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CyanoEXpress: A web database for exploration and visualisation of the integrated transcriptome of cyanobacterium *Synechocystis sp.* PCC6803

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Abstract:
Synechocystis sp. PCC6803 is one of the best studied cyanobacteria and an important model organism for our understanding of photosynthesis. The early availability of its complete genome sequence initiated numerous transcriptome studies, which have generated a wealth of expression data. Analysis of the accumulated data can be a powerful tool to study transcription in a comprehensive manner and to reveal underlying regulatory mechanisms, as well as to annotate genes whose functions are yet unknown. However, use of divergent microarray platforms, as well as distributed data storage make meta-analyses of *Synechocystis* expression data highly challenging, especially for researchers with limited bioinformatic expertise and resources. To facilitate utilisation of the accumulated expression data for a wider research community, we have developed CyanoEXpress, a web database for interactive exploration and visualisation of transcriptional response patterns in *Synechocystis*. CyanoEXpress currently comprises expression data for 3073 genes and 178 environmental and genetic perturbations obtained in 31 independent studies. At present, CyanoEXpress constitutes the most comprehensive collection of expression data available for *Synechocystis* and can be freely accessed.

Availability: http://cyanoexpress.sysbiolab.eu

Key words: Cyanobacteria, *Synechocystis* sp. PCC6803, Gene expression, Meta-analysis, Cluster, Microarray, Transcriptome, Database

Background:

Photosynthetic organisms have the fascinating capacity to harvest energy from sun light, and thereby provide for the majority of other life forms on earth. Cyanobacteria are believed to have been the first organisms which carried out photosynthesis and crucially contributed to the accumulation of oxygen in the atmosphere. Today, cyanobacteria are the main contributors to global biomass production and are responsible for up to 50% of the CO_2 fixation in oceans. Besides their ecological importance for the environment, cyanobacteria promise to be key instruments in the generation of renewable biofuels **[1].** Among the various cyanobacteria, *Synechocystis* sp. PCC 6803 has been most intensively studied. *Synechocystis* can be easily genetically manipulated and can grow under both photohetero- and photoautotrophic conditions, making it a model organism for scientists around the world. Importantly, it was the first photosynthetic organism to be sequenced **[2].** The complete sequence served as a basis for the development of several microarray platforms that can monitor expression changes for the full set of *Synechocystis* genes. Examination of generated microarray data has helped to decipher transcriptional regulation and has provided important clues to the functional relevance of specific genes. Unfortunately, the small number of samples and conditions tested in a typical microarray study limits the capacity to dissect interrelated regulatory mechanisms. Here, the integration of additional expression data from other studies can be helpful. Although public repositories offer ample expression data nowadays, utilization of these data for meta-analysis is by no means an easy task. Data needs to be collect from various repositories, that employ different query systems and data formats. Furthermore, care must be taken in their integration, as the different microarray platforms and analysis programs used in the original studies can cause additional heterogeneity in expression data. This is especially relevant in the case for *Synechocystis*. Plenty of genome-wide expression data sets have been generated over the last decades but they are dispersed at different locations and derived by different microarray technologies, hindering their direct use in meta-analyses.

Scope of CyanoEXpress:

To facilitate the use of amassed *Synechocystis* expression data, we have collected publicly available raw microarray data, processed them in standardised manner and made the integrated data set accessible on our CyanoEXpress web-server. At present, 645 genome-wide (partially replicated) microarray measurements carried out in 31 independent studies have been evaluated and incorporated into CyanoEXpress **Table 1 (see supplementary material)** The integrated data set includes expression changes due to various environmental and genetic perturbations. In total, CyanoEXpress provides over 178 expression entries for 3073 *Synechocystis* genes, which accounts for 82.5% of the genes annotated in the Cyanobase **[3].** A detailed meta-analysis of the data presented in CyanoEXpress will be published elsewhere.

Data collection and implementation of web-server:

Raw expression data were collected from the NCBI Gene Expression Omnibus **[4],** EBI ArrayExpress **[5]** and KEGG Expression database **[6].** Data processing was carried out using the R/Bioconductor platform **[7].** Datasets were individually processed and normalized by OIN (Optimised Intensity-dependent Normalisation) **[8, 9].** This method performs an optimized local regression of logged fold changes across the intensity range to correct for potential dye bias. Log_2 fold changes were calculated with respect to the controls used in individual experiments (which were primarily *Synechocystis* wild-type cultures grown under standard conditions). Processed expression data were filtered to eliminate genes for which less than 80% of the measurements were available. To support identification of co-expression and potential co-regulation, hierarchical clustering of genes was carried out on the filtered data set using the software Cluster 3.0 **[10].** Clustering was based on complete linkage, with the Spearman correlation as similarity measure. Additionally, clustered expression matrices were generated for sub-sets of the microarrays and genes: (i) Expression data for all genes, but only for perturbations of environmental conditions, excluding data from genetic manipulation of *Synechocystis*; and (ii) expression data from all measurements, but only for a sub-set of functionally related genes (e.g., genes associated with photosynthesis and respiration). The former sub-set can aid examination of expression changes occurring in the *Synechocystis* wild-type, whereas the latter subsets allow a more focused inspection of the regulation of particular processes. Functional annotation of genes was obtained from Cyanobase **[3].** The clustered expression matrices can be visualized and queried through a Common Gateway Interface (CGI)-based application, which is a modified version of the GeneXplorer software **[11].** CyanoEXpress is currently running on an Apache web server under an Ubuntu Linux operating system.

Usage of CyanoEXpress:

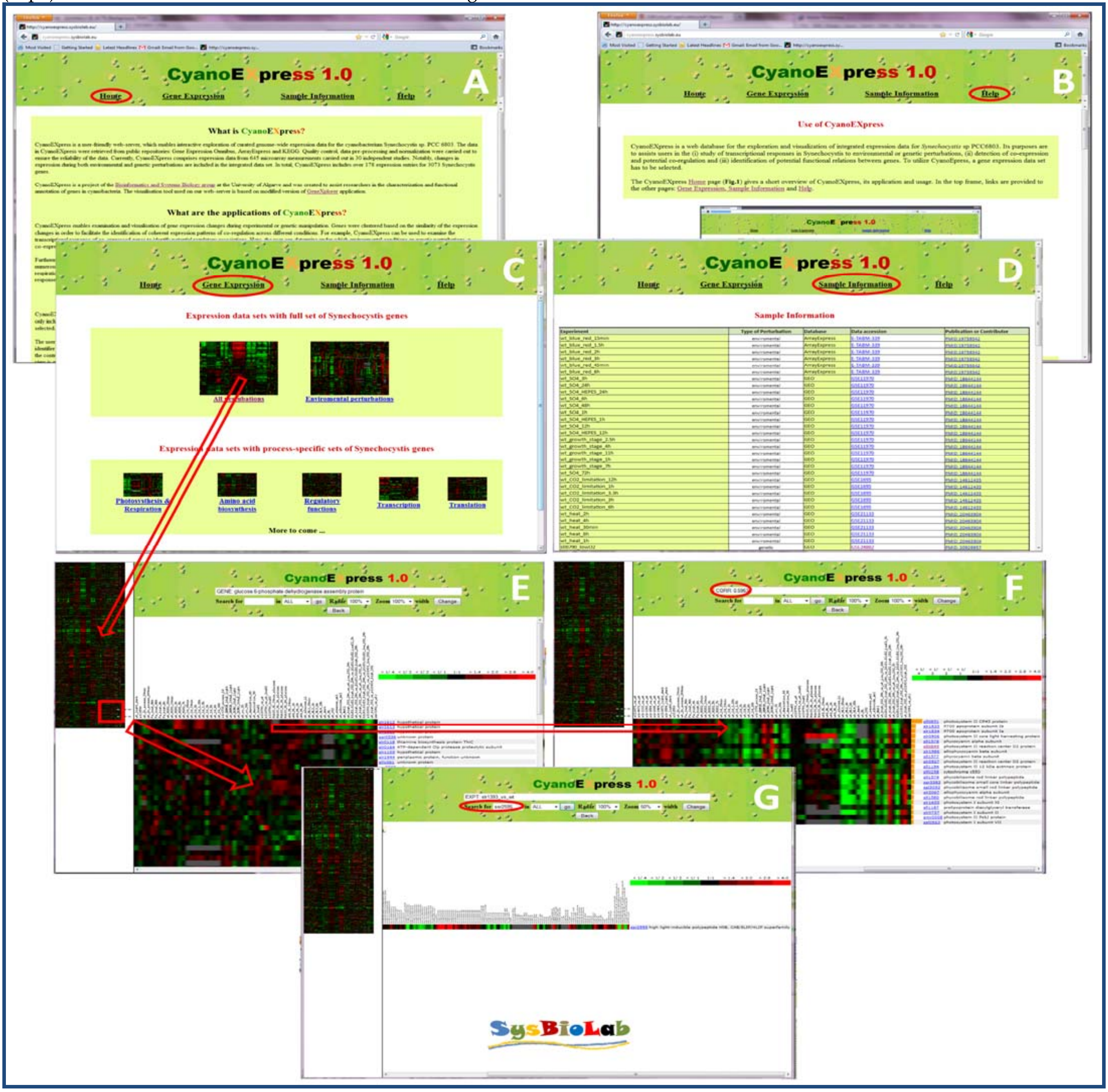
The CyanoEXpress web-site (http://cyanoexpress.sysbiolab.eu) has a simple structure: from the *Home* page **(Figure 1A)** the user can easily navigate through the different sections. A Help page provides instructions for CyanoEXpress **(Figure 1B)**. Information about the integrated microarray data sets is given on the *Sample Information* page **(Figure 1D).** Links are provided to the repositories from where the data sets were obtained, and to the publications in which the experiments are described**.**

To visualize the clustered expression data, the user chooses one of the integrated datasets available on the *Gene Expression* page **(Figure 1C).** Here, the user can select whether they access the full set of microarrays measurements (with all perturbations included) or only measurements with environmental perturbations. Alternatively, expression data sub-sets for genes associated with specific functions can be selected (i.e., Photosynthesis and Respiration, Amino Acid Biosynthesis, Regulatory function, Transcription and Translation). Upon selection, a miniature heat map of the expression data is displayed on the left side of the page. Using the miniature map, a range of genes can be selected **(Figure 1E)** red square, and their expression pattern will be enlarged in a separated HTML frame (*Zoom*). The names and identifiers of the genes are given on the right side of the enlarged expression heat map. Gene identifiers are linked to the Cyanobase for following up study of the corresponding genes. The user can also query the database for specific genes, whose expression changes will be displayed. This can be done by entering a unique gene identifier or name in the *Search for* text field **(Figure 1G),** red circle. Expression changes are colour-coded by a green to red colour gradient, corresponding to decreased (green) or increased (red) expression with respect to the control sample. No differences in expression are represented by black squares, while grey squares indicate no available data. The overall graphical view is provided as a matrix formed by rows representing genes, and columns representing the environmental conditions or genetic perturbations. Finally, a simple mouse-click on a specific row generates a display of genes, which show a high correlation with the gene corresponding to that particular row **(Figure 1F).**

Potential applications:

CyanoEXpress enables examination and visualisation of gene expression changes for various experimental or genetic manipulations**.** The clustering of genes facilitates identification of coherent expression patterns, which can indicate co-regulation across different conditions. For example, we found that transcripts of several subunits of NADH dehydrogenase complexes tend to be tightly clustered and display specific differential regulation upon environmental perturbations, despite originating from different operons. This observation suggests the existence of common regulatory mechanisms.

Furthermore, CyanoEXpress can be used to assign un-annotated genes to functions based on their co-expression with genes of known functions. This feature is advantageous given that numerous *Synechocystis* genes are yet to be assigned functions. For instance, we found that the expression of *sll1735* (cupS), for which no functional annotation has been given in Cyanobase, is correlated with genes encoding for NADH dehydrogenase subunits 4 and 5, and is similarly strongly up-regulated under CO_2 limitation. Such co-expression indicates a potential functional association of *sll1735* with NADH dehydrogenase.



What is CyanoEXpress?

CyanoEXpress is a user-friendly web-server, which enables interactive exploration of curated genome-wide expression data for the cyanobacterium Synechocystis sp. PCC 6803. The data in CyanoEXpress were retrieved from public repositories: Gene Expression Omnibus, ArrayExpress and KEGG. Quality control, data pre-processing and normalization were carried out to ensure the reliability of the data. Currently, CyanoEXpress comprises expression data from 645 microarray measurements carried out in 30 independent studies. Notably, changes in expression during both environmental and genetic perturbations are included in the integrated data set. In total, CyanoEXpress includes over 178 expression entries for 3073 Synechocystis genes.

CyanoEXpress is a project of the Bioinformatics and Systems Biology group at the University of Algarve and was created to assist researchers in the characterization and functional annotation of genes in cyanobacteria. The visualisation tool used on our web-server is based on modified version of GeneXplorer application.

What are the applications of CyanoEXpress?

CyanoEXpress enables examination and visualisation of gene expression changes during experimental or genetic manipulation. Genes were clustered based on the similarity of the expression changes in order to facilitate the identification of coherent expression patterns of co-regulation across different conditions. For example, CyanoEXpress can be used to examine the

Use of CyanoEXpress

CyanoEXpress is a web database for the exploration and visualization of integrated expression data for *Synechocystis* sp PCC6803. Its purposes are to assists users in the (i) study of transcriptional responses in Synechocystis to environmental or genetic perturbations, (ii) detection of co-expression and potential co-regulation and (iii) identification of potential functional relations between genes. To utilize CyanoEpress, a gene expression data set has to be selected.

The CyanoEXpress Home page (**Fig.1**) gives a short overview of CyanoEXpress, its application and usage. In the top frame, links are provided to the other pages: Gene Expression, Sample Information and Help.

Expression data sets with full set of Synechocystis genes

All perturbations | Enviromental perturbations

Expression data sets with process-specific sets of Synechocystis genes

Photosynthesis & Respiration | Amino acid biosynthesis | Regulatory functions | Transcription | Translation

More to come ...

Sample Information

Figure 1: CyanoEXpress website. An introduction to CyanoEXpress is provided on its *Home* page **(A)** and instructions for its use are given on the *Help* page **(B).** Data sets can be selected on the *Gene Expression page* **(C)** and information regarding the integrated microarray studies can be found on the *Samples information page* **(D).** After selection of the data set, the CyanoEXpress can display the expression changes across different perturbations for a set of clustered genes **(E),** the co-expression and correlation with a selected gene **(F)** or the expression data for single gene upon query **(G).**

Future development:

CyanoEXpress will be routinely updated and expanded as new datasets become available. An option for the direct download of expression data for selected gene sets will be implement in the near future. Finally, upcoming versions of CyanoEXpress will include a wider range of biological processes, for which specific expression matrices will be provided.

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Supplementary material:

Table 1: Overview of data included in CyanoEXpress

Number of studies	31
Number of microarrays	645
Number of genes	3073
Number of conditions	178
Number of genetic mutations	69
Number of environmental conditions	109