Data in Brief

Transcriptional profiling of thymidine-producing strain recombineered from Escherichia coli BL21

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A B S T R A C T

DNA microarrays were used to compare the expression profiles of a thymidine overproducing strain (BLT013) and its isogenic parent, Escherichia coli BL21(DE3), when each was grown under well-defined thymidine production conditions with glycerol as carbon source. Here we describe the experimental procedures and methods in detail to reproduce the results and provide resource to be applied to similar engineering approach (available at Gene Expression Omnibus database under GSE69963). Taken together, the microarray data provide a basis for new testable hypotheses regarding enhancement of thymidine productivity and attaining a more complete understanding of nucleotide metabolism in bacteria.

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69963.

2. Experimental design, materials and methods

2.1. Construction of thymidine producing strain BLT013

Using PCR-mediated homologous recombination, integration and disruption of target genes were performed in E. coli BL21(DE3) [1]. PCR fragments for disruption were amplified using plasmid DNA constructs that contained a FRT-flanking selectable marker (cat). In case of PCR fragments for integration, T4 thymidylate synthase (tdΔI) and PBS2 TMP phosphohydrolase were each amplified from sources by PCR with flanking primers that included 6-bp restriction site extensions and ligated to each other into pETduet (Novagen). Similarly, the T4 nrdCA operon and T4 nrdB were amplified by PCR. The intron region of nrdB was removed to construct mature nrdB using a Gibson assembly kit (NEB). Uridine kinase (udk) and dCTP deaminase (dcd) were also each amplified from E. coli PCR with flanking primers that included 6-bp restriction site extensions, and ligated to each other with an RBS to create an artificial operon (udk-dcd). Each DNA fragment was ligated into pETduet, and then each expression cassette was prepared for chromosomal integration after amplification using each flanking primer set. Then, each expressing cassette under control of the T7 promoter was inserted to the region between FRT site and target gene homologous region. We summarized the structure of PCR fragments for constructing BLT013 in Supplementary Fig. S1. The PCR products were transformed into electrocompetent cells harboring pKD20, which encodes the bacteriophage λ Red operon. Colonies were selected on LB agar plates containing ampicillin (50 μg/ml) or chloramphenicol (30 μg/ml). Successful gene replacement with the antibiotic marker was confirmed by PCR. The antibiotic marker was eliminated using helper plasmid pCP20 encoding the FLP recombinase. The comparative genotype of final strain BLT013 to BL21(DE3) was shown in Table 1.

2.2. Bacterial culture conditions

For seed culture, a suspension of cells was inoculated into a 250-ml flask containing 50 ml of Luria-Bertani (LB) medium (5 g/l yeast extract, 10 g/l bactotryptone, and 10 g/l NaCl) and incubated at 37 °C and
250 rpm for 8 h. For flask culture, a 5-ml aliquot of seed culture broth was transferred to a 500-ml baffled flask containing 50 ml of production medium (60 g/l glycerol, 10 g/l CaCO₃, 10 g/l yeast extract, 0.4 g/l MgSO₄·7H₂O, 14.8 g/l soyate, and trace elements) [2] and then incubated at 34 °C and 250 rpm until the cells reached exponential phase.

2.3. RNA isolation and microarray

Total cellular RNA was extracted from mid-log phase cells with a QIAGEN RNEasy Mini Kit (Qiagen, Valencia, CA) as described by the manufacturer. RNase-free DNasel (Takara Bio, Shiga, Japan) was used during the isolation procedure to eliminate possible DNA contamination. The integrity of bacterial total RNA was checked by capillary electrophoresis with an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA).

For control and test RNAs, the synthesis of target cRNA probes and hybridization were performed using Agilent’s Low Input Quick Amp WT Labeling Kit (Agilent Technology, USA) according to the manufacturer’s instructions. Then, the transcription master mix was prepared as the manufacturer’s protocol (4× Transcription buffer, 0.1 M DTT, NTP mix, 50% PEG, RNase-Out, Inorganic pyrophosphatase, 0.1 M EDTA). Transcription of dsDNA was performed by adding the transcription master mix to the dsDNA reaction samples and incubating at 40 °C for 2 h. Amplified and labeled complementary RNA (cRNA) was purified on RNase mini column (Qiagen) according to the manufacturer’s protocol. Labeled cRNA target was quantified using ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). After checking labeling efficiency, each of cyanine 3-labeled and cyanine 5-labeled cRNA target were mixed and the fragmentation of cRNA was performed by adding 10× blocking agent and 25× fragmentation buffer and incubating at 60 °C for 30 min. The fragmented cRNA was resuspended with 2× hybridization buffer and directly pipetted onto assembled Mycroarray (E. coli BL21-D23) 30 K Microarray including five additional foreign genes; PBS2 TMP phosphohydrolase (CAT ATG TAA TTA TAG AAA CAG CAC AAA CAG AAG AGA AAG CAG TAT), T4 nrdA (GGG GTT GTC AAT CAG CTT CAG CGA GTA CAT ATT ACC CCG AGA), T4 nrdB (CCG CAA GAA GTA CAA CTG TCA TCT TAC CTT GGT GCA CAG ATT GAT), T4 nrdC (CAG GTA TTT GCT CCT GAT GGA AGT CAT ATT GGT GGA TTT GAC CAA), T4 tddA (ACA ACT ATG TAT CAC CTC CTA TTA AAG GAA AGA TGG CGG TGT) (ref). The arrays hybridized at 57 °C for 17 h using Agilent Hybridization oven (Agilent Technology, USA). The hybridized microarrays were washed as the manufacturer’s washing protocol (Agilent Technology, USA).

2.4. Data acquisition and analysis

The hybridization images were analyzed by Agilent DNA microarray Scanner (Agilent Technology, USA) and the data quantification was performed using Agilent Feature Extraction software 10.7 (Agilent Technology, USA). The average fluorescence intensity for each spot was calculated and local background was subtracted. All data normalization and selection of fold-changed genes were performed using GeneSpring GX 7.3.1 (Agilent Technology, USA). Genes were filtered with removing flag-out genes in each experiment. Intensity-dependent normalization (LOWESS) was performed, where the ratio was reduced to the residual of the Lowess fit of the intensity vs. ratio curve [3,4]. The averages of normalized ratios were calculated by dividing the average of normalized signal channel intensity by the average of normalized control channel intensity. The genes showing P values < 0.05 were considered to be significant.

3. Discussion

Here we described the data of differentially expressed genes in thymidine producer HLT013 compared to parental strain BL21(DE3). We investigated the impact of engineering for thymidine producer by enhancing pyrimidine nucleotide biosynthetic pathway. Especially, transcriptome comparison of the HLT013 with parental strain BL21 grown in thymidine production media revealed the specific effect on the arginine biosynthetic pathway as well as nucleotide biosynthetic pathway by disrupting carAB repressors [2]. This microarray data could provide resources to be employed for comparative analysis in E. coli that have the enhanced ability to produce thymidine.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.08.012.

References

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