Genomics 110 (2018) 201–209

Contents lists available at ScienceDirect

Genomics

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

Comparative transcriptomics of *Pleurotus eryngii* reveals blue-light regulation of carbohydrate-active enzymes (CAZymes) expression at primordium differentiated into fruiting body stage

Chunliang Xie, Wenbing Gong, Zuohua Zhu, Li Yan, Zhenxiu Hu, Yuande Peng*

Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, People's Republic of China

ARTICLE INFO

Keywords:
Blue light
Carbohydrate-active enzymes
High-throughput sequencing approach
Primordium differentiated into fruiting body

ABSTRACT

Blue light is an important environmental factor which could induce mushroom primordium differentiation and fruiting body development. However, the mechanisms of *Pleurotus eryngii* primordium differentiation and development induced by blue light are still unclear. The CAZymes (carbohydrate-active enzymes) play important roles in degradation of renewable lignocelluloses to provide carbohydrates for fungal growth, development and reproduction. In the present research, the expression profiles of genes were measured by comparison between the *Pleurotus eryngii* at primordium differentiated into fruiting body stage after blue light stimulation and dark using high-throughput sequencing approach. After assembly and compared to the *Pleurotus eryngii* reference genome, 11,343 unigenes were identified. 539 differentially expressed genes including white collar 2 type of transcription factor gene, A mating type protein gene, MAP kinase gene, oxidative phosphorylation associated genes, CAZymes genes and other metabolism related genes were identified during primordium differentiated into fruiting body stage after blue light stimulation. KEGG results showed that carbon metabolism, glycolysis/gluconeogenesis and biosynthesis of amino acids pathways were affected during blue light inducing primordia formation. Most importantly, 319 differentially expressed CAZymes participated in carbon metabolism were identified. The expression patterns of six representative CAZymes and laccase genes were further confirmed by qRT-PCR. Enzyme activity results indicated that the activities of CAZymes and laccase were affected in primordium differentiated into fruiting body under blue light stimulation. In conclusion, the comprehensive transcriptome and CAZymes of *Pleurotus eryngii* at primordium differentiated into fruiting body stage after blue light stimulation were obtained. The biological insights gained from this integrative system represent a valuable resource for future genomic studies on this commercially important mushroom.

1. Introduction

The mushrooms development could be divided into three different stages, including hyphal knot, primordium and fruiting body [1]. The primordium differentiated and fruiting body formation is focused as a popular topic in mycological study, which has been illustrated in several model species such as *Coprinopsis cinerea* [2,3], *Schizophyllum commune* [4], *Agaricus bisporus* [5,6], *Boletus edulis* [7] and *Flammulina velutipes* [8,9]. The process of primordium differentiated and fruiting body formation in life cycle of mushrooms is mediated by cellular processes, genetic, physiological and environmental factors. Several genes which participate in this process have been demonstrated previously. For instance, ubiquitin–proteasome, cytochrome P450, hydrophobin, *Noxs* [10], *dst* [11,12], *Ubc2* [13] and *eln2* [14] showed different expression among primordium differentiated and fruiting body formation. In addition, genes participated in MAPK, cAMP and ROS signals were found differentially expressed during these stages [13,15].

Beside the genetic factor, the environmental factor such as light could also affect fruiting body differentiation [16,17]. Several researches showed that blue light could induce differentiation and development of fruiting body in *Hypsizygus marmoreus* [18], *Pleurotus ostreatus* and *Coprinus cinereus* [11,19].Several blue light receptor were found and cloned successfully. The *WC-1/2* of *Schizophyllum commune* [5], *dst1* and *dst2* genes in *Coprinus cinereus* [11,12], *phrA* and *phrB* from *Lentinula edodes* [20,21], *Cmwc-1* in different strains of *Cordyceps*

Abbreviations: AAs, auxiliary activities; CDS, coding DNA sequence; CEs, carbohydrate esterases; CBMs, carbohydrate-binding modules; COG, Cluster of Orthologous Groups; DEGs, differentially expressed genes; FDR, false discovery rate; FPKM, Fragments Per Kilobase of transcript per Million; GO, Gene Ontology; GHs, glycosyl hydrolases; GTs, glycosyl transferases; KEGG, Kyoto Encyclopedia of Genes and Genomes; MnP, manganese peroxidase; NR, non-redundant; PLs, polysaccharide lyases; RH, relative humidity; SRA, Sequence Read Archive

* Corresponding author.
E-mail address: ibfcpyd313@126.com (Y. Peng).

http://dx.doi.org/10.1016/j.ygeno.2017.09.012
Received 31 May 2017; Received in revised form 10 September 2017; Accepted 27 September 2017
Available online 29 September 2017

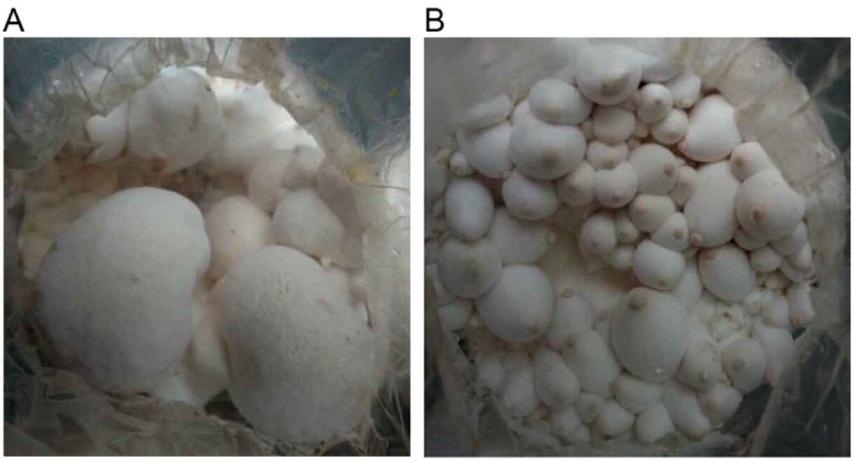

Fig. 1. Primordium differentiated into fruiting body of *P. eryngii* by comparison between the blue light group and the dark group. Dark control (a) and blue light stimulation (b).

militaris [22], and *Slwc-1* from *Sparassis latifolia* were encoded as putative photoreceptor for blue light [23]. In addition to the blue light receptor, it is also found that primary metabolic pathways in oyster mushroom mycelia were regulated induced by blue light stimulation [24]. However, the molecular mechanism of primordium differentiated and fruiting body formation induced by blue light is limited in other non-model commercial mushrooms.

Carbohydrate-active enzymes (also known as CAZy enzymes or CAZymes) are involved in the hydrolysis of plant cell wall polysaccharides, and play an important role in substrate degradation processes [25]. The carbohydrates from degraded lignocellulosic substrate, such as mono and oligosaccharides, can be utilized as nutrition for fungal development and reproduction. Previous research showed that CAZymes play important roles on the sclerotial formation of *Wolfiporia cocos* [26]. In addition, cellulase gene expressions of *Trichoderma reesei* were modulated by blue light. However, it remains unclear whether other CAZymes are involved in fruiting body differentiation and development induced by blue light.

Pleurotus eryngii (*P. eryngii*) is an edible and medicinal white-rot fungus which has been planted extensive in the Mediterranean, central Europe, central Asia, and north Africa due to its remarkable flavor, high nutritional value and numerous medicinal features [27]. In factory production, blue light is used to stimulate fruiting body development in *P. eryngii*. However, the mechanisms of *P. eryngii* primordium differentiation into fruiting body induced by blue light are still unclear. For better understanding the blue light photoresponse on the primordium development in *P. eryngii*, the transcriptomes under dark or blue light were examined. The undifferentiated fruiting body or differentiated fruiting body was measuring and the RNA-seq was performed by using Illumina technology, resulting in the identification of differentially expressed genes. Our previous research showed that *P. eryngii* can also produce various CAZymes to transform all components of plant biomass, including cellulose, hemicellulose, and lignin [28,29]. However, it is still unclear whether blue light induces the expression of CAZymes in primordium differentiated into fruiting body stage. So the expression level and enzyme activity of CAZymes during fruiting body development were also discussed. qRT-PCR was also used to confirm the expression profiles of the CAZymes genes. This transcriptomic information could promote our understanding of the genetic and molecular mechanisms of the development of fruiting body under blue light.

2. Materials and methods

2.1. P. eryngii cultivation

The *P. eryngii* (CICC50126) was obtained from the inquiry network for microbial strains of China. Ramie stalks from Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences were collected and used as main component of substrates. *P. eryngii* was cultured in solid medium containing 50% mixture of ramie stalks, 21% cottonseed hulls, 21% wheat bran, 6% corn meal, 1% sucrose and 1% calcium carbonate. The ratio of material to water was 1:1.5. The mixed substrates were packed in mushroom culture packages, sterilized, and cooled to room temperature. Pre-cultured *P. eryngii* was inoculated onto the top of the substrates in culture packages. To obtain a uniform spread of the hypha in the substrates, packages were kept at 20–24 °C, 65–70% relative humidity (RH) in the dark. When the mycelium colonized the substrate completely within 40 days, the culture packages were moved to an environment at 10–15 °C, 90–95% RH. Bags were then either exposed to a 12 h/day or dark regime with blue light LED illumination (455 nm, 80 μmol m^{-2} s^{-1}) for 10 days to induce fruiting body differentiation (blue light) or maintained for 10 days in darkness (control).

2.2. RNA isolation, cDNA library preparation and Illumina sequencing

To obtain the expression changes of *P. eryngii* induced by blue light, two cDNA libraries were prepared including dark and blue light irradiated group. First, the total RNA extracted from pooled undifferentiated fruiting body (Fig. 1A) or differentiated fruiting body (Fig. 1B) obtained from 15 culture bags of each sample (blue light and dark control) was isolated using TRIZOL reagent (Takara, Japan) according to the manufacturer's protocol. The quality of the isolated RNA was detected by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). For each group, to minimize biological variations, individual RNAs from three random samples were equally pooled for library construction and labeled as sample A. Sample B was prepared as sample A, and both samples(A and B) were processed separately.

The cDNA libraries were prepared using TruSeq RNA sample preparation kit from Illumina (San Diego, CA). Shortly, the mRNA was separated out using oligo(dT) beads from 5 μg total RNA. The RNAs were cut into 200 bp in fragmentation buffer. Then, mRNA was used to synthesize the cDNA and following end repair, A-base addition and ligation as Illumina's instruction. The short fragments with 200 read lengths were chosen for PCR amplification. Finally, the cDNA libraries were sequenced by Beijing Biomarker Technologies (Beijing, China) using Illumina HiSeq 2500 sequencer.

2.3. De novo assembly and annotation

The sequenced paired-end reads were first trimmed the adaptor sequences and the low quality bases were discarded. Subsequently, the clean data were used to conduct de novo assembly by Trinity (http://trinityrnaseq.sourceforge.net/) using the default parameters to obtain contigs. The contigs were connected to generate sequences which