Microbiological Research 192 (2016) 292–299

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Transcriptomic analysis for elucidating the physiological effects of 5-aminolevulinic acid accumulation on *Corynebacterium glutamicum*

Xiaoli Yu [b], Haiying Jin [b], Xuelian Cheng [a], Qian Wang [a,*], Qingsheng Qi [b]

[a] National Glycoengineering Research Center, Shandong University, Jinan, 250100, People's Republic of China
[b] State Key Laboratory of Microbial Technology, Shandong University, Jinan, 250100, People's Republic of China

ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form 10 July 2016
Accepted 3 August 2016
Available online 4 August 2016

Keywords:
5-Aminolevulinic acid
Corynebacterium glutamicum
Heme biosynthesis
Transcriptomic analysis

ABSTRACT

5-Aminolevulinic acid (ALA), the committed intermediate of the heme biosynthetic pathway, attracts close attention among researchers because of its potential applications to cancer treatment and agriculture. Overexpression of heterologous *hemA* and *hemL*, which encode glutamyl-tRNA reductase and glutamate-1-semialdehyde aminotransferase, respectively, in *Corynebacterium glutamicum* produces ALA, although whether ALA accumulation causes unintended effects on the host is unknown. Here we used an integrated systems approach to compare global transcriptional changes induced by the expression of *hemA* and *hemL*. Metabolic pathway such as glycolysis was inhibited, but tricarboxylic acid cycle, pentose phosphate pathway, and respiratory metabolism were stimulated. Moreover, the transcriptional levels of certain genes involved in heme biosynthesis were up-regulated, and the data implicate the two-component system (TCS) HrrSA was involved in the regulation of heme synthesis. With these understandings, it is proposed that ALA accumulation stimulates heme synthesis pathway and respiratory metabolism. Our study illuminates the physiological effects of overexpressing *hemA* and *hemL* on the phenotype of *C. glutamicum* and contributes important insights into the regulatory mechanisms of the heme biosynthetic pathways.

© 2016 Published by Elsevier GmbH.

1. Introduction

The nonpathogenic, aerobic gram-positive soil bacterium *Corynebacterium glutamicum* attracts great interest and is the subject of intensive studies (Eggeling and Bott, 2005; Burkovski, 2008), because it is used for large-scale biotechnological production of amino acids, predominantly L-glutamate and L-lysine as well as other commercially useful compounds such as organic acids, diamines, and biofuels (Becker and Wittmann, 2012). Moreover, *C. glutamicum*, which is related to *Mycobacterium tuberculosis*, serves as a model for research on the complex cell walls of the *Corynebacterineae* (Varela et al., 2012).

Heme biosynthesis in *C. glutamicum* occurs downstream of the pathway that generates 5-aminolevulinic acid (ALA) from glutamate. Evidence indicates a potential role for ALA production by *C. glutamicum* via the C5 pathway in the presence of higher accumulations of heme (Yu et al., 2015). Moreover, heme plays an essential role as a cofactor for various enzymes, in particular, for those involved in the electron transport chain (Möbius et al., 2010). Although the synthesis of heme is required to capture energy through respiration, an excess of this critical cofactor is toxic to bacteria. In *C. glutamicum*, the two-component system HrrSA plays a central role in the control of heme homeostasis to overcome heme toxicity (Eggeling and Bott, 2005; Bott and Brocker, 2012). When heme is existent, HrrSA promotes heme degradation and the synthesis of the heme-containing cytochrome bc1-aa3 supercomplex, and decreases heme synthesis (Bott and Brocker, 2012). Because heme is important for maintaining cellular homeostasis, ALA production should induce changes in the overall metabolism and regulation of recombinant strains of *C. glutamicum*. Therefore, a global method to analyze gene expression should facilitate the identification of mechanisms that regulate heme metabolism.

Here we used RNA-seq to study transcriptomic-level changes in gene expression of a *C. glutamicum* strain that overexpresses *hemA* and *hemL* (encoding glutamyl-tRNA reductase and glutamate-1-semialdehyde aminotransferase, respectively), leading to higher levels of ALA and the accumulation of heme. Here we analyzed total RNAs isolated from exponentially growing cells (48 h). The transcriptomic data showed that the glycolysis pathway was down-regulated while the tricarboxylic acid cycle (TCA), pentose phosphate pathway (PPP), and respiratory metabolism were up-regulated. In addition, the two-component system HrrSA was stimulated to regulate the up-regulated heme biosynthesis. Taken together, the transcriptomic data in our study contribute to a new

* Corresponding author.
E-mail address: qiqi20011983@gmail.com (Q. Wang).

http://dx.doi.org/10.1016/j.micres.2016.08.004
0944-5013/© 2016 Published by Elsevier GmbH.

understanding of how the overexpression of *hemA* and *hemL* affects metabolic regulatory mechanisms.

2. Methods

2.1. Strains and plasmids

The bacterial strains and plasmids used in this study are described in Supplementary Table S1. The parental *C. glutamicum* strain ATCC 13032 was transformed with the plasmid pECXK99E, and the transformant is designated PECXK99E. *C. glutamicum* strain ATCC 13032 transformed with the plasmid pSEAL that expresses $hemA^{M}$ from *Salmonella arizona* and *hemL* from *Escherichia coli* is designated strain SEAL. Disruption of *hrrA* was performed using the nonreplicable integration vector pK-JL, which was constructed from pK18mobsacB (Jiang et al., 2013). To construct pK-JL-Δ*hrrA*, the flanking sequences of *hrrA* (712 bp and 726 bp) were amplified from the genomic DNA of *C. glutamicum* ATCC13032 using the primers hraF/hraR and hrbF/hrbR (Supplementary Table S2), respectively. The products were ligated to *Bam*HI-digested pK-JL using Gibson assembly (Gibson et al., 2009). The nonreplicable integration vector pK-JL-Δ*hrrA* was used to transform *C. glutamicum* to disrupt *hrrA* through homologous recombination and two-step selection using kanamycin resistance as a selective marker along with the *sacB* system (Schäfer et al., 1994). The engineered strain designated *C. glutamicum* ATCC13032 Δ*hrrA* was transformed with pSEAL to generate strain SEAL2. *E. coli* DH5α was used for DNA manipulations and plasmid construction.

2.2. Media and culture conditions

LB medium contained 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl was used for generating recombinant DNAs and seed cultures. BHIS medium contained 2.5 g/L beef extract, 5 g/L tryptone, 5 g/L NaCl, 18.5 g/L brain heart infusion, and 91 g/L sorbitol was used electrophoretic transformation of *C. glutamicum*. Solid media contained 1.5% (w/v) agar. A modified minimal medium (CGXII) (20 g of $(NH_4)_2SO_4$, 5 g urea, 1 g KH_2PO_4, 1 g K_2HPO_4, 0.25 g $MgSO_4{\cdot}7H_2O$, 42 g MOPS (3-morpholinopropanesulfonic acid), 10 mg $CaCl_2$, 0.01 mg $FeSO_4{\cdot}7H_2O$, 10 mg $MnSO_4{\cdot}H_2O$, 1 mg $ZnSO_4{\cdot}7H_2O$, 0.2 mg $CuSO_4$, 0.02 mg $NiCl_2{\cdot}6H_2O$, and 0.02 g sodium citrate) (pH 7.0) was used to produce Ala. The seed culture specifically contained 10 mg $FeSO_4{\cdot}7H_2O$, and glucose was also added as the sole carbon source. 5.0 μg/L biotin was used for the seed culture while 1.0 μg/L biotin was applied for the main culture. All recombinant strains were cultured at 30 °C and shaken at 120 rpm. Gene expression was induced by adding isopropyl-β-D-thiogalactopyranoside (0.25 mM final). Kanamycin (50 μg/mL) was added to the medium for selection.

2.3. Analytical methods

Cell concentrations were measured at 600 nm (OD_{600}) using a UV-1700 spectrophotometer (Shimadzu, Japan). Residual glucose concentrations were detected using a glucose-glutamate analyzer SBA-40C (Biology Institute of Shandong Academy of Sciences, China). The production of ALA was analyzed using Modified Ehrlich's Reagent after the cultures were centrifuged (Burnham, 1970), and a fluorescence assay was used to measure heme concentrations (Sinclair et al., 2001). To determine the rates of growth and glucose consumption, cells were grown in CGXII with 40 g/L glucose as the sole carbon source and were collected during exponential growth. The concentrations of lactate and acetate were measured using a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) equipped with an HPX-87H column (300 mm × 7.8 mm, Bio-Rad, USA) and a refractive index (RI) detector (Shimadzu RID-10A). Samples analyzed using HPLC were filtered through a 0.22-mm pore syringe filter. The mobile phase (0.5 mM H_2SO_4) was delivered at 0.6 mL/min at 65 °C. An Orion RDO meter (Thermo Fisher Scientific, USA) was used to determine oxygen uptake rates (OURs). Each culture (50 mL) was harvested during the exponential growth phase (48 h), and the OD_{600} was recorded. The cells were pelleted by centrifugation and washed once with sterilize phosphate-buffered saline, pH 7.5, and then diluted to 0.5 OD_{600}, and 100 mL of the diluted cell suspension was cultured in medium containing 40 g/L glucose at 30 °C with shaking at 200 rpm. The slopes of curves of the dissolved oxygen (DO) concentration vs time were determined from 70% to 20% air saturation. OURs were determined according to the OD_{600} of the cell suspension and presented as mg O_2 consumed per OD_{600} biomass per minute (Tsai et al., 2002).

2.4. Transcriptomic analysis

RNA-seq was performed by BGI Tech Company (Shenzhen, China) using an Illumina HiSeq sequencer (Illumina). Total RNAs from *C. glutamicum* PECXK99E and SEAL cultures collected during exponential growth (48 h) in CGXII medium were prepared using TRIzol reagent (Invitrogen, USA). Each sample was analyzed at least in duplicate (PECXK99E1, PECXK99E2, SEAL1 and SEAL2). RNA was treated with RNase-free DNase I (Takara, Japan) to prevent contamination by traces of genomic DNA, and ribosomal RNAs were removed using the RiboZero rRNA removal kit (Epicenter, USA) for gram-positive organisms before sequence analysis. The quality and quantity of RNAs were measured using the BioAnalyzer 2100 system (Agilent, USA), and RNA was fragmented and used as a template for PCR using random primers. Strand-specific cDNA libraries were prepared with the mRNA-seq Sample Prep kit (Illumina) and standard techniques for Illumina sequencing. An Agilent 2100 Bioanaylzer and an ABI StepOnePlus Real-Time PCR System were used to determine the representation and quality of the library. Suitable libraries were sequenced using an Illumina HiSeq 2000 according to the manufacturer's protocols. Raw sequence data were processed using software supplied by Illumina (Langmead and Salzberg, 2012).

The raw reads were subjected to quality control to determine if a resequencing step was required, and raw reads were subsequently filtered to prepare clean reads. SOAPaligner/SOAP2 (Li et al., 2009) was used to align the reads to the reference *C. glutamicum* ATCC 13032 genome sequence acquired from the database of the United States National Center for Biotechnology Information. Gene expression levels were estimated using the Reads Per Kilobase Transcriptome Per Million Mapped Reads method (Mortazavi et al., 2008). Expression analysis of two samples was performed using DEGseq software (Wang et al., 2010). A false-discovery rate ≤ 0.001 (Benjamini and Yekutieli, 2001) and the absolute value of the $\log_2$ ratio ≥ 1 were applied as threshold values to define a significant difference in gene expression levels. Gene ontology (GO) analysis of differentially expressed genes (DEGs) was performed to identify biological process, cellular components, and molecular function using Web Gene Ontology Annotation Plotting (BGI WEGO, http://wego.genomics.org.cn/cgi-bin/wego/index.pl) and the GO Analysis Toolkit and Database for the Agricultural Community (agriGO, http://bioinfo.cau.edu.cn/agriGO/index.php) (Du et al., 2010) with a corrected p value ≤ 0.05 as a threshold. The Kyoto Encyclopedia of Genes and Genomes pathways database was used to perform pathway enrichment analysis of DEGs (Kanehisa et al., 2012).

2.5. Quantitative real-time PCR (qRT-PCR)

For total RNA extraction, the samples were taken at 48 h after inoculation, and total cellular RNA was extracted with RNAeasy

Download English Version:

https://daneshyari.com/en/article/2091880

Download Persian Version:

https://daneshyari.com/article/2091880

Daneshyari.com