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Light-regulated synthesis of extra- and intracellular enzymes related to wood degradation by the white rot fungus *Cerrena unicolor* during solid-state fermentation on ash sawdust-based medium

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The light-dependent metabolism of the white rot basidiomycete *Cerrena unicolor* FCL139 has already been demonstrated using transcriptomic and Biolog-based approaches. To further analyze the influence of light on *C. unicolor* wood degradation, we measured the activity of an array of CAZymes (carbohydrate-active enzymes) and enzymes involved in the redox system of fungal cells associated with lignolysis. Extra- and intracellular enzymatic extracts were obtained from solid-state ash sawdust *C. unicolor* cultures cultivated for 14 days under red, blue, green, or white light conditions, or in the dark. Light greatly influenced the synthesis of MnP, total cellulases, endo-1,4-β-glucanase, endo-1,4-β-xylanase, catalase, and superoxide dismutase. The production of MnP and catalase was evidently stimulated by white light. It is also worth noticing that blue light caused a gradual increase in the activity of total cellulases throughout the entire period of *C. unicolor* growth. Moreover, endo-1,4-β-glucanase showed the highest activity on day 13 of fungus cultivation and the production of laccase and β-glucosidase appeared to be the least influenced by light. However, the strongest activity of the endo-1,4-β-xylanase was observed in the dark. It seemed that light not only influenced the regulation of the synthesis of the wood-degrading enzymes at different levels, but also acted indirectly by affecting production of enzymes managing harmful lignin by-products causing oxidative stress. The ability of the fungus to decompose woody plant material is clearly influenced by environmental factors.

Key words: light conditions, *Cerrena unicolor*, lignin-modifying enzymes, cellulases, superoxide dismutase, catalase

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Abbreviations: CAZYmes, carbohydrate-active enzymes; FPA, filter paper assay; LME, lignin-modifying enzymes; MnP, manganese-dependent peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase

INTRODUCTION

The lignocellulosic material, the main biomass component, consists of cellulose, hemicellulose, and lignin in various proportions (Jeffries, 1994; Pérez *et al.*, 2002; Sánchez, 2009; Janusz *et al.*, 2017). In nature, it can be degraded by a variety of microorganisms that produce a set of hydrolytic and unique oxidative enzymes working synergistically (Blanchette *et al.*, 1989; Pérez *et al.*, 2002). Complete degradation of cellulose requires the cooperative action of a number of cellulases, xylanolytic and accessory enzymes (Eriksson *et al.*, 1990; Jeffries, 1994; Pérez *et al.*, 2002). Lignin-modifying (LME) and lignin-degrading auxiliary (LDA) enzymes are involved in lignin degradation (Janusz *et al.*, 2017). It has also been postulated that some enzymes lacking direct lignin-degrading activities, such as superoxide dismutases (SODs) and catalase, are indispensable in lignolysis. The main role of these enzymes would be to protect their producers from the toxic effects of harmful oxygen species produced during lignin transformation processes (Leonowicz *et al.*, 2001). The main organisms responsible for lignocellulose degradation are the wood-degrading fungi, with the most-known types of brown rot, soft rot, and white rot (Blanchette *et al.*, 1989). Each individual type of the rot fungus produces a specific set of enzymes, degrades different plant material, and thus colonizes diverse ecological niches (Eriksson *et al.*, 1990). Therefore, understanding the physiology and metabolic aptitudes of wood-degrading fungi and the ability to influence their metabolism may have a far-reaching impact not only on future research, but also lead to practical solutions.

Environmental factors greatly affect the decay caused by wood-rotting fungi (Blanchette *et al.*, 1989), and light is undoubtedly one of the most important signals influencing their metabolism (Grassi *et al.*, 2018; Pawlik *et al.*, 2019b). Sunlight serves as a source of energy or information and can be crucial for successful competition, survival, and development of an organism in nature. In fungi, light controls developmental decisions, physiological adaptations, stress response, and the circadian clock, which is reflected by metabolic changes (Carlile, 1965). Fungi can sense light with a set of discrete photoreceptor proteins, that include flavin-based blue-light, retinal-based green-light (such as rhodopsin), and linear tetrapyrrole-based red-light sensors (Fischer *et al.*, 2016), suggesting that they can detect specific light wavelengths covering the whole white light spectrum and actively respond to these signals.

Cerrena unicolor, a mossy maze polypore, is a globally distributed wood-degrading basidiomycete that causes extensive white rot (Enebak & Blanchette, 1989; Roody, 2003). *C. unicolor* strains are also well described as potential bioproducers of industrially relevant enzymes and other bioactive compounds of medical importance (Janusz *et al.*, 2007; Hibi *et al.*, 2012; Belova *et al.*, 2014; Mizerska-Dudka *et al.*, 2015; Sulej *et al.*, 2015). A recent RNAseq-based transcriptomic analysis has provided new

insights into the differentially expressed *C. unicolor* genes associated with diverse metabolic and signaling pathways employed by the fungus in response to different lighting conditions (Pawlik *et al.*, 2019a). It has also been demonstrated that the *C. unicolor's* metabolic aptitudes related to utilization of carbon sources and its chemical sensitivity are also light-dependent (Pawlik *et al.*, 2019b). The dependence of the synthesis of several fungal enzymes on lighting conditions has been analysed as well (Janusz *et al.*, 2016). However, that study was performed using a synthetic mineral (LH) medium, and so far the synthesis of specific enzymes involved in wood decay in the *C. unicolor* fungus grown on natural sawdust medium has not been studied. Moreover, the transcriptomic and Biolog-based analyses are insufficient to fully characterize the light-dependent processes occurring in *C. unicolor*. There is still a need to confirm the presence of certain wood-related activities and to complement the study with further enzymatic measurements. Given the ability of the fungus to degrade wood material and synthesize biotechnologically significant compounds, a better understanding of light-dependent production of individual enzymes from a natural substrate may be extremely valuable. Furthermore, it can contribute to the development of biotechnological applications of *C. unicolor* and understanding of biochemical changes induced in the fungus by various light conditions.

In this paper we present a comparative analysis of the ability of *C. unicolor* FCL139 grown in different lighting conditions to synthesize enzymes associated with wood degradation.

MATERIALS AND METHODS

Fungal strain and cultivation conditions. The strain used in this study was *C. unicolor* FCL139 from the culture collection of the University of Regensburg, in Regensburg, Germany, deposited in the Fungal Culture Collection (FCL) of the Department of Biochemistry, Maria Curie-Sklodowska University, in Lublin, Poland (Janusz *et al.*, 2012). The stock cultures were maintained on 4% (w/v) malt extract agar (Difco, BD, USA) slants. The slants were inoculated with mycelia, incubated at 28°C for 10 days, and then used for seed culture inoculation. About 5 mm^2 of the slants were punched out with a sterilized cutter and used as inoculum. Then, the mycelia of each strain were transferred into a 100-ml liquid Lindeberg-Holm (LH) medium (1952). The seeds were cultivated in the dark at 28°C. Next, the ten-day-old mycelia were homogenized in a disperser homogenizer (IKA, Staufen, Germany) and used as a standard inoculum. Solid-state lignocellulose *C. unicolor* cultures were grown at 28°C on 1 g of sterile ash sawdust (wood particles <4 mm) soaked with 9 ml of distilled water in Erlenmeyer flasks placed in incubators (KT 115, Binder, Germany) equipped with illumination LED cassettes for 14 days. The following lighting variants were applied: white (4000–4750 K), green (510-520 nm), blue (465–470 nm), red (620–625 nm) light, and darkness. Continuous lighting conditions (20 lux) were provided throughout the entire period of *C. unicolor* cultivation.

Preparation of sawdust enzymatic extracts. Extracellular enzymes extracts were obtained from solid-state lignocellulose *C. unicolor* cultures. For this purpose, 10 ml of deionized water was added to each Erlenmayer flask, and the sawdust medium overgrown with the mycelium was gently crushed with a glass rod. Next, the suspension was stirred for 5 minutes using a magnetic stirrer and the enzymatic extract was separated from the residues by filtration using Whatman No. 1 qualitative filter paper.

Intracellular extracts were prepared using 4 g of ash sawdust overgrown with the mycelium. The samples were homogenized on ice (4 min processing cycle of 30 s pulses per min, 80% amplitude) in 15 ml of ice-chilled distilled water in an ultrasonic homogenizer (Vibra-Cell, Sonics and Materials, Inc., USA). Immediately after sonication, each sample was filtered through Miracloth and the homogenates were centrifuged (10 000×*g*, 15 min, 4°C). Clear supernatant fractions were then aliquoted, frozen, and kept at –20°C.

Clear extra- and intracellular enzymatic filtrates were used as a source of the crude enzymatic preparation subjected to further measurements as described below.

Wood-related enzymatic activity assays. All the spectrophotometric measurements were performed using an Infinite 200 Pro microplate reader (Tecan, Germany) in triplicates in three biological replications.

Aryl-alcohol dehydrogenase [EC 1.1.1.91, AAD] activity was determined by measuring NADPH-dependent reduction of veratraldehyde (3,4-dimethoxybenzaldehyde) at pH 6.0 (Muheim *et al.*, 1991; Guillén & Evans, 1994). One unit of AAD activity was defined as the amount of enzyme that oxidizes one μmole of NADPH to NADP per minute at 30°C.

Cellobiose dehydrogenase [EC 1.1.3.25, CDH] activity was measured by following the decrease in absorbance of the electron acceptor 2,6-dichloroindophenol at 520 nm (ε^M=6.8 mM^{-1} cm^{-1}), pH 4.5 and 30°C (Baminger *et al.*, 2001; Karapetyan *et al.*, 2006). This assay measures the activity of an intact enzyme and that of the catalytically active flavin domain. The final enzyme activity was expressed as nanokatals per liter (nkat/l).

Laccase [EC 1.10.3.2] activity was measured spectrophotometrically at 525 nm with syringaldazine as a substrate (Leonowicz & Grzywnowicz, 1981). One unit of laccase activity was defined as the amount of the enzyme needed to catalyze the production of one nanomole of colored product (quinone, ε^M=65 000 M^{-1} cm^{-1}) per second at 25°C and pH 5.5. The activity was expressed as nanokatals per liter of the enzymatic filtrate (nkat/l).

Catalase [EC 1.11.1.6] activity was determined indirectly using catalase assay kit, according to the producer's protocol (Sigma-Aldrich, USA) (Fischer *et al.*, 2016). It is based on the measurement of the hydrogen peroxide substrate remaining after the enzyme's action on 3,5-dichloro-2-hydroxybenzenesulfonic acid. In the presence of horseradish peroxidase (HRP), a red quinoneimine dye is formed which absorbs 520 nm light. One unit of catalase was defined as the amount of the enzyme needed to catalyze the decomposition of 1 μmole of hydrogen peroxide to oxygen and water per minute at pH 7.0 at 25°C, at a substrate concentration of 50 mM hydrogen peroxide.

Manganese-dependent peroxidase [EC 1.11.1.13, MnP] activity was determined with a method described by Wariishiand others (Wariishi *et al.,* 1992) modified by Hofrichter and others (Hofrichter *et al.,* 1999). The reaction was initiated by adding H_2O_2, and the rate of Mn^{3+}-malonate complex formation was monitored by measuring the increase in absorbance at 270 nm (ε^M=11 590 M^{-1} cm^{-1}). The enzyme activity was expressed as nanokatals per litre (nkat/l).

Lignin peroxidase [EC 1.11.1.14, LiP] activity was determined using the oxidation of veratryl alcohol method as described by Arora and Gill (Arora & Gill, 2001). The reaction was initiated by H2O2, and the change in ab-

sorbance was monitored at 310 nm (ε^M=9 300 M^{-1} cm^{-1}). The enzyme activity was expressed as nanokatals per litre (nkat/l).

Superoxide dismutase [EC 1.15.1.1, SOD] activity was measured spectrophotometrically at 450 nm using WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt as a substrate with an indirect assay method based on xanthine oxidase as described in the SOD activity determination kit (Sigma-Aldrich, USA). The SOD activity was expressed as percentage of inhibition.

Endo-1,4-β-glucanase [EC 3.2.1.4, endo-cellulase] activity was determined using 4,6-*O*-(3-ketobutylidene)-4-nitrophenyl-β-D-cellopentaoside substrate in an automated assay procedure as described in the CellG5 method (Megazyme, Ireland) (Mangan *et al.*, 2016). One unit of endo-β-1,4-glucanase activity (CellG5 Unit/ml) equals the amount of enzyme required to release 1 μmole of *p*-nitrophenol per minute in the assay conditions.

Endo-1,4-β-xylanase [EC 3.2.1.8, endo-xylanase] activity was determined using 4,6-O-(3-ketobutylidene)-4-nitrophenyl-β-D-45-glucosyl-xylopentaoside colorimetric reagent with an automated assay procedure according to the XylX6 method (Megazyme, Ireland) (Mangan *et al.*, 2017). One unit of endo-1,4-β-xylanase activity (XylX6 Unit/ml) is equal to the amount of enzyme required to release 1 μmole of *p*-nitrophenol per minute under test conditions.

β-1,4-glucosidase [EC 3.2.1.21] activity was measured at 400 nm with *p*-nitrophenyl-β-D-glucopyranoside as a substrate (Ghose & Bisaria, 1987). One unit of β-glucosidase activity (U/ml) was expressed as the amount of enzyme required to release 1 μmole of *p*-nitrophenol per minute in the assay conditions.

β-glucuronidase [EC 3.2.1.31] activity was measured at 400 nm with *p*-nitrophenyl-β-D-glucuronide as a substrate (Kuroyama *et al.*, 2001). One unit of β-glucuronidase activity (U/ml) was expressed as the amount of enzyme required to release 1 μmole of *p*-nitrophenol per minute under assay conditions.

β-xylosidase [EC 3.2.1.37] activity was measured at 400 nm with *p*-nitrophenyl-β-D-xylopyranoside as a substrate (John & Schmidt, 1988). One unit (U/ml) of xylosidase activity was expressed as the amount of enzyme required to release 1 μmole of *p*-nitrophenol per minute under assay conditions.

β-mannanase [EC 3.2.1.78] activity was determined using 0.5% (w/v) locust bean gum as a substrate (Naganagouda *et al.*, 2009). The amount of reducing sugars produced in the enzyme reaction was measured as D-mannose reducing equivalents with the 3,5-dinitrosalicylic acid method (Miller, 1959) at 540 nm. One unit of mannanase activity (U/ml) was expressed as the amount of enzyme that liberates 1 μmole of mannose during 1 minute at 50°C.

Total cellulase activity was measured with the filter paper method (FPA) using 50 mg of Whatman No. 1 filter paper as described by (Ghose, 1987). The reducing sugars released were measured using the 3,5-dinitrosalicylic acid method (Miller, 1959). One unit of cellulases activity (U/ml) was expressed as the amount of enzyme that liberates 1 μmole of glucose during 1 minute at 50°C.

Statistical analysis. All measurements were performed in triplicates in three independent biological replications. All results are expressed as the mean ± S.D. (standard deviation) for nine measurements (n=9).

RESULTS

To detect and measure the activity of white rot basidiomycete *C. unicolor* FCL139 enzymes engaged in wood degradation in different lighting conditions, an array of CAZymes (carbohydrate-active enzymes, including cellulolytic, hemicellulolytic, lignin-degrading enzymes) and enzymes involved in the redox system of fungal cells associated with ligninolysis was tested in the sawdust medium cultures under red, blue, green, white light and darkness conditions. Extra- and intracellular enzymatic extracts were obtained from solid-state ash sawdust *C. unicolor* cultures cultivated for 14 days in controlled lighting conditions.

In general, the synthesis of specific wood-degrading enzymes, i.e.: laccase, MnP, β-glucosidase, endo-1,4-β-

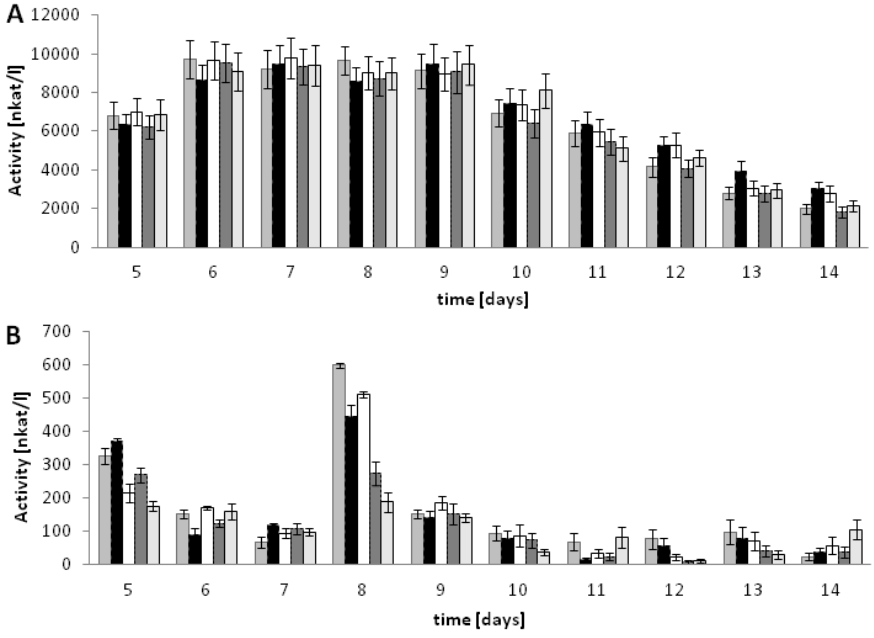

Figure 1. Extracellular activities of lignolytic enzymes (A) laccase and (B) MnP produced by *C. unicolor* during 14 days of cultivation on ash sawdust medium in the dark (■) or under white (■), red (□), blue (■), or green (■) lighting conditions.

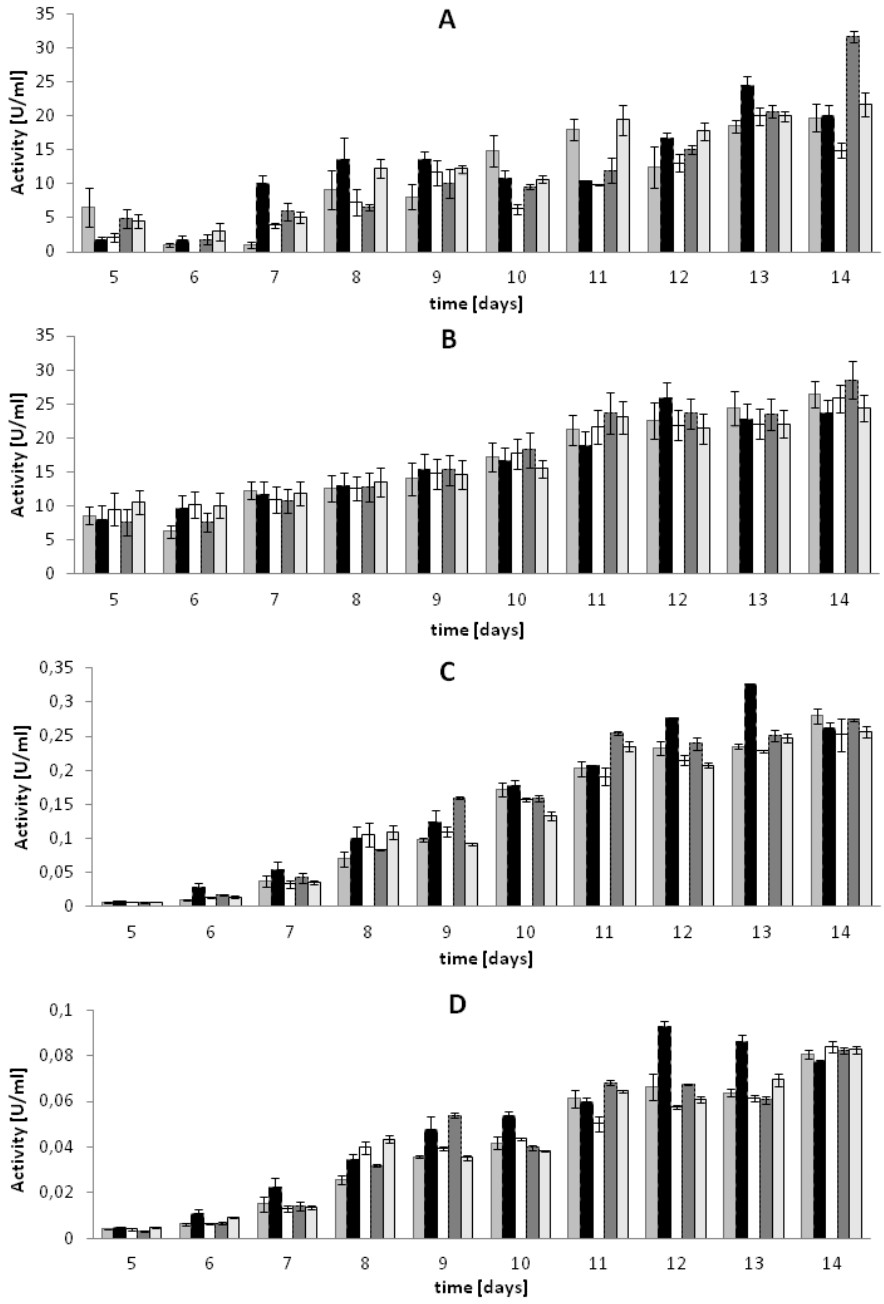

Figure 2. Cellulo- and xylanolytic activities of (A) total cellulases, (B) β-glucosidase, (C) endo-β-1,4-glucanase, and (D) endo-β-1,4-xylanase produced by *C. unicolor* during 14 days of cultivation on ash sawdust medium in the dark (■) or under white (■), red (□), blue (■), or green (■) lighting conditions.

glucanase, endo-1,4-β-xylanase, and total cellulases took place in all lighting variants. When the ash sawdust medium was used as a source of nutrients, the production of laccase, MnP, β-glucosidase, endo-1,4-β-glucanase, endo-1,4-β-xylanase, SOD, catalase, and total cellulolytic activity in *C. unicolor* was observed regardless of the lighting conditions. In turn, low enzymatic activities or no activity was observed for AAD, CDH, LiP, β-glucuronidase, β-xylosidase, and β-mannanase (data not shown). *C. unicolor* was not able to synthesize these enzymes effectively when the sawdust medium was used.

The highest activities for lignin-modifying enzymes (LME) were observed from the 6th to 9th day of cultivation for laccase and on 8th day of cultivation for MnP (Fig. 1). The cellulolytic and xylanolytic activities gradually increased over time and reached their maximum on cultivation days 11 to 14 (Fig. 2). The synthesis of SOD was highly variable. Nonetheless, the SOD inhibition rate ranged from 61 to 81% during the entire period of *C. unicolor* cultivation. The highest inhibition rate was present under red light cultivation conditions (73–81%) and the lowest value was found for blue light cultivation conditions (61–76%) (Fig. 3A). In turn, catalase activities remained at a high level from culture day 5 to 9 (Fig. 3B).

In general, light influenced the synthesis of MnP, total cellulases, endo-1,4-β-glucanase, endo-1,4-β-xylanase, catalase, and SOD. The synthesis of MnP was stimulated by white light, and its maximal enzymatic activity (598 nkat/l) was observed on day 8 of the culture. High MnP activities were also noticed under the red light (510 nkat/l) and dark (444 nkat/l) conditions (Fig. 1B). Strong

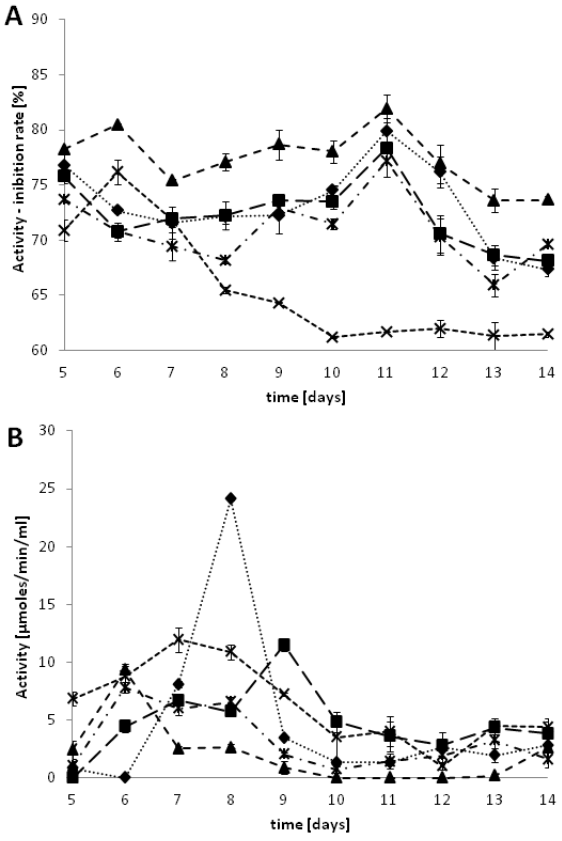

Figure 3. Intracellular activities of the enzymes involved in the fungal redox system. (A) SOD and (B) catalase produced by *C. unicolor* during 14 days of cultivation on ash sawdust medium in the dark (■) or under white (◆), red (▲), blue (×), or green (✶) lighting conditions.

stimulation of enzymatic activity by white light was also proved for catalase, and its maximal activity (24 U/ml) was noted on day 8 of *C. unicolor* cultivation. Moreover, the blue light and dark conditions also significantly increased catalase activity on the 7–8th and the 9th day of culture, respectively (Fig. 3B).

A large variety of enzymatic profiles was obtained for the total saccharifying cellulases activity assessed with the FPA method (Fig. 2A). Darkness and green light maximally stimulated cellulolytic activity on culture day 8, when compared to red and blue lighting conditions, on day 9 when compared to white lighting conditions, and on day 12 when compared to white, red, and blue lighting conditions. Moreover, on the 13th day of fungus cultivation in the dark, the cellulases activity was increased compared to all lighting conditions. Meanwhile, on the 10th and 11th day of cultivation the increase in cellulases synthesis was due to the white vs. dark, red, blue, and green light and white and green vs. dark, red, and blue light conditions, respectively. It is worth noticing that blue light caused a gradual increase in total cellulases activity throughout the entire period of *C. unicolor* cultivation, in the range from 5 U/ml on day 5 to 31 U/ml on day 14.

The specific enzymes engaged in cellulose degradation showed varied levels of activity in the later period of fungus cultivation, starting from day 11. Endo-1,4-β-glucanase (endo-cellulase) showed the highest activity on day 13. In turn, these activities were 25–30% lower when other than dark lighting conditions were applied (Fig. 2C). Slight light-dependent differences in β-glucosidase synthesis were also proved for various culture days (Fig. 2B). Of all the cellulose degrading enzymes tested, light under these cultivation conditions had the least effect on the production of laccase and β-glucosidase (Fig. 1A and 2B). Laccase reached its maximal activity of about 9 000–10 000 nkat/l between day 6 and 9 in all the culture lighting variants.

The greatest effect of light on enzymatic activity was observed for the xylanolytic enzyme, i.e. endo-1,4-β-xylanase (endo-xylanase). The strongest stimulation of its activity was noted in *C. unicolor* cultivated in the dark. Endo-xylanase was produced most efficiently on days 12 and 13, with maximal activity reaching about 0.09 U/ml. In comparison, the activities reached under other lighting conditions were in the range of 0.05–0.06 U/ml, that is over 30% lower (Fig. 2D).

DISCUSSION

The kingdom of fungi comprises of species living in various ecological niches, but the life of them all depends on the bioavailability of organic matter, oxygen, water, and light. Light has focused special attention in recent years, as it affects metabolism and behaviour of fungi in a different way than in known living organisms. In contrast to plants, fungi use light as a source of information and not as a source of energy (Carlile, 1965). As suggested before, fungi can sense a full spectrum of light through a variety of photoreceptors. Our previous research proved that *C. unicolor,* like many other fungal organisms, is potentially sensitive to a variety of light wavelengths (Pawlik *et al.*, 2019a). Also, the influence of light on *C. unicolor* laccase, cellobiose dehydrogenase, and proteases synthesis in two different media (mineral and cellulose-containing) has been analysed (Janusz *et al.*, 2016). The results obtained in this study clearly indicate that fungal enzyme activity is not only affected by lighting conditions but also relies on medium composition. Therefore, it seems that light may exert a different effect on fungal metabolism depending on the availability of nutritional substances in the environment. Hence, in the optimal nutritional conditions, the growth-related metabolism should be approximately equal in the light and in the dark, but alterations in these conditions would affect the metabolic processes differently depending on the lighting status (Carlile, 1965). It has been proved that light regulates gene expression in *Trichoderma reesei* and the genes encoding CAZYmes are clustered together in the genome (Schmoll, 2018a). Therefore, it is hard to compare different fungal organisms growing in different media in the same lighting conditions. Moreover, since fungi have several photoreceptors, diverse reactions may be observed to different light wavelengths. Blue and red light greatly influence gene expression and transcriptional machinery, but it is still unclear how green light transmembrane photoreceptors affect fungal metabolism (Yu & Fischer, 2019). Furthermore, the multiyear research on *Neurospora crassa* suggests that also epigenetics and post-translational modification must be considered (Proietto *et al.*, 2015). Therefore, it is hard to understand which photoreceptor is responsible for enzyme synthesis when only white and dark variants are used.

The gradual increase in the *C. unicolor* cellulolytic and xylanolytic activities over cultivation time may be related to the use of a natural sawdust medium and its bioavailability of cellulosic and hemicellulosic polymers for fungal degradation. The FPA method used for total saccharifying cellulases activity measurement gave back

a large diversity of enzymatic profiles, which is not surprising since the method measures the overall cellulose hydrolyzing capacity of enzymatic extracts. These results proved that production of cellulases by *C. unicolor* may be regulated, inter alia, by green light, and it seems that a detailed mechanism of such regulation cannot be revealed at this stage of research and further analyses are required. Since the green-light photoreceptor may be a proton pump (Bieszke *et al.*, 1999), it is possible that the fungus is changing pH in its close environment, thus activating extracellular enzymes. In our previous research, a slight increase in β-glucosidase transcripts was observed when *C. unicolor* was grown in the same medium in green lighting conditions (Pawlik *et al.*, 2019a). The results obtained previously suggested that, of all the wavelengths tested, green light affected the lowest number of wood-degrading enzymes. However, among them was one cellulase, and it is probable that this light wavelength may also regulate enzyme synthesis at the gene expression level. Moreover, the highest activities of cellobiose dehydrogenase were observed for the fungus maintained in cellulose-based medium (Janusz *et al.*, 2016). It should be noted that even if CDH is engaged in cellulose degradation, no activities of this enzyme were detected in the ash sawdust medium, irrespective of the lighting conditions.

Our results showed that both white and red light boosted up MnP activities, which agrees with the transcriptomic analysis and supports the idea that red light regulates enzyme synthesis transcriptionally. Similarly, the transcriptomic and biochemical assays showed related results for laccase, whose activity did not seem to be influenced by light when the fungus was grown in ash sawdust, in contrast to the Lindeberg and Holm medium, when differential regulation of laccase synthesis was observed as different lighting conditions were applied (Janusz *et al.*, 2016). It is possible that laccase production by *C. unicolor* is strictly regulated by carbon repression, which is easily observed in glucose-containing medium (Janusz *et al.*, 2013). It has been shown in *T. reesei* that cellulases synthesis in darkness is regulated by YPR2 transcription factors, which also involved synthesis of carbon catabolite repressor CRE1 (Hitzenhammer *et al.*, 2019). The same repressor has been proved to influence the synthesis of lignolytic enzymes (Janusz *et al.*, 2013), cellulases (Bischof *et al.*, 2016), and hemicellulases (Amore *et al.*, 2013). Moreover, it may also affect fungal growth, virulence, morphology, and utilisation of non-glucose sources (Adnan *et al.*, 2018).

It should be emphasized that light may be considered as a stress factor for microbial organisms and can provide information that the cell has been exposed to hazardous conditions (UV or drying). Fungal blue-light photoreceptors have been proposed as integrators of light and oxidative stress (Osorio-Concepcion *et al.*, 2017). Therefore, the blue light may rather cause a cascade of several processes associated with reaction to stress than the synthesis of enzymes related to carbohydrate metabolism (Schmoll, 2018b). In fungi, exposure to light appears to prepare cells for oxidative stress by upregulating the expression of catalase and superoxide dismutase (Andries *et al.*, 2016; Igbalajobi *et al.*, 2019). Moreover, it is possible that both light and oxidative stress regulate the asexual-sexual switch *via* light-responsive and ROS (reactive oxygen species) pathways, as already proved in the model fungus *N. crassa* (Wang *et al.*, 2018). In the same fungus, cellular ROS levels were managed by one catalase isoform, whose expression varied as a function of circadian clock (Yoshida *et al.*, 2011). Other possible processes related to light and SOD are conidiation and synthesis of carotenoids (Yoshida *et al.*, 2008). In the case of wood-degrading fungi, oxidative stress may be a result of the accumulation of lignin degradation products, and light-driven up-regulation of the enzymes described above may be helpful to cope with high ROS levels (Leonowicz *et al.*, 2001).

In summary, light affects wood degradation by fungal organisms to the same extent as the entire microbial metabolism. It seems that this applies not only to the multi-level regulation of the synthesis of wood-degrading enzymes, but also indirectly to the production of enzymes that manage the harmful by-products of lignin degradation that cause oxidative stress. However, compared to model organisms *N. crassa* or *T. reesei*, little is known about the effect of light on *C. unicolor* metabolism. Future studies using different techniques can help to address this issue.

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