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Data in Brief

Transcriptional profiling of CcpE-regulated genes in *Staphylococcus aureus*

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ABSTRACT

The transcriptional regulator CcpE is an important citrate-sensing regulator that modulates metabolic state, virulence factor expression, and bacterial virulence of *Staphylococcus aureus* (Ding et al., 2014 [1]). In this article, we report detailed methods for genome-wide transcriptional profiling of CcpE-regulated genes generated for the research article "Metabolic sensor governing bacterial virulence in *Staphylococcus aureus*" (Ding et al., 2014 [1]). All transcriptional profiling data was deposited to Gene Expression Omnibus (GEO) database under accession number GSE57260.

Specifications	
Organism/cell line/tissue	*Staphylococcus aureus* Newman
Sex	N/A
Sequencer or array type	Affymetrix *S. aureus* Genome Array
Data format	Raw and analyzed
Experimental factors	*ccpE* deletion mutant versus wild-type Newman strain
Experimental features	Microarray comparison was preformed to identify genes differentially expressed in *ccpE* deletion mutant compared to wild-type Newman in TSB medium without glucose
Consent	N/A
Sample source location	Shanghai, China

1. Direct link to deposited data

Microarray data is accessible under the following link: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57260.

2. Experimental design, materials and methods

2.1. Bacterial strains and growth conditions

The strains used for transcriptome analysis are *Staphylococcus aureus* Newman and its Δ*ccpE* derivative [1]. Overnight cultures of *S. aureus* Newman and its *ccpE* deletion mutant (Δ*ccpE*) were washed and diluted 100-fold in fresh TSB medium (without glucose) in a 20-ml tube with a tube volume-to-medium volume ratio of 5:1 and in triplicates. The liquid culture was grown at 37 °C for about 6 h ($OD_{600} \approx 5.0$) with shaking, 250 rpm of aeration.

2.2. RNA extraction

Total RNA was immediately stabilized with an RNAprotect Bacteria Reagent (Qiagen, Valencia, CA) following the manufacturer's instructions. The *S. aureus* cells were harvested by centrifugation and suspended in 1 ml 1× TE (10 mM Tris, pH 8.5, 1 mM EDTA) buffer containing lysostaphin (final concentration of 50 μg/ml), suspensions were incubated at 37 °C for 30 min. The bacteria were subsequently disrupted by mechanical disruption (FastPrep®-24 Instrument from MP Biomedicals) and RNA extraction was performed as described before [4,5] through the use of an RNeasy Mini kit (Qiagen, Valencia, CA) following the manufacturer's instructions. DNase digestion was performed according to the manual provided with an RNeasy Mini kit. The concentration of RNA was checked on a spectrophotometer and the quality of RNA was checked using formaldehyde agarose gel electrophoresis and Agilent RNA analysis kit (Agilent technologies).

2.3. cDNA preparation and labeling

The cDNA preparation and labeling were performed by CapitalBio Corp (http://www.capitalbio.com/index.asp, Beijing, China) according to the manufacturer (Affymetrix, Santa Clara, CA) with some minor modifications. In short, total RNA (10 μg) was mixed with 10 μl random

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primers (75 ng/μl) and 2 μl diluted poly-A RNA controls and nuclease free water was added if necessary to keep the final volume of the annealing mixture to 30 μl according to the standard Affymetrix protocol (GeneChip Expression Analysis Technical Manual, Chapter 4 Prokaryotic Target Preparation. 702232, Rev.3). The annealing mixture was kept at 70 °C for 10 min and then at 25 °C for 10 min, and hold at 4 °C. 30 μl of master mix for cDNA synthesis [consisting of 12 μl 5× first strand buffer, 6 μl 100 mM DTT, 3 μl 10 mM dNTPs, 1.5 μl SUPERase-IN (20 U/μl) RNase inhibitor and 7.5 μl SuperScript II reverse transcriptase (200 U/μl)] was added to the annealing mixture and the reaction mix was incubated at the following temperatures: 25 °C for 10 min; 37 °C for 60 min; 42 °C for 60 min; 70 °C for 10 min (in order to inactive SuperScript II) and chill to 4 °C. Following the incubation, the mRNA from the reaction mixture was degraded by adding 20 μl of 1 N NaOH and placing the reactions at 65 °C for 30 min. To neutralize the NaOH in reactions, 20 μl of 1 N HCl was added. The cDNA mixture was purified by using a MinElute PCR Purification Kit (Qiagen) and the purified cDNA product was analyzed by reading the absorbance under 260 nm for the quantification (1.0 A_{260} unit = 33 μg/ml of single-stranded DNA). cDNA was end-labeled (3′) using a GeneChip labeling reagent (Affymetrix, P/N 900542) and following the manufacturer's protocol.

2.4. Hybridization and washing

The labeled cDNA (0.5–7.0 μg) was then hybridized to the Affymetrix GeneChip *S. aureus* genome array (Affymetrix, Cat. no. 900514) for 16 h at 50 °C through the use of the GeneChip hybridization oven at 60 rpm. Washing, staining, and scanning were performed using the Affymetrix GeneChip system at CapitalBio Corp (http://www.capitalbio.com/index.asp, Beijing, China). Standard Affymetrix protocols were utilized.

2.5. Microarray data analysis

Microarray slides were scanned and pre-analyzed using GeneChip® Operating Software (GCOS) version 1.2 (Affymetrix) and GeneSpring GX version 7.3 (Agilent Technologies, Inc., Santa Clara, CA) at CapitalBio Corp. Raw data files were also deposited on GEO under the accession number GSE57260. The microarray data were further normalized using Robust Multi-array Average (RMA) [6,7]. Gene expression analysis was performed using three biological replicate mRNA samples for each strain which were analyzed with SAM (Significance Analysis of Microarrays) software [8]. For differentially expressed genes, fold change ≥2 or ≤0.5 and q-value ≤5% was used as standard.

Conflict of interest

The authors declare no conflict of interest.

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