**Lentinus crinitus RESPONSE TO BLUE LIGHT ON CARBOHYDRATE-ACTIVE ENZYMES**

**EFEITO DA LUZ AZUL NA PRODUÇÃO DE ENZIMAS ATIVAS SOBRE CARBOIDRATOS DE Lentinus crinitus**

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ABSTRACT: Fungi are capable of sensing light from ultraviolet to far-red and they use light as a source of information about the environment anticipating stress and adverse conditions. *Lentinus crinitus* is a lignin-degrading fungus which produces laccase and other enzymes of biotechnological interest. The effect of blue light on fungal enzymatic activity has been studied; however, it has not been found studies on the effect of the blue light on carbohydrate-active enzymes and on mycelial biomass production of *L. crinitus*. We aimed to investigate carbohydrate-active enzymes activity and mycelial biomass production of *L. crinitus* cultivated under continuous illumination with blue light-emitting diodes. The blue light reduced the total cellulase, pectinase and xylanase activities but increased the endoglucanase activity. Blue light reduced the mycelial growth of *L. crinitus* in 26% and the enzymatic activity-to-mycelial biomass ratio (U mg⁻¹ dry basis) increased in 10% total cellulase, 33% endoglucanase, and 16% pectinase activities. Also, it is suggested that *L. crinitus* has a photosensory system and it could lead to new process of obtaining enzymes of biotechnological interest.


**INTRODUCTION**

All lifeforms are capable to obtain and decode information in their environment. Light is an abiotic environmental factor that regulates essential processes in several organisms including fungi (CASAS-FLORES; HERRERA-ESTRELLA, 2016). Fungi use light as information about the environment being also capable of sensing light from ultraviolet to far-red light (TISCH; SCHMOLL, 2010). Light might affect physiological responses of a fungus such as growth and growth direction, asexual and sexual reproduction, pigmentation, the circadian clock, secondary metabolism among others (IDNURM; HEITMAN, 2005). Fungi have photoreceptor systems where specific photoreceptive receptors respond to different light wavelengths (GLUKHOVA et al., 2014). The main ones are those photoreceptors that contain the chromophores flavin, retinal, and tetrapyrrole (FISCHER et al., 2016). Long-term light effects are related to great changes of fungal gene expression pattern, but it is still not clear how the coordinated activation and repression of several genes occur (FISCHER et al., 2016).

The blue-light response has been studied, notably in the fungal model of *Neurospora crassa*. The mechanisms of fungal blue light phototransduction require the wc-1 and wc-2 genes, named after the presence of a white collar (WC) of non-pigmented hyphae in mutant strains (RODRIGUEZ-ROMERO et al., 2010). Upon blue light exposure, the proteins WC-1 and WC-2 interact and form the white collar complex (WCC). WC-1 has a flavin-binding domain, protein-protein interaction domains (Per-Arnt-Sim) to interact with WC-2 to form WCC, and GATA-type zinc finger DNA binding domains also present in WC-2. Thus,
WCC operates as a photoreceptor and a transcription factor that binds with light-response elements from the promoters of light-regulated genes affecting their expression (CORROCHANO, 2007; FULLER; LOROS; DUNLAP, 2015). Blue light response is conserved among fungi and WCC has orthologs found in Ascomycota, Basidiomycota, and Mucoromycotina (SCHMOLL, 2018).

The effect of light on the fungal enzyme activity has been studied such as cellulase from Neurospora crassa (SCHMOLL et al., 2012), lignin peroxidase from Phanerochaete chrysosporium (RAMIREZ et al., 2010), laccase and cellubiose dehydrogenase from Cerrena unicolor, Phlebia lindneri, and Pycnoporus sanguineus (HERNÁNDEZ et al., 2016; JANUSZ et al., 2016), and carbohydrate-active enzymes from Pleurotus eryngii (XIE et al., 2018) with varied responses of fungi to light, indicating that more explanations are needed on light-perception mechanisms and signal transduction in these microorganisms.

Carbohydrate-active enzymes (CAZymes) are involved in breakdown, biosynthesis, or modification of glycoconjugates, oligo- and polysaccharides and, CAZymes known as cell wall degrading enzymes, are directly involved in the hydrolysis of polysaccharides of the plant cell wall (ZHAO et al., 2013). This group of enzymes have become relevant mainly due to the fact that lignocellulosic biomass is an emerging source of fuels and chemicals, and because its depolymerization is usually required before it can be used in biotechnological applications (LÓPEZ et al., 2018).

The plant cell wall is organized as a structure of cellulose microfibrils embedded in a matrix made of hemicellulose, pectin, lignin, and proteins (SORIEUL et al., 2016). White-rot fungi are capable to degrade all the components of cell wall because they express an enzymatic system with different CAZymes and oxidoreductases (MALI et al., 2017). The cellulase complex contains enzymes that act synergically on the depolymerization of cellulose to glucose as endo-1,4-β-D-glucanases (EC 3.2.1.4), exo-1,4-β-D-glucanases (EC 3.2.1.91), and β-glucosidas (EC 3.2.1.21) (SINGHANIA et al., 2010). Because of the structure and variable organization of hemicellulose, its complete degradation depends on the action of a hydrolase consortium that includes endo-1,4-β-xylanase (EC 3.2.1.8), exo-1,4-β-xylosidases (EC 3.2.1.37), α-L-arabinofuranosidase (EC 3.2.1.55), endo-α-1,5-arabnananse (EC 3.2.1.99), endo-β-1,4-mannanases (EC 3.2.1.78), α-β-1,4-mannosidase (EC 3.2.1.25), α-glucoronidases (EC 3.2.1.139), and hemicellulolytic esterases as acetyl xylan esterase (EC 3.1.1.72) and acetyl mannan esterase (EC 3.1.1.6) (SHALLOM; SHOHAM, 2003). Pectin hydrolysis by fungi also occurs by the activity of several pectinolytic enzymes classified according to their catalytic reaction as polygalacturonases, pectin esterases, and pectin lyases (JAYANI; SAXENA; GUPTA, 2005). Polygalacturonase (1-4-α-D-galacturonic acid glycanohydrolase, EC 3.2.1.15) degrade the polygalacturonic acid chain by randomly cleaving 1,4-α-glycosidic bonds between two galacturonic acid residues releasing monosaccharide or oligogalacturonides (SUNNOTE; NIGAM, 2002).

Lentinus crinitus (L.) Fr. (Basidiomycota) is a white-rot fungus with pantropical occurrence that grows in decaying wood (SILVA; GIBERTONI, 2006). It is known to produce enzymes of biotechnological interest such as laccase (VALLE et al., 2014; SANTANA et al., 2018), cellulase, and xylanase (CAMBRI et al., 2016) under different cultivation systems (MARIM et al., 2018). Its ability to decolorize dyes (ALMEIDA et al., 2018) and produce antioxidant compounds (UMEO et al., 2016) was also reported. However, there is a lack of studies on the effect of light on the enzymatic activity or any metabolism aspect of this fungus. In our study, we investigated how the activity of carbohydrate-active enzymes and the mycelial biomass growth of L. crinitus are affected by blue light. The understanding of how light affects L. crinitus contributes to increase the knowledge of light impact and regulation to this fungus, aiming the potential development of biotechnological applications and/or optimization processes for enzyme production.

MATERIAL AND METHODS

Microorganism and inoculum

Lentinus crinitus (L.) Fr. (Polyporaceae) strain U9-1 from the culture collection of the Graduate Program in Biotechnology Applied to Agriculture of the Paranaense University was used in the assays. The GenBank accession number of the internal transcribed spacer (ITS) of ribosomal DNA sequence is MG211674 (MARIM et al., 2018).

The mycelial biomass was cultivated on 20 g L⁻¹ malt extract agar (MEA; Himedia®) medium at 28 ± 1 °C in the dark. Three MEA disks (6 mm diameter) with mycelia without sectoring were used as inoculum (VALLE et al., 2014).
Mycelial biomass growth and enzymatic activity under blue light

Fungal cultivation to obtain enzymatic activity and mycelial biomass yield was in conical flasks (250 mL) with 100 mL of autoclaved (121 °C for 20 min) malt extract (ME, 20 g L\(^{-1}\), Himedia\(^{®}\)) medium. After inoculation, the flasks with ME liquid medium were kept at 28 ± 1 °C for 15 days without agitation. Biomass yield was done under continuous blue light wavelength (450-495 nm) using light-emitting diodes (LED). The LED intensity was set at 20 μmol m\(^{-2}\) s\(^{-1}\). Cultivation in the dark was used as control and the flasks were covered to prevent any light exposition. After 15 days with or without blue light, mycelial biomass was separated from the cultivation medium with a filter (Whatman\(^{®}\) grade 1) by vacuum filtration, washed, and dried at 60 °C until constant mass, and the filtered cultivation medium was used for enzymatic activities.

Cellulase assay

The total cellulolytic activity and endoglucanase activity were determined according to Ghose (1987). Total cellulase (FPase) activity was determined with filter paper (1 x 6 cm; 50 mg; Whatman\(^{®}\) grade 1) strips as substrate. The enzymatic reaction mixture contained one filter paper strip, 500 µL sodium citrate buffer (0.05 M, pH 4.8), and 250 µL ME. The mixture was incubated for 60 min at 50 °C and the release of reducing groups from filter paper was determined with 3,5-dinitrosalicylic acid (DNS) reagent assay (MILLER, 1959). FPase activity was expressed in international units (U) and was defined as the amount of enzyme required to release 1 μmol of glucose per minute.

Endoglucanase (CMCase) activity was determined with 2% carboxymethyl cellulose (CMC; mass/volume) in 0.05 M sodium citrate buffer (pH 4.8) as substrate. The enzymatic reaction was set mixing 250 µL CMC and 250 µL ME. The mixture was incubated for 30 min at 50 °C and the release of reducing groups from filter paper was determined with DNS reagent assay (MILLER, 1959). CMCase activity was expressed in international units (U) and was defined as the amount of enzyme required to release 1 μmol of glucose per minute.

Pectinase assay

Pectinase (polygalacturonase) activity was determined with citric pectin as substrate (BIZ et al., 2016). A reaction mixture was assembled containing 250 µL citric pectin (1% mass/volume) in sodium acetate buffer (0.2 M, pH 4.5) and 250 µL ME. The mixture was incubated for 20 min at 30 °C and the release of reducing groups from pectin was determined with DNS reagent assay (MILLER, 1959). Pectinase activity was expressed in international units (U) and was defined as the amount of enzyme required to release 1 μmol of D-galacturonic acid per minute.

Xylanase assay

Xylanase activity was determined using 1% xylan from birchwood (mass/volume) in sodium citrate buffer (0.05 M, pH 5.3) as substrate (BAILEY; BIELY; POUTANEN, 1992). The xylan solution (900 µL) was mixed with 100 µL ME, incubated for 5 min at 50 °C and the release of reducing groups from xylan was determined with DNS reagent assay (MILLER, 1959). Xylanase activity was expressed in international units (U) and was defined as the amount of enzyme required to release 1 μmol of xylose per minute.

Statistical analysis

The assays had a completely random design with three replications. The results were evaluated using Student's unpaired t-test and significant differences among arithmetic means and standard deviation were determined at 5% probability.

RESULTS AND DISCUSSION

The cultivation under blue light reduced the total cellulase activity in 15%, pectinase in 8%, and xylanase in 33%, but increased endoglucanase activity in 13% compared to the cultivation in the dark (Table 1). The decrease of mycelial biomass growth of \textit{L. crinitus} cultivated under blue light was 26% compared to the cultivation in the dark (Table 1). However, enzymatic activity-to-mycelial biomass ratio was greater under blue light with the increase in 33% endoglucanase activity, and 16% pectinase compared to the production in the dark, except for total cellulase and xylanase activities which were the same for the cultivation under light or in the dark.

Specific light wavelengths may affect growth, secondary metabolism and metabolite production in fungi (IDNURM; HEITMAN, 2005). Most of other reports on the effect of light are on the metabolism of ascomycetes and there are fewer studies on basidiomycetes (JANUSZ et al., 2016). In addition, more relevant studies that approach the effects of light on the enzymatic expression were carried out with the genus \textit{Trichoderma} (SCHMOLL, 2018). Our study is the first report...
about the effect of blue light on *L. crinitus* mycelial biomass production and enzymatic activity.

### Table 1. Enzymatic activity, mycelial biomass (average ± standard deviation), and enzymatic activity-to-mycelial biomass ratio of *Lentinus crinitus* cultivated in malt extract liquid medium (20 g L\(^{-1}\)), static, under blue light or in the dark, for 15 days.

<table>
<thead>
<tr>
<th>Enzymatic activity or Mycelial biomass</th>
<th>Enzymatic activity (U mL(^{-1}))</th>
<th>Enzymatic activity-to-Mycelial biomass ratio (U mg(^{-1}) dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in the dark</td>
<td>in blue light</td>
</tr>
<tr>
<td>Total cellulase (FPase)</td>
<td>2.0 ± 0.01(^a)</td>
<td>1.7 ± 0.1(^b)</td>
</tr>
<tr>
<td>Endoglucanase (CMCase)</td>
<td>2.3 ± 0.08(^b)</td>
<td>2.6 ± 0.1(^a)</td>
</tr>
<tr>
<td>Pectinase</td>
<td>11.5 ± 0.2(^a)</td>
<td>10.6 ± 0.3(^b)</td>
</tr>
<tr>
<td>Xylanase</td>
<td>8.7 ± 0.5(^b)</td>
<td>5.8 ± 0.7(^a)</td>
</tr>
<tr>
<td><strong>Mycelial biomass (mg mL(^{-1}))</strong></td>
<td>2.3 ± 0.1(^a)</td>
<td>1.7 ± 0.1(^b)</td>
</tr>
</tbody>
</table>

Different letter on the same line indicate significant difference between the cultivation in the dark and under blue light according to Student’s t-test \((p \leq 0.05)\).

Cultivation of *L. crinitus* under blue light increased endoglucanase activity and reduced activity of other evaluated CAZymes in the enzymatic extract. On the other hand, the enzymatic activity related to mycelial biomass was greater for endoglucanase and pectinase, and for xylanase and total cellulase the activity was the same as for the one in the dark. This suggests that there was a greater cellulolytic and pectinolytic activity of the mycelium when *L. crinitus* was cultivated under blue light, probably due to modifications of the metabolism and/or increase in enzymes expression. In addition, considering that the mycelium grew less under blue light, it is evident that a higher enzymatic activity was produced by a less developed mycelium. Although gene expression analyzes have not been conducted, literature data and our results support the assumption that blue light can modify CAZymes expression of *L. crinitus*. Xie et al. (2018) that investigated the gene expression of carbohydrate-active enzymes on primordium differentiation into fruiting body of *Pleurotus eryngii* after blue light exposition observed that 319 carbohydrate-active enzymes were differentially expressed and endoglucanase, exoglucanase, beta-glucosidase, and pectin lyase had their expression and enzymatic activity increased.

These results suggest that the activity and expression of *L. crinitus* CAZymes may be affected by a photosystem similar to *N. crassa* WC photosystem. WC system of response to blue light encompasses mainly two genes (*wc-1* and *wc-2*), whose products get linked to promoters of light-regulated genes and act as a transcription factor complex activated in response to blue light (TISCH; SCHMOLL, 2010). Although there are not data on *L. crinitus* genome yet, the genome analysis of other fungi indicates the presence of homologous sequences to WC system in enzyme expression regulating regions. Sano et al. (2009) identified an equivalent of *wc-2* gene in cDNA of *Lentinula edodes (phrB)* and a deduced PHRB protein contained DNA binding consensus sequence complementary to the light responsive elements (LRE) found in a tyrosinase gene promoter. Gyalai-Korpos et al. (2010) screened gene promoters that codify enzymes involved in plant cell wall degradation of *Trichoderma reesei* when searching for LRE and found at least one LRE in promoters from 7 genes that codify endoglucanases and from four genes that codify xylanases. This suggests that *L. crinitus* may have a system of CAZyme regulation, mainly due to the effect of blue light on the activity of these enzymes.

As mentioned before, blue light significantly reduced mycelium biomass growth of *L. crinitus* in liquid cultivation. This species is a robust fungus that can grow over a wide range of substrates (ALMEIDA et al., 2018), temperatures and pH of the cultivation media (MARIM et al., 2018), however, blue light negatively influenced the biomass yield. Our results are similar to the one reported by Nakano et al. (2010) who cultivated *Pleurotus ostreatus* in Petri dishes under blue light with suppression of mycelial biomass growth according to light intensity. Ramirez et al. (2010) reported that the cultivation of *Phanerochaete chrysosporium* in liquid medium and under different LED affected mycelial biomass production with decrease of 3.5% under blue light. On the other hand, Zapata et al. (2009) cultivated *Ganoderma lucidum* in liquid medium under continuous lighting.
with different wavelengths, and observed that the blue light increased mycelial biomass production in 23%. The available data in the literature on the effects of light on the vegetative growth of basidiomycete mycelial biomass demonstrate that the responses to light are complex and depend on each fungus, but for the majority the response to light involves the integration of multiple light inputs by photosensitive proteins (FULLER; LOROS; DUNLAP, 2015). Light properties such as wavelength, intensity, dose of irradiation can vary widely which makes it difficult to compare results and the fungal sensitivity to it and throughout different fungal development stages (POYEDINOK et al., 2008).

The fungal metabolic responses to light conditions in the environment have an ecological role that allows the fungal adaptation to stressful or adverse conditions. Thus, it is important to explore and know aspects of _L. crinitus_ physiology under varied conditions of luminosity in order to develop cultivation strategies, aiming the production of mycelial biomass and specific enzymes.

**CONCLUSIONS**

Blue light on _L. crinitus_ cultivation reduces the mycelial biomass growth but increases the enzymatic activity-to-mycelial biomass ratio of endoglucanase and pectinase when compared to the cultivation condition in darkness.

The enzymatic activity-to-mycelial biomass ratio is the same for total cellulase and xylanase when compared to the cultivation condition in darkness.

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**RESUMO:** Fungos são capazes de sentir a luz com comprimentos de onda que variam do ultravioleta ao infravermelho e usam a luz como fonte de informação sobre o ambiente, antecipando condições adversas e de estresse. _Lentinus crinitus_ é um fungo ligninolítico que produz lacase e outras enzimas de interesse biotecnológico. O efeito da luz azul na atividade enzimática de fungos já foi estudado, contudo, ainda não há estudos sobre o efeito da luz azul na produção de enzimas ativas sobre carboidratos (CAZymes, carbohydrate-active enzymes) e de biomassa micelial de _L. crinitus_. O objetivo deste estudo foi investigar a atividade de CAZymes e a produção de biomassa micelial de _L. crinitus_ cultivado sob iluminação contínua com luz azul. _L. crinitus_ foi cultivado em meio extrato de malte, sem agitação, na ausência de luz e sob luz contínua fornecida por diodos emissores de luz azul. A luz azul reduziu a atividade de cellulase total, pectinase e xilanase, mas aumentou a atividade de endoglucanase. A luz azul reduziu o crescimento micelial de _L. crinitus_ em 26% e aumentou a razão atividade enzimática/biomassa micelial (U mg⁻¹ em base seca) de celularase total em 10%, endoglucanase em 33% e pectinase em 16%. Além disso, sugere-se que _L. crinitus_ possua um sistema fotossensitivo que poderia ser explorado para a otimização de bioprocessos que visam a obtenção de enzimas de interesse biotecnológico.


**REFERENCES**


