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Transcriptome analysis of *Streptococcus pneumoniae* D39 in the presence of cobalt

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**ABSTRACT**

Cobalt (Co^{2+}) is an important transition metal ion that plays a vital role in cellular physiology of bacteria. The role of Co^{2+} in the regulation of several genes/operons in *Streptococcus pneumoniae* has recently been reported [1]. The data described in this article relate to the genome-wide transcriptional profiling of *Streptococcus pneumoniae* D39, either in the presence or absence of 0.5 mM Co^{2+} in chemically defined medium (CDM) using DNA microarray analysis. Genes belonging to a broad range of cellular processes such as virulence, transport and efflux systems, stress response and surface attachment were differentially expressed in the presence of Co^{2+}. We used transcriptional lacZ assays and electrophoretic mobility shift assays (EMSAs) to confirm our results [1]. The dataset is publicly available at the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE57696.

**Keywords:** Co^{2+}, PsaR, *Streptococcus pneumoniae*, Microarray

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**1. Specifications**

| Organism/cell line/tissue | Streptococcus pneumoniae strain D39 |
|---------------------------|-------------------------------------|
| Sex                       | N/A                                 |
| Sequeror or array type    | Oligo-based DNA microarray          |
| Data format               | Raw and processed                   |
| Experimental factors      | N/A                                 |
| Experimental features     | Differentially expressed genes were identified by microarray comparison of D39 wild-type grown in CDM + 0 mM Co^{2+} to D39 wild-type grown in CDM + 0.5 mM Co^{2+} in CDM |
| Consent                   | N/A                                 |
| Sample source location    | Groningen, The Netherlands          |

**2. Direct link to deposited data**

The raw and processed DNA microarray dataset has been deposited in the Gene Expression Omnibus (GEO) database and can be accessed under following link: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57696.

**3. Experimental design, materials and methods**

**3.1. Objective of the experiment**

Our objective was to investigate the impact of Co^{2+} on the gene expression of *S. pneumoniae*.

**3.2. Strains and growth conditions**

*S. pneumoniae* D39 serotype 2 strain (cps2), obtained from the laboratory of Prof. Peter Hermans, was used in this study [2]. The chemically defined medium (CDM) was treated with 1% Chelex 100 Resin (Bio-Rad) to ensure a metal depleted environment (medium). 50 ml of cell culture of *S. pneumoniae* D39 was grown in the CDMchelex either with or without 0.5 mM Co^{2+} at 37 °C in replicates. Cells were collected at an optical density of 0.2–0.25 (i.e. mid-exponential growth phase) at 600 nm (OD_{600}) by centrifugation for 1 min at 4 °C. The cell pellets were maintained at −80 °C if not processed immediately.

**3.3. Total RNA extraction and removal of ribosomal RNA**

Total RNA from the samples were isolated as described [3]. In short, cell pellets were resuspended in 400 μl of nuclease free water (DEPC-treated), after which 50 μl of 10% SDS, 500 μl of phenol/chloroform (1:1) and 500 mg glass beads were added and lysed by beat beater in the screw-capped tubes. Total RNA was isolated by the combination of
the Macoid method and the RNA isolation Kit (Roche) from lysed cells. DNA contamination was eliminated from the RNA sample by treatment with 2U of RNase free DNase I (Invitrogen, Paisley, United Kingdom). A NanoDrop Spectrophotometer (NanoDrop Technologies, Inc.) was used to determine the RNA concentration and sample quality was assessed using an Agilent RNA analysis kit (Agilent technologies).

3.4. cDNA preparation, hybridization and data acquisition
15 μg of RNA was mixed with 2 μl random nonamers (1.6 μg/μl) to prepare the annealing mixture. The volume of the annealing mixture was kept at 18 μl by the addition of nuclease free water (DEPC-treated), if required. The reaction mixture was kept at 70 °C for 5 min following 10 min cooling step at room temperature. 12 μl of master mix was prepared for each sample by the addition of 6 μl 5X first strand buffer [250 mM Tris–HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2], 3 μl 0.1 M DDT, 1.2 μl 25X AA-DUTP/nucleotide mix, and 1.8 μl Superscript III reverse transcriptase. The master mix was added to the annealing mixture carefully, and incubated at 42 °C for 2–16 h. After incubation, the reaction mixture was treated with 3 μl of 2.5 M NaOH at 37 °C for 15 min to remove the mRNA from the reaction mixture. After that 15 μl of 2 M HEPES free acid was added in the reaction mixture to neutralize the NaOH. The cDNA mixture was purified by the DNA purification Kit (NucleoSpin, Gel and PCR clean-up kit), following the manufacturer’s protocol. cDNA samples were labeled with DyLight-550 and DyLight-650 in dye-swap.

We combined the equal quantities of labeled cDNAs (max 30% difference), and dried the samples using the vacuum concentrators at high temperature (approx. 40 min) until the volume was smaller than 7 μl. The dried samples were dissolved in 7 μl H2O and incubated at 94 °C for 2 min. Finally, hybridization was performed with labeled cDNA for 16 h at 45 °C in Ambion Slidehyb #1 hybridization buffer on in house built super amine glass slides (Array-It, SMMBC) containing ampiclon of on average 600 bp representing 2087 ORFs of S. pneumoniae TIGR4 [4] and 184 ORFs specific for S. pneumoniae R6 [5]. 0.5 pmol/μl was taken as the minimum concentration of DyLight550 or DyLight650 in a total eluted volume of 50 μl. After hybridization, slides were washed using freshly prepared wash-buffers I, II and III and scanned at appropriate wavelengths in the scanner as described before [3].

3.5. Microarray data analysis
The microarray scanned slides were analyzed in GenePix Pro 6.0 Microarray Acquisition and by Analysis Software [6]. Raw data files were deposited on GEO under the accession number GSE57696. After initial analysis, the normalization and processing of the data was performed using different Microprep software package (Table 1). Statistical analyses were performed as described previously [7]. Finally, Cyber-T was used to analyze the data generated using Microprep for the identification of statistically significant differentially expressed genes. False discovery rates (FDRs) were calculated as described [8]. For differentially expressed genes, p < 0.001 and FDR < 0.05 were taken as a standard. Genes exhibiting a fold change ≥2.0 and a p-value < 0.05 were considered differentially expressed. Software packages mentioned in Table 1 were used for further data interpretation.

4. Discussion
Here, we have investigated the impact of Co2+ on the global gene expression of S. pneumoniae D39 by DNA microarray analysis. Transcriptome comparison of D39 wild-type grown in CDM with 0 mM Co2+ to same strain grown in CDM with 0.5 mM Co2+, revealed the impact of Co2+ on the gene expression of S. pneumoniae D39. 24 genes were downregulated (Table 2) and 14 genes were upregulated (Table 3). The PsrA regulon (pscA, psbBCA and ptrA), the cbi operon, and the nrd operon were highly downregulated in the absence of Co2+, suggesting the role of Co2+ in the regulation of these systems. This was further confirmed by β-galactosidase assays, metal accumulation assays and electrophoretic mobility shift assays (EMSA) [11]. The expression of some other genes was also altered in our transcriptome analysis and further investigations are required to clear the role of Co2+ in the regulation of these genes.

| Software       | Purpose                                      | URL                          |
|----------------|----------------------------------------------|------------------------------|
| Microprep [8]  | A cDNA microarray data pre-processing framework | http://www.molgenrug.nl/index.php/molgensoftware |
| CyberT         | Amplementation of a variant of t-test        | http://bioinformatics.biol.rug.nl/cybert/index.shtml |
| Genome2D [9]   | A visualization tool for the rapid analysis of bacterial transcriptome data | http://genome2d.molgenrug.nl/ |
| FIVA [10]      | Functional Information Viewer and Analyzer extracting biological knowledge from transcriptome data of prokaryotes | http://bioinformatics.biol.rug.nl/standalone/fiva/ |
| Projector [11] | A webserver for prediction of prophylacte promoter elements and regulons | http://bamins2.cmbi.ru/website/software/projector2/projector2_start.php |
| PePPER [12]    | Automatic contig mapping for gap closure purposes | http://pepper.molgenrug.nl/ |

Table 1: Summary of computational tools used to analyze DNA microarray data.
Table 3
Summary of upregulated genes in transcriptome comparison of S. pneumoniae D39 wild-type grown in CDM plus 0 mM Co²⁺ and CDM plus 0.5 mM Co²⁺.

| Gene tag  | Function                                      | Ratio | P-value |
|-----------|-----------------------------------------------|-------|---------|
| SPD0801   | Hypothetical protein                          | 2.01  | 1.29E-05|
| SPD0910   | Serine hydroxymethyltransferase               | 2.17  | 1.69E-11|
| SPD1018   | Immunoglobulin A1 protease precursor          | 2.08  | 2.29E-06|
| SPD1039   | Phosphoenolpyruvate-protein phosphotransferase| 2.05  | 5.54E-11|
| SPD1053   | Galactose-6-phosphate isomerase, LacA subunit | 2.45  | 5.83E-07|
| SPD1294   | Hypothetical protein                          | 2.65  | 1.98E-11|
| SPD1355   | Hypothetical protein                          | 2.10  | 7.71E-01|
| SPD1466   | ABC transporter, ATP-binding protein          | 2.02  | 4.65E-09|
| SPD1588   | Hypothetical protein                          | 2.29  | 3.09E-07|
| SPD1598   | Hypothetical protein                          | 2.16  | 2.62E-08|
| SPD1596   | Tryptophan synthase, alpha subunit            | 2.24  | 2.36E-08|
| SPD1727   | Hypothetical protein                          | 2.39  | 2.61E-07|
| SPD1728   | Hypothetical protein                          | 2.26  | 1.25E-10|

a Gene numbers refer to D39 locus tags.
b D39 annotation/TIGR4 annotation [5,13].
c Ratios (0 mM Co²⁺ /0.5 mM Co²⁺).

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