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Genome-wide transcriptome analysis of hydrogen production in the cyanobacterium *Synechocystis*: Towards the identification of new players

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ABSTRACT

We report the development of new tools and methods for facile integration and meaningful representation of high throughput data generated by genome-wide analyses of the model cyanobacterium *Synechocystis* PCC6803, for future genetic engineering aiming at increasing its level of hydrogen photoproduction. These robust tools comprise new oligonucleotide DNA microarrays to monitor the transcriptomic responses of all 3725 genes of *Synechocystis*, and the SVGMapping method and custom-made templates to represent the metabolic reprogramming for improved hydrogen production. We show, for the first time, that the AbrB2 repressor of the hydrogenase-encoding operon, also regulates metal transport and protection against oxidative stress, as well as numerous plasmid genes, which have been overlooked so far. This report will stimulate the construction and global analysis of hydrogen production mutants with the prospect of developing powerful cell factories for the sustainable production of hydrogen, as well as investigations of the probable role of plasmids in this process.

1. Introduction

Energy production and consumption has become a major environmental issue [1]. In this context, cyanobacteria, the only known prokaryotes capable of oxygenic photosynthesis, are receiving a growing attention for the sustainable production of the clean fuel hydrogen, due to their (i) simple nutritional requirements, (ii) robustness, (iii) metabolic plasticity, and (iv) the powerful genetics of some model strains. This is true for the unicellular cyanobacterium *Synechocystis* sp. PCC6803 (hereafter *Synechocystis*) which possesses a small genome (about 4 Mb; Table 1), comprising a circular chromosome and seven plasmids (CyanoBase: http://genome.kazusa.or.jp/cyanobase [2,3];, easily manipulable [4–7]. The pentameric hydrogenase enzyme (HoxEFUYH) is encoded by a heptacistronic operon, which also contains three open

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Table 1 – Number of chromosomal and plasmidic genes of *Synechocystis* which are up-regulated or down-regulated in the Δ*abrB2* mutant as compared to the WT strain (fold change: FC > 2; *p*-value < 0.01%) growing under standard conditions.

Name	Size (bp)	Genes	Deregulated genes	%	Up regulated	FC up max	Down regulated	FC down max
Chromosome	3,573,471	3317	250	7.5	219	+9.36	31	−5.45
pSYSM	119,895	132	33	25	32	+58.22	1	−3.33
pSYSX	106,004	110	41	37.3	41	+77.22	0	–
pSYSA	103,307	106	8	7.5	6	+5.37	2	−5.31
pSYSG	44,343	49	1	2	1	+3.35	0	–
pCC5.2	5214	6	–	–	–	–	–	–
pCA2.4	2378	2	–	–	–	–	–	–
pCB2.4	2345	3	–	–	–	–	–	–
Total	3,956,957	3725	333	8.9	299	+77.22	34	−5.45

reading frames of unknown function (*sll1222*, *ssl2420* and *sll1225* in CyanoBase). The active Hox enzyme, matured by the HoxW protease [8], and assembled using the six-subunits HypABCDEF complex [9–13] produces H_2 through the reversible reaction $2H^+ + 2\ e^- \leftrightarrow H_2$ (with a bias to H_2 production) [14,15]. It uses NAD(P)H as the source of electrons originating from photosynthesis and/or sugar catabolism, as well as a nickel-iron cluster and several iron-sulfur centers as redox cofactors [15].

Besides the assembly and activity of the Hox enzyme, it is also important to thoroughly study the mechanisms controlling the expression of the *hox* operon, in order to better understand the role of the Hox enzyme in the global metabolism of the cell, and possibly develop novel tools to enhance the photoproduction of H_2. The *hox* operon is controlled by at least three transcription factors, the LexA (Sll1626) and AbrB1 (Sll0359) proteins acting positively [11,13] and the AbrB2 factor (Sll0822 in CyanoBase) acting negatively [12]. Indeed, we recently found that AbrB2 represses the transcription of the *hox* operon through binding to the *hox* operon promoter [16]. Mutants totally or partially depleted of any of these regulatory proteins were analyzed with DNA microarray, which however were not pan-genomic in covering (at most) only 2852 chromosomal genes out of 3317 (about 85%) and none of the 408 plasmidic genes of as yet unknown functions [12,17,18]. Consequently, we developed truly pan-genomic (oligonucleotide) DNA microarrays to study the genome-wide transcriptome responses to environmental stresses and/or analyse of *Synechocystis* strains engineered for the production of biofuels. In addition, we have developed SVGMapping a R package to facilitate the analysis of large numbers of *omics* data, and visualize them onto custom-made templates depicting genome organization, metabolic pathways and cellular structures. Using these tools we characterized and compared the transcriptome profiles of our Δ*abrB2* deletion mutant, with an increased level of hydrogen production [16], by comparison with the WT strain. We report that AbrB2 is a master regulator that regulates (mostly negatively) a large number of chromosomal genes operating in regulation, metal transport and protection against oxidative stress, as well as numerous plasmid genes of as yet unknown function. These findings suggest that the regulation of hydrogen production might be rather complex, and that plasmid encoded functions, which have been overlooked so far, might be involved in hydrogen production.

2. Materials and methods

2.1. Culture conditions and RNA isolation

Synechocystis PCC6803 wild-type (WT) and Δ*abrB2* [16] were grown at 30°C under continuous white light (2500 lux; 31.25 μE m^{-2} s^{-1}) on BG11 medium enriched with 3.78 mM Na_2CO_3 (MM) as previously described [17]. 50 μg ml^{-1} kanamycin (Km) was added to maintain the Δ*abrB2* mutant strain.

RNA isolations were repeatedly (three times) isolated from exponentially growing cultures of the WT strain and Δ*abrB2* mutant with RNeasy Midi kit (Qiagen) as we previously described [17].

2.2. Design of pangenomic oligonucleotide DNA microarray

Sequentially using the Earray and OligoArray softwares (https://earray.chem.agilent.com and http://berry.engin.umich.edu/oligoarray2_1) we designed new and truly pan-genomic oligonucleotide DNA microarrays (TranSyn6803 project) where the protein coding sequence (CS) of each chromosomal and plasmid genes is represented by one to three non-overlapping 60 mer oligonucleotides, depending on CS length. Hence, long CS (≥500 bp) are represented by three oligonucleotides, which correspond to the beginning, middle and end of the CS; middle and end of the CS; whereas shorter CS (500 and 120 bp) and small CS (≤120 bp) are represented by two and one oligonucleotides, respectively.

2.3. Microarray experiments and analysis

One dye swap was carried out for each biological replicate. cDNA synthesis by reverse transcriptase superscript II kit, dNTP and aminoallyl-dUTP (Invitrogen), cDNA purification with QIAquick PCR Purification kit (Qiagen), tagging with Cy™Dye Post-labeling Reactive Dye Pack (Life Sciences), and synthesis of DNA microarray, and hybridization and scans were accomplished by using Agilent Technologies.

Microarray signals and data were analyzed with the Limma package of the R/Bioconductor software [19]. Microarray spot intensities were normalized by subtracting the background and using the LOWESS method with the smooth parameter set to 0.33 as recommended [20]. Normalized measures served to compute the ratios of Cy3/Cy5 intensity and the associated log2-transform (denoted log2-ratios) for each gene. Then, to