Microarray studies on the effect of silencing tynA in Escherichia coli K-12

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To study the biological role of the tynA gene product of Escherichia coli, a primary amine oxidase (ECAO, E.C. 1.4.3.21), the tynA gene was genetically silenced by conjugation with a kanamycin resistance cassette. We used a microarray method to compare the mRNA expression in the modified strain (ΔtynA) to that in the wild type (wt) strain at the time of induction of ECAO expression (0 h) as well as 1 h and 4 h after the induction. These data in brief describe the different experimental conditions, sample preparation, data collection and analysis of the conducted microarray experiment. The differential expression of genes in the studied strains 1 h after the induction of ECAO expression is described. The microarray data have been deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO Series accession number GSE65385.

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1. Direct link to deposited data

(http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65385). Accession number GSE65385.

2. Experimental design, materials and methods

Escherichia coli (E. coli) K-12 harbors a tynA gene, which encodes for a periplasmic primary amine oxidase (ECAO, E.C. 1.4.3.21). We constructed a genetically modified E. coli K-12 strain unable to express ECAO (ΔtynA, [1]). Both wild type (wt) and ΔtynA strains were cultured in conditions, which induce the expression of ECAO, to be able to compare the expression profiles of ΔtynA and wt bacteria. We analyzed the samples at the start of the induction as well as 1 h and 4 h after the induction. We have published a research article describing the construction of the ΔtynA strain together with the full interpretation of the effect of the deletion of tynA on the expression of genes at the 4 h time point [1]. Here the methods used are described in more detail as well as the list of differentially expressed genes 1 h after the induction of ECAO is presented.

2.1. Constructing the ΔtynA strain

Detailed information about the generation of the modified ΔtynA strain is published [1]. In brief, we introduced a kanamycin cassette into the tynA gene by homologous recombination utilizing a bacterial conjugation to silence the gene.

2.2. Bacterial culture conditions

For the microarray sample preparation, we inoculated wt and ΔtynA bacteria in 5 ml of modified M9-lactose medium (0.2 M Na2HPO4, 0.2 M KH2PO4, 90 mM NaCl, 0.2 M NH4Cl, 1 mM MgSO4, 0.2% lactose, 0.1 mM CaCl2, and 1 mM Thiamine–HCl) at 30 °C, 250 rpm, and 16 h. Then 0.5 ml of bacterial culture was withdrawn for the RNA extraction to be used as the control sample for the induction (0 h, see below). The rest of the culture was renewed in a fresh, pre-warmed modified M9-lactose medium supplemented with 0.5 mM CuSO4 and 5 mM phenylethylamine (PEA) 1:20 to induce the expression of the tynA gene. Bacterial cultures were then further cultivated at 30 °C and 250 rpm. The bacterial samples (0.5 ml) were withdrawn for the RNA extraction at 1 and 4 h (A600 = 0.3)
3. Results

This report describes the detailed sample preparation conditions together with the details of the microarray experiment and analysis. In our experimental setup we compared the expression of genes of wt E. coli K-12 and its genetically modified ΔΔtynA strain at time points 0 h, 1 h and 4 h. The analysis of differentially expressed genes 4 h after induction has been published in our primary paper [1]. Here we briefly describe the differences between two strains 1 h after the induction of ECAO expression, which was not described in our primary publication.

At the start of the induction of the tynA gene (0 h), we detected no difference between tynA gene expression between the wild type and ΔΔtynA strains (normalized expression level of tynA was 2.48 and 2.68 for the wt and tynA strains, respectively). Because we were only interested in the effect of the lack of tynA expression, we did not analyze the differentially expressed genes at this time point. However, the existing data are freely available (see link to the deposited data).

As expected, the transfer of bacteria to PEA containing medium led to the increasing expression of tynA in the wt strain as a function of time (normalized expression levels were 37.73 and 422.78 from the time point 1 h to 4 h, respectively), meanwhile the normalized expression in the ΔΔtynA remained at background level (2.04 and 1.98, respectively). When the gene expression of the ΔΔtynA strain was compared to the wt strain at the 1 h time point, we detected the down-regulation of only a few genes above the threshold of −2.5 fold change (FC, Table 1). In addition to our target gene, tynA, the gene encoding for the next enzyme in the PEA pathway, feedB, was also down-regulated (Table 1). However, in comparison to the situation at the 4 h time point, where we were able to see the down-regulation of the whole phenylalanine utilization pathway, we detected no down-regulation of genes in the paaABCDEGFIJKXX operon [1]. In contrast, we observed a slight down-regulation of fhuf, ferric ion reductase, wcq, a putative UDP-glucose lipid carrier transferase and one hypothetical protein, which were not down-regulated 4 h after the induction (Table 1). The differences in the differentially expressed genes between 1 h and 4 h after the induction was not surprising, as the activity of ECAO reached a plateau 2 h after the induction, and was at the 1 h time point less than 25% of the activity at 4 h after the induction [1].

In the list of up-regulated genes, we observed only five genes, whose expression was up-regulated in the ΔΔtynA strain 1 h after the induction when compared to the wt strain (Table 2). The FC values remained low, under 3.5, and none of the up-regulated genes at this time point was up-regulated at the 4 h time point.

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| Gene symbol | Gene | FC |
|-------------|------|----|
| tynA        | Tyramine oxide, copper containing | −19.49 |
| feedB       | Phenylacetalddehyde dehydrogenase | −4.08 |
| fhuf        | Ferric ion reductase involved in ferric hydroximate transport | −2.97 |
| c062J       | Hypothetical protein ybcY precursor | −2.68 |
| wcq         | Putative UDP-glucose lipid carrier transferase | −2.62 |
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References

[1] H. Elovaara, T. Huusko, M. Maksimow, K. Elima, G.G. Yegutkin, M. Skurnik, U. Dobrindt, A. Siitonen, M.J. McPherson, M. Salmi, S. Jalkanen, Primary amine oxidase of Escherichia coli is a metabolic enzyme that can use a human leukocyte molecule as a substrate. PLoS One 10 (11) (2015), e0142367.

[2] Affymetrix, Affymetrix GeneChip expression analysis technical manual. Affymetrix, Santa Clara, CA, 2000.

[3] R.C. Gentleman, V.J. Carey, D.M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. Irizarry, F. Leisch, C. Li, M. Maechler, A.J. Rossini, G. Sawitzki, C. Smith, G. Smyth, L. Tierney, J.Y. Yang, J. Zhang, Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5 (10) (2004) R80.

[4] R. R Development Core Team, R, A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2006, ISBN 3-900051-07-0 (URL http://www.R-project.org).

[5] I. Hovatta, K. Kimppa, A. Lehmussola, T. Pasanen, J. Saarela, I. Saarikko, J. Saharinen, P. Tiilikainen, T. Tolvanen, M. Tolvanen, M. Vihinen, G. Wong, DNA microarray data analysis. 2006.

[6] G. Smyth, Limma: linear models for microarray data. in: R. Gentleman, S. Carey, R. Dudoit, R. Irizarry, W. Huber (Eds.), Bioinformatics and Computational Biology Solutions Using R and Bioconductor, Springer, New York 2005, pp. 307–420.

Table 2
Differentially up-regulated (FC > 2.50) genes in ΔtynA vs. wt E. coli 1 h after the induction of ECAO expression.

| Gene symbol | Gene                          | FC  |
|-------------|-------------------------------|-----|
| ydiE        | Hypothetical protein          | 3.13|
| tnaC        | Tryptonase leader peptide     | 3.07|
| metN        | t- Methionine transporter ATP-binding subunit | 2.88|
| c2375       | Hypothetical protein          | 2.83|
| yddM        | Predicted DNA-binding transcriptional regulator | 2.57|