jian [a], Jing Hu [a], Xiang Xiao [a,b,*]

[a] State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, PR China
[b] State Key Laboratory of Ocean Engineering, School of Naval Architecture, Ocean and Civil Engineering, Shanghai Jiao Tong University, Shanghai, PR China

ARTICLE INFO

Article history:
Received 12 April 2015
Accepted 21 April 2015
Available online 30 April 2015

Keywords:
CRP
Deep-sea
Microarray
Shewanella
Gene regulation

ABSTRACT

The cAMP receptor protein (CRP) is a conserved regulator in bacteria and involved in regulation of energy metabolism, such as glucose, galactose, and citrate (Green et al., 2014 [1]). As an important catabolite activator protein, it has been well characterized in model microorganism such as *Escherichia coli*. However, our understanding of the roles of CRP in deep-sea bacteria is rather limited. To indentify the function of CRP, we performed whole genome transcriptional profiling using a custom designed microarray which contains 95% open reading frames of *Shewanella piezotolerans* WP3, which was isolated from West Pacific sediment at a depth of 1914 m (Xiao et al., 2007 [2]; Wang et al., 2008 [3]). Here we describe the experimental procedures and methods in detail to reproduce the results (available at Gene Expression Omnibus database under GSE67731 and GSE67732) and provide resource to be employed for comparative analyses of CRP regulon and the regulatory network of anaerobic respiration in microorganisms which inhabited in different environments, and thus broaden our understanding of mechanism 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	*crp* gene mutants *vs.* wild-type strain
Experimental features	Whole genome analysis to identify genes response to *crp* gene deletions at 20 °C
Consent	N/A
Sample source location	Shanghai, China

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67731.
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67732.

* Corresponding author at: State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, PR China. Tel.: +86 21 34207206.
E-mail address: xoxiang@sjtu.edu.cn (X. Xiao).

2. Experimental design, materials and methods

2.1. Construction of crp gene deletion mutants

Two *crp* deletion mutants were constructed by the method as described previously [4]. First, the upstream and downstream fragments flanking both sides of the cAMP binding domain (D1) and DNA binding domain (D2) of *crp* gene were amplified with PCR primer pairs (Supplemental Table 1), respectively. These two fragments were used as templates in a second fusion PCR, resulting in a fragment with a deletion in the *crp* gene. Then, the PCR products were cloned into pRE112, yielding pRE112-*crpD1* and pRE112-*crpD2*, respectively. These plasmids were transformed into *Escherichia coli* WM3064 and then moved into *Shewanella piezotolerans* WP3 (hereafter referred to as WP3) by two-parent conjugation. The transconjugant was selected by chloramphenicol resistance and verified by PCR. The WP3 strains with pRE112-*crpD1* and pRE112-*crpD2* inserted into the chromosome were plated on 2216E agar medium supplemented with 10% sucrose. The successful *crp* deletion mutants were screened and confirmed by PCR (Table 1).

2.2. Bacterial culture conditions

For aerobic cultivation, the WP3 strains were cultured in modified 2216E marine medium (2216E) (5 g/l tryptone, 1 g/l yeast extract,

http://dx.doi.org/10.1016/j.gdata.2015.04.019

Table 1
Bacterial strains used in microarray study.

Strain	Description	Source
WP3	Wild-type strain of *Shewanella piezotolerans* WP3, GenBank accession number CP000472	Lab stock
WP3Δ*crpD1*	WP3, deletion mutant of cAMP binding domain of *crp* gene	This study
WP3Δ*crpD2*	WP3, deletion mutant of DNA binding domain of *crp* gene	This study

0.1 g/l $FePO_4$, 34 g/l NaCl). The single clone of WP3 strains was inoculated into a 5 ml test tube, and then the culture was diluted 1000-fold in the same medium with shaking (220 rpm) at 20 °C. The culture was. For anaerobic cultivation, an oligotrophic medium (0.1 g/l tryptone, 0.2 g/l yeast extract, 34 g/l sodium chloride, 4.8 g/l HEPES, and 3.4 g/l sodium lactate) was dispensed into serum bottles gassed with O_2-free nitrogen. After the media were autoclaved, fumarate (20 mM) was added as electron acceptor. 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) and *crp* gene mutants (WP3Δ*crpD1* and WP3Δ*crpD2*) was collected immediately when the cells reached exponential phase ($OD_{600} \approx 1.2$) under the aerobic condition (Fig. 1A). 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.

2.3. RNA isolation

Total RNA was isolated from the WP3 cultures with TRI reagent-RNA/DNA/protein isolation kit (Molecular research center, Cincinnati, USA) according to the manufacturer's instructions as described previously [5,6]. The quality of RNA samples was determined by running a 1.0% TAE (Tris-Acetate-EDTA) agarose gel (Fig. 1B). 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). In general, the ratios of 260 nm/280 nm > 2 and 260 nm/280 nm ≈ 2.2 indicate that the RNA is pure and could be used for the follow-up microarray analysis.

2.4. WP3 custom microarray designing

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; and (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).

2.5. Preparation of fluorescent dye-labeled DNA and hybridizations

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) according to 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 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

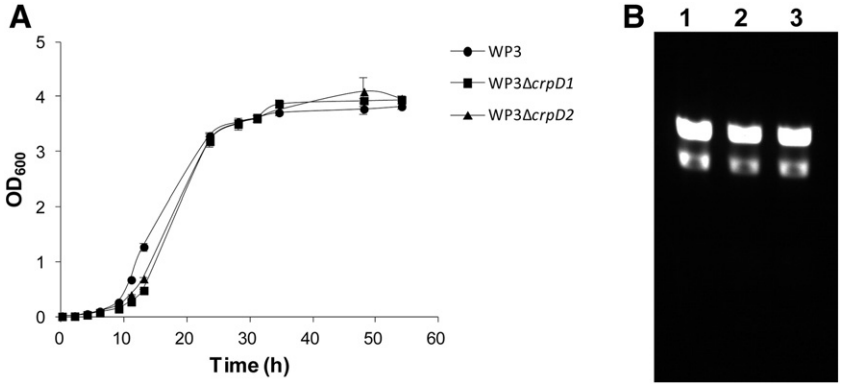

Fig. 1. (A) Growth curve of WP3ΔcrpD1 and WP3ΔcrpD2 at 20 °C. The assays were performed in 2216E medium and oligotrophic medium, respectively. The average values and standard deviations displayed by the error bars resulted from three replicates. (B) Electrophoresis of total RNA of WP3 wild-type strain and crp gene mutants. Lane 1: WP3, lane 2: WP3ΔcrpD1, and lane 3: WP3ΔcrpD2.

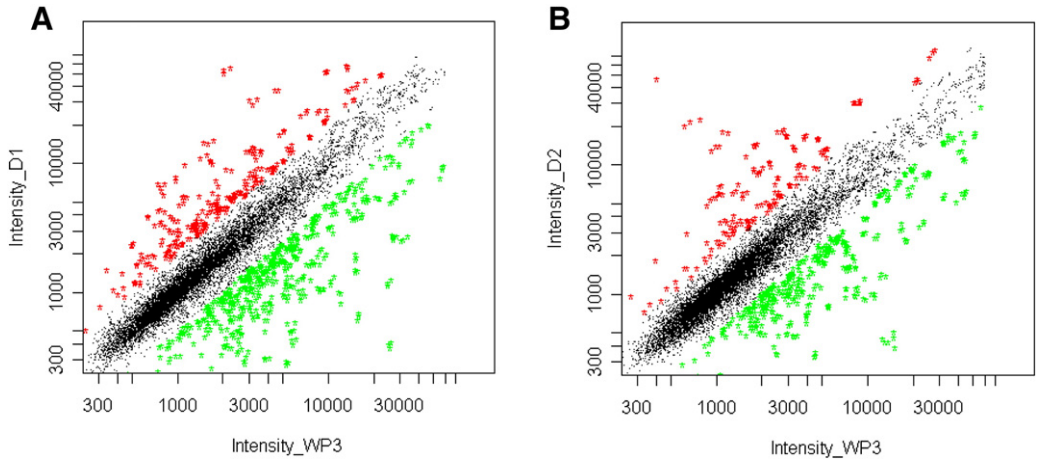

Fig. 2. Overview of gene expression by compares WP3-WT with WP3ΔcrpD1 (A) and WP3ΔcrpD2 (B) in the present experiment. X and Y axes present the intensity of gene transcription in WP3-WT and crp gene mutants, respectively. The black dots indicate genes with no significant change of transcriptional level, while red and green dots indicate up-regulated and down-regulated genes, respectively.

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.

2.6. 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 [7]. Microarray spots with P values < 0.001 in the F-test were regarded as differentially expressed genes (DEGs) (Fig. 2). In addition, all of the DEGs were confirmed with Significance Analysis of Microarrays (SAM) software [8].

3. Discussion

Here we describe the data of differentially expressed genes in two *crp* gene deletion mutants compared to wild-type strain using our custom designed genome-wide *S. piezotolerans* array. The *Shewanella* species are well-known for their versatile respiration ability and widely distributed in aquatic environment including deep-sea [2,3,9,10]. Meanwhile, the CRP protein is the key transcriptional regulator of anaerobic respiration [1,11]. Thus further investigations are required to clear the function of CRP 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.2015.04.019.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Grant No. 31290232), China Ocean Mineral Resources R & D Association (Grant No. DY125-15-T-04) and the National Natural Science Foundation of China (Grant No. 41306129).

References

[1] J. Green, et al., Cyclic-AMP and bacterial cyclic-AMP receptor proteins revisited: adaptation for different ecological niches. Curr. Opin. Microbiol. 18 (2014) 1–7.
[2] X. Xiao, et al., *Shewanella psychrophila* sp. nov. and *Shewanella piezotolerans* sp. nov., isolated from west Pacific deep-sea sediment. Int. J. Syst. Evol. Microbiol. 57 (Pt 1) (2007) 60–65.
[3] F. Wang, et al., Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium *Shewanella piezotolerans* WP3. PLoS One 3 (4) (2008) e1937.
[4] H. Jian, X. Xiao, F. Wang, Role of filamentous phage SW1 in regulating the lateral flagella of *Shewanella piezotolerans* strain WP3 at low temperatures. Appl. Environ. Microbiol. 79 (22) (2013) 7101–7109.
[5] F. Wang, et al., Role and regulation of fatty acid biosynthesis in the response of *Shewanella piezotolerans* WP3 to different temperatures and pressures. J. Bacteriol. 191 (8) (2009) 2574–2584.
[6] H. Jian, et al., Dynamic modulation of DNA replication and gene transcription in deep-sea filamentous phage SW1 in response to changes of host growth and temperature. PLoS One 7 (8) (2012) e41578.
[7] *Wu, H.*, et al., MAANOVA: a software package for the analysis of spotted cDNA microarray experiments. The Analysis of Gene Expression Data, 2003: p. 313–341.
[8] V.G. Tusher, R. Tibshirani, G. Chu, Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. U. S. A. 98 (9) (2001) 5116–5121.
[9] H.H. Hau, J.A. Gralnick, Ecology and biotechnology of the genus *Shewanella*. Annu. Rev. Microbiol. 61 (2007) 237–258.
[10] J.K. Fredrickson, et al., Towards environmental systems biology of *Shewanella*. Nat. Rev. Microbiol. 6 (8) (2008) 592–603.
[11] D.A. Saffarini, R. Schultz, A. Beliaev, Involvement of cyclic AMP (cAMP) and cAMP receptor protein in anaerobic respiration of *Shewanella oneidensis*. J. Bacteriol. 185 (12) (2003) 3668–3671.