Microbiological Research 207 (2018) 256–268

Contents lists available at ScienceDirect

Microbiological Research

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

Comparative transcriptomic analysis of *Cerrena unicolor* revealed differential expression of genes engaged in degradation of various kinds of wood

Grzegorz Janusz[a,*], Andrzej Mazur[b], Jerzy Wielbo[b], Piotr Koper[b], Kamil Żebracki[b], Anna Pawlik[a], Beata Ciołek[a], Andrzej Paszczyński[c], Agnieszka Kubik-Komar[d]

[a] Department of Biochemistry, Maria Curie-Skłodowska University, Akademicka 19 St., 20-033, Lublin, Poland
[b] Department of Genetics and Microbiology, M. Curie-Skłodowska University, Akademicka 19 St., 20-033, Lublin, Poland
[c] School of Food Science, Food Research Center, University of Idaho, 709 S Deakin St, Moscow, ID, USA
[d] Chair of Applied Mathematics and Informatics, Lublin University of Life Sciences, Akademicka 13 St., 20-950, Lublin, Poland

ARTICLE INFO

Keywords:
Cerrena unicolor
White rot fungi
Transcriptome
RNA-seq

ABSTRACT

To explore the number of enzymes engaged by *Cerrena unicolor* FCL139 for wood degradation, the transcriptomes of the fungus growing on birch, ash, maple sawdust and the control liquid medium were analyzed. Among 12,966 gene models predicted for the *C. unicolor* genome, 10,396 all-unigenes were detected, of which 9567 were found to be expressed in each of the tested growth media. The highest number (107) of unique transcripts was detected during fungus growth in the control liquid medium, while the lowest number (11) – in the fungal culture comprising maple saw dust. Analysis of *C. unicolor* transcriptomes identified numerous genes whose expression differed substantially between the mycelia growing in control medium and each of the sawdust media used, with the highest number (828) of upregulated transcripts observed during the fungus growth on the ash medium. Among the 294 genes that were potentially engaged in wood degradation, the expression of 59 was significantly ($p < .01$) changed in the tested conditions. The transcripts of 37 of those genes were at least four times more abundant in the cells grown in all sawdust media when compared to the control medium. Upregulated genes coding for cellulases and, to a lower extent, hemicellulases predominated during fungus growth on sawdust. Transcripts encoding cellulolytic enzymes were the most abundant in mycelia grown on birch and maple while lower number of such transcripts was detected in fungus growing on ash. The expression pattern of lignolytic activities-coding genes was strongly dependent on the type of sawdust applied for fungus growth medium.

1. Introduction

Plant biomass is the most abundant renewable source of organic matter on Earth (Kirk, 1984). Its main component consists of a lignocellulose fraction of plant cell walls, which is, however, highly recalcitrant to biodegradation and is a major bottleneck in the cellulosic biofuel production processes. Nevertheless, in nature, there are numerous organisms, mainly fungi, capable of degrading lignocellulose through a set of polysaccharidases and/or lignin-degrading enzymes. Polysaccharidases, hydrolyze the carbohydrate components of the plant cell wall, releasing oligomeric sugars that are further metabolized inside hyphal cells, thus providing chemical energy for the metabolic processes and growth. White-rot fungi are well known to effectively utilize all components of lignocellulose-containing tissue, and as such, play a key role in global carbon cycling and formation of soil organic matter (Blanchette, 1995). In addition, plant cell wall-degrading enzymes have the potential for a broad range of biotechnological applications and are very attractive tools for the development of new strategies concerning industrial processes, including sustainable production of paper, food products, and many chemicals including fuels (Malherbe and Cloete, 2002). Fungal enzymatic machinery may be successfully used as alternative combination of enzymes in production of biofuels (bioethanol, biogas and pyrolysis) and animal feed, as well as biopulping and biobleaching (Isroi et al., 2011).

Commonly known as a mossy maze polypore, *Cerrena unicolor* is a wood-degrading fungus of the *Polyporaceae* family (Enebak and Blanchette, 1989; Roody, 2003) bearing the general features of the fungi belonging to the genus *Trametes* (Ko and Jung, 1999). The most

Abbreviations: AO, alcohol oxidase; CDH, cellobiose dehydrogenase; DyP, dye-decolorizing peroxidase; FCL, fungal culture collection in lublin; LDA, lignin-degrading auxiliary enzymes; LiP, lignin peroxidase; LME, lignin-modifying enzymes; MnP, manganese peroxidase; POD, ligninolytic class II peroxidases; SSG, solid-state growth; VP, versatile peroxidase

* Corresponding author.
E-mail address: gjanusz@poczta.umcs.lublin.pl (G. Janusz).

https://doi.org/10.1016/j.micres.2017.12.007
Received 7 December 2017; Accepted 16 December 2017
Available online 20 December 2017
0944-5013/

studied among them is *Trametes versicolor*. *C. unicolor* usually lives on hardwoods such as *Aesculus*, *Acer*, *Fagus*, or *Quercus* in Europe, Africa, and South America and is an aggressive saprophytic organism which also attacks living trees causing extensive white rot decay in hardwood forests (Krieglsteiner, 2000). Strains of *C. unicolor* are also well known as efficient producers of industrially relevant laccase (Janusz et al., 2007; Lisova et al., 2010) and bioactive compounds of pharmacological interest (Mizerska-Dudka et al., 2015).

The genome of *Cerrena unicolor* 303 has been recently sequenced and annotated with ESTs data (http://genome.jgi.doe.gov/programs/fungi/index.jsf). However, no transcriptomic data focusing on differential gene expression were available so far. Thus, relatively little was known about the global expression profile of genes especially the ones engaged in lignocellulose degradation and the mechanism employed by fungus for wood decomposition with respect to various growth conditions.

The emergence of high throughput, next-generation sequencing techniques has helped to improve the efficiency of novel gene discovery and has provided insight into the exploration and understanding of gene expression, allowing in turn to accelerate the description of complex biological machineries used by fungi to decompose wood. Since the first published transcriptome study (Velculescu et al., 1997), an increasing number of genomes and transcriptomes of wood decaying fungi have been reported (Martinez et al., 2004; Martinez et al., 2009; Ohm et al., 2010; Chen et al., 2012; Fernandez-Fueyo et al., 2012; Suzuki et al., 2012; Yu et al., 2012; Rohr et al., 2013; Hori et al., 2014; Kuuskeri et al., 2016; Yang et al., 2017). In contrast to the data gained from the genome sequence analysis demonstrating overall metabolic potential of organism, the transcriptome reflects the dynamic gene expression snapshot in response to various environmental conditions, establishing a link between an genetic information stored in the genome and biophysical/metabolic characteristics of the cell (Meijueiro et al., 2014). Transcriptomic data further supported by proteomic approaches have provided new insights into polysaccharide lytic monooxygenase (LPMO) or dye-decolorizing peroxidase and contributed to better understanding of the molecular biology of previously discovered peroxidases or laccases (Martinez et al., 2009; Fernandez-Fueyo et al., 2012; Hori et al., 2014; Janusz et al., 2017). These findings also clearly showed the huge complexity of enzymatic systems engaged by fungi for wood degradation, demonstrating necessity of further research in the field, before the pathways will be fully understood and biotechnologically explored. The complexity of the wood degradation process results from the interactions of enzymes with low molecular compounds engaged in wood decomposition (Evans et al., 1994). Moreover, wood-degrading enzymes, especially lignolytic ones, may play divergent functions inside fungal cells which are not necessarily directly related to lignocellulose decomposition (Nagai et al., 2003; Kumar et al., 2015). Millions of years of evolution allowed fungi to develop very precise strategies for decomposing different species of wood. Besides of the ability to grow on dead woods, *C. unicolor* belongs to the group of fungi capable of growth on some species of living hardwood trees (e.g., *Fraxinus excelsior*, *Betula* sp., *Acer* sp.).

In this paper, using RNA-based sequencing technology, the whole transcriptomes of *C. unicolor* FCL139 cultivated in SSG (solid-state growth) conditions on sawdust substrates were analyzed and compared with the transcriptome of fungus grown on mineral medium. This approach demonstrated several differences in the qualitative and quantitative production of enzymes potentially engaged in lignocellulose degradation, suggesting various adaptive strategies employed in wood degradation by *C. unicolor*.

2. Materials and methods

2.1. Medium and growth conditions

The *C. unicolor* FCL139 strain was obtained from the culture collection of the Regensberg University. The fungal culture was maintained in 2% (w/v) malt agar slants. As an inoculum, pieces of agar culture were grown in the Lindenberg and Holm (LH) medium (Lindeberg and Holm, 1952) in a non-agitated conical flasks for 7 days at 28 °C. The LH medium had the following composition (1 L): 10 g glucose, 1 g L-asparagine, 480 mg Na_2HPO_4, 470 mg KH_2PO_4, 500 mg $MgSO_4 \times 7H_2O$, 12 mg $Mn(CH_3COO)_2 \times 4H_2O$, 3.14 mg $Zn(NO_3)_2 \times 6H_2O$, 3.9 mg $CuSO_4 \times 5H_2O$, 3.2 mg $FeCl_3 \times 6H_2O$, 50 mg $Ca(NO_3)_2 \times 4H_2O$, 100 mg yeast extract. The pH was adjusted to 6.5 with 5 M HCl. Next, the mycelial mats were subsequently collected and homogenized in a disperser (IKA, Poland). The fragmented mycelial culture (1% v/v) was used as a standard inoculum for further studies. Solid-state lignocellulose cultures were grown at 25 °C on 1 g of sawdust of ash, maple, or birch wood particles soaked with 8 mL of distilled water in glass Erlenmeyer flasks. Wood shavings (*Betula pendula*, *Fraxinus excelsior*, *Acer* platanoides) were dried, milled to sawdust and sieved to final size < 4 mm. A control mycelium was grown in static conditions at 25 °C in Lindeberg and Holm liquid medium, and the inoculum consisted of 1 mL freshly homogenized mycelium obtained from actively growing liquid culture (Lindeberg and Holm, 1952). All media were autoclaved at 121 °C, 30 min.

2.2. RNA extraction and sequencing

Fifty mg of *C. unicolor* mycelia grown in triplicate for eight days in Lindeberg and Holm medium and the ash, maple, or birch sawdust were frozen in liquid nitrogen and hand ground to a fine powder with a pre-chilled mortar and pestle. Polyadenylated (polyA) mRNA was directly isolated using oligo (dT) magnetic beads. The Dynabeads mRNA DIRECT Kit (Ambion, Waltham, MA) designed for simple and rapid isolation of polyA mRNA directly from the crude lysate was employed for polyA mRNA extraction following the manufacturer's protocol. After homogenization of hyphae, the DNA shearing step (allowing reduction of viscosity) was performed by forcing the lysate through a 21 gauge needle 3–5 times using a 2 mL syringe. The mRNA quantity and quality were evaluated using a Qubit 2.0 fluorimeter and a Qubit RNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA), as well as an Agilent 2100 Bioanalyzer and an RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA). The transcriptome library for each sample was prepared using a Solid Total RNA-Seq Kit (Applied Biosystems, Waltham, MA) following the manufacturer protocol for low-input RNA samples. The median size of the mRNA fragments after RNase III fragmentation was 193 base pairs (bp). The median size of the amplified cDNA whole transcriptome libraries was 270 bp. Each of the 12 constructed cDNA libraries (3 replicates per fungal growth conditions) was sequenced using the Solid 5500 platform. Sequencing data were derived from the P1 end of the template in the Solid templated bead, using forward ligation chemistry (FWD1 Seq. Primers) in a single run. The processing of original images to sequences and base-calling for the obtained 75 bp single-end reads were performed by the LifeScope pipeline (version 1.6) (LifeTechnologies).

2.3. NGS data analysis

The 75 bp raw reads were filtered to obtain high-quality reads by the removal of adaptor sequences before assembly and mapping to the reference genome. Filtered and processed results of individual cDNA replicates reads of *C. unicolor* are summarized in Table 1. Adapter trimming and removal of low-quality reads was performed with cutadapt (Martin, 2011). The *C. unicolor* rRNAs have been identified de novo with Barrnap 0.7 (https://github.com/Victorian-Bioinformatics-Consortium/barrnap). Based on these predictions, rRNA sequences were fetched from the *C. unicolor* genome using a custom Python script, and the rRNA-mapping reads were excluded from the sequencing results using Bowtie 2 (Langmead and Salzberg, 2012). The *C. unicolor* v1.1 (Cerun2) genome assembly, downloaded from the U.S. Department