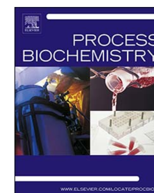
Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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



Integrated transcriptomic and metabolomic analysis of *Rhizopus oryzae* with different morphologies

Qing Xu[a], Yongqian Fu[b,*], Shuang Li[c], Ling Jiang[d], Guan Rongfeng[e], He Huang[a,*]

[a] State Key Laboratory of Material-Oriented Chemical Engineering, School of Pharmaceutical Sciences, Nanjing Tech University,No. 30 Puzhu Road, Nanjing 211816, China
[b] Institute of Biomass Resources, Taizhou University, Jiaojiang, Zhejiang 318000, China
[c] School of Pharmaceutical Sciences, Nanjing Tech University, No. 30 Puzhu Road, Nanjing 211816, China
[d] College of Food Science and Light Industry, Nanjing Tech University,No. 30 Puzhu Road, Nanjing 211816, China
[e] Laboratory for Advanced Technology in Environmental Protection of Jiangsu Province, Yancheng Institute of Technology, Yancheng 224051, China

ARTICLE INFO

Keywords:
Rhizopus oryzae
Transcriptomics
Metabolomics
Morphology

ABSTRACT

In trying to discover the potential genes or strategies used to regulate the morphology of *Rhizopus oryzae*, cells in different morphological states were subjected to transcriptomic and metabolomic analyses. The results showed that the tricarboxylic acid cycle was down-regulated in the pellet sample, whereas the metabolism of pyruvic acid, unsaturated fatty acids, and amino acids, as well as the synthesis of sugar alcohols, were up-regulated. Moreover, an increase of oxidative stress markers was detected during pellet formation. In response to this stress, *R. oryzae* up-regulated the aforementioned pathways and induced a number of antioxidant enzymes. In addition, *R. oryzae* adapted its cell wall composition to reduce the surface area exposed to the environment. Following these findings, hemicellulose hydrolysate was added into the culture medium, which led to the formation of uniform pellets over a broad pH range. This study might provide clues to deduce the unequivocal general relationships between process variables and fungal morphology.

1. Introduction

When grown in submerged culture, fungi exhibit different morphologies, ranging from mycelia to pellets or even clumps [1], and a particular morphological form can be favorable for the fermentation of a particular product. For example, the mycelial form is preferred for enzyme production, whereas the pellet form is preferred for the production of organic acids. In addition, the mycelial form increases the viscosity of the broth, whereas with the pellet form the viscosity remains comparatively low [2]. Thus, great efforts have been made to manipulate the morphology of industrial fungi [3,4].

Rhizopus oryzae in the morphological pellet form is considered the best producer of fumaric acid [2]. Recent studies on morphology control of *Rhizopus* spp. mostly focused on the optimization of physical or chemical culture conditions. By optimizing the culture condition regarding temperature, medium composition and agitation rate, Zhou et al. obtained the pellet morphology in *Rhizopus delemar* NRRL 1526, and by so doing enhanced the production of fumaric acid by 46.13% [5]. Das et al. investigated the effects of substrate concentration, pH, inoculum size and temperature on the morphology of *R. oryzae* 1526, and discovered the optimal conditions for pellet formation [6].

As previously reported, any particular morphological type is the result of both the genotype of the organism and the environment in which it is cultured [7]. The genes *pod*B, *swo*A and *swo*F of *Aspergillus* have been identified as having roles in the establishment and maintenance of hyphal polarity, and the inactivation of these genes resulted in swollen hyphae [8]. Similarly, a class III chitin synthase of *Penicillium chrysogenum* has been observed to play an important role in branching, and silencing this gene resulted in shorter and more branched hyphae [9]. However, there are few reports concerning the effects of genes on the morphology of *Rhizopus* spp., necessitating the empirical optimization of cultivation parameters, which can be erratic and laborious.

Transcriptomics and metabolomics, which focus on the comprehensive analysis of intracellular responses to different environmental conditions in biological systems, are powerful tools used to build our understanding of the genomic and metabolic differences between the physiological states an organism finds itself in [10–12]. Here, a quantitative transcriptomics approach utilizing RNA-Seq technology was applied to reveal the global differences of *R. oryzae* with different morphologies. A comparison of the transcriptomics and metabolomics data was made to confirm the target genes related to different morphologies. Based on these parameters, a strategy which enables

* Corresponding authors.
E-mail addresses: uyq@tzc.edu.cn (Y. Fu), biotech@njut.edu.cn (H. Huang).

http://dx.doi.org/10.1016/j.procbio.2017.10.001
Received 25 April 2017; Received in revised form 28 September 2017; Accepted 2 October 2017
1359-5113/ © 2017 Elsevier Ltd. All rights reserved.

pellet formation under a broad set of conditions was successfully developed.

2. Materials and methods

2.1. Strain and growth conditions

Rhizopus oryzae N6, a derivative of *R. oryzae* ATCC 20344, was used throughout this study. To obtain spores, *R. oryzae* N6 was cultured on potato-dextrose agar slants at 35 °C for seven days. The spores were suspended in sterilized water and filtered to remove the mycelium. The spore suspension was maintained at 4 °C until use.

For seed cultures in flasks, 1 mL of seed suspension (10^7 spores/mL) was added into 50 mL of seed culture medium (30 g/L glucose, 2 g/L urea, 0.6 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.11 g/L $ZnSO_4 \cdot 7H_2O$, and 0.0088 g/L $FeSO_4 \cdot 7H_2O$), which was incubated at 35 °C for 28 h under constant orbital shaking at 200 rpm. In order to induce pellet formation, the pH of the medium was set to 2.5 with 2 M H_2SO_4 at the beginning of culture. To obtain a filamentous morphology, the initial pH was adjusted to 3.0.

For seed culture in the 5 L fermenter, 8 mL of seed suspension was added into 3 L of seed culture medium (30 g/L glucose, 2 g/L urea, 0.6 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.11 g/L $ZnSO_4 \cdot 7H_2O$, and 0.0088 g/L $FeSO_4 \cdot 7H_2O$, with or without hemicellulose hydrolysate) and incubated at 35 °C and 200 rpm for 28 h. The pH of the media was set to different values using 2 M H_2SO_4 at the beginning of cultivation.

2.2. RNA sequencing and data analysis

R. oryzae was incubated in culture media with different initial pH values for 16 h, after which the mycelia were harvested by centrifugation at 12,000 rpm and 4 °C for 1 min; the pellets were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

The mRNA was extracted according to a published procedure [13]. The cDNA library was constructed using the Truseq™ RNA sample prep kit (Illumina, CA, USA). RNA sequencing was conducted using the Illumina HiSeq 2000 platform provided by BGI. Tech, Shenzhen, China. After sequencing, the primary data were subjected to quality control, with the right data filtered into clean reads, and aligned with the reported genome sequences of *R. oryzae* (http://www.ncbi.nlm.nih.gov/genome/?term=Rhizopus%20delemar) using SOAPaligner/SOAP2 (performed by BGI. Tech, Shenzhen, China). No more than five mismatches were allowed in the alignment. Each sample was analyzed in three biological replicates.

2.3. Analysis of differential gene expression levels

The gene expression levels were calculated using the RPKM (reads per kilobase of exon region per million mapped reads) value. The *p* value was used to determine the significance of the gene expression differences. The FDR method was used to correct for false negatives and false positives. Genes were regarded as being differentially expressed if the data met the requirement "FDR ≤ 0.001and Log2Ratio ≥ 1".

2.4. Analysis of metabolic pathways

Genes play important roles in certain biological functions. Pathway-based analysis could help to further understand the biological function of genes. The KEGG database was used to perform pathway enrichment analysis of differentially expressed genes. First, all differentially expressed genes were mapped to the KEGG pathways, after which the gene number for each pathway was calculated and significantly enriched pathways were identified.

Table 1
Primers used for real-time PCR.

protein	gene	primer
asparagines synthase	RO3G-15613	FW: GACTCAGACCTCCAGCATACCACA
		Rev: CTGAAGGGAATCACGCAACAG
arginase	RO3G-01881	FW: GATGTAGACGACGGTGAAAAGAAG
		Rev: GATCTCGGTTGGGATTGATGTGA
pyruvate kinase	RO3G-12744	FW: GCTCTCTGGTGAAACAGCGAA
		Rev: CTCGGTAGGTAGCGGTGTAAG
glucose-6-phosphate dehydrogenase	RO3G-17071	FW: GTTGGCTATGCTCGCTCAGA
		Rev: CATCAACTTCAAGATGGGATTGGA
fructose-1,6-bisphosphatase	RO3G-13347	FW: GTCTGTCGGTAATGGCGTCA
		Rev: CATGGTAATCTTGGGGTGTGTCA
agmatinase	RO3G-08948	FW: GCATACGCCCGTCTATCTTTCCA
		Rev: GTGCAACTTCCACCACATCCAATC
isocitric dehydrogenase	RO3G-04614	CTGCTGATGGTTCAGAGCCTAGAA
		GGAGGGCAAGACGGAAGCAA

2.5. Quantitative real-time PCR analysis

RNA samples were collected for transcriptome analysis under the conditions described above. mRNA extraction and cDNA synthesis was done according to published methods [13]. The resulting cDNA was used as a template for real-time PCR, which was conducted using the Power SYBR® Master Mix (Invitrogen, Waltham, MA, USA). For each gene, at least three technical replicates were analyzed. The Oligo-dT primers used for cDNA synthesis are listed in Table 1.

2.6. Analysis of biomass and extracellular metabolites

Extracellular acids and ethanol were measured using high-performance liquid chromatography according as reported by Fu et al. [14]. Glucose concentrations were analyzed using an SBA-80C biosensor analyzer (Institute of Biology, Shandong Academy of Sciences, Shandong, China) [15]. To determine the biomass yields, *R. oryzae* cells were harvested via filtration, washed, and dried at 70 °C to a constant weight.

2.7. Extraction and analysis of intracellular metabolites

After 16 h of cultivation, *R. oryzae* cells were quenched in 60% (v/v) methanol pre-cooled to −40 °C, centrifuged at 12,000 rpm for 2 min, and the final deposition used for the extraction of intracellular metabolites. The extraction and derivatization procedures were done according to a previous report [16], as follows: The deposition was ground into a powder mixed with liquid nitrogen, 50% methanol was used as the extraction buffer, and 10 μL ribitol (0.1 mg/mL) was used as the internal standard. After lyophilization, 50 μL of methoxamine hydrochloride (20 mg/mL in pyridine) and 80 μL of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide were added to the lyophilized sample and derivatized. The final solution was stored at −80 °C until gas chromatography–mass spectrometry (GC–MS) analysis. At least four replicates were used for each sample.

For GC–MS analysis, a capillary column (30 m × 0.25 mm × 0.25 μm, DB-5 MS, Agilent, Santa Clara, CA, USA) was employed, and helium with a flow rate of 1.0 mL/min was used as the carrier gas. The analysis conditions were the same as reported by Liu et al. [16].

Metabolites were identified by comparing the mass fragmentation patterns with the National Institute of Standards and Technology mass

Download English Version:

https://daneshyari.com/en/article/6495559

Download Persian Version:

https://daneshyari.com/article/6495559

Daneshyari.com