SHORT COMMUNICATION

A NEW SUSTAINABLE APPROACH FOR LACCASE PRODUCTION AND BIOREMEDIATION

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ABSTRACT

Background: *Fungal laccase is a ligninolytic enzyme with great biotechnological interests into bioremediation, but the optimization need of their production conditions on an industrial scale difficult its commercial viability. Thus, a promising alternative is the utilization of agroindustrial wastes and here we propose açaí (Euterpe sp.) wastes as an alternative substrate from Amazon rainforest.* ***Methods:*** *We cultivated the fungus in solid-substrate fermentation method using açaí wastes (bagasse and seed) as substrate. We performed a synthetic dyes decolorization activity assay using Reactive Black 5, Reactive Blue 4, AB129, Acid Red 1 and RBBR as substrates.* ***Results:*** *We report that white rot fungus grows well in the solid substrate and the maximum laccase activity was in 21st day of cultivation. Laccase was able to degrade all synthetic dyes tested, with better activity on Acid Blue 129 and RBBR.* ***Conclusions:*** *Our work demonstrate that the white rot fungus cultivation in açaí wastes provides a significant laccase activity and also produces an enzymatic complex able to degrade synthetic dyes, what provides useful insights into the development of industrial laccase production and the sustainable commercial exploitation of agroindustrial waste products from Amazon rainforest.*

KEY WORDS
Ligninolytic enzymes, bioremediation, synthetic dyes, white rot fungi, Euterpe

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INTRODUCTION

Laccase is a ligninolytic enzyme that have attracted great attention due to their potential to recovery environments contaminated by chemical compounds coming from industrial processes and which are inappropriately disposed, such as synthetic dyes mainly related to textile and paper industries [1-3]. In nature laccase can be produced by a wide range of conditions and by a diverse array of bacteria, plants and fungi [4]. The white rot fungi are efficient ligninolytic enzymes producers and for this reason have been the subject of numerous scientific works, which have tried to manipulate their enzyme production for commercial exploitation [5-6].

At present, the key limitation impeding to large scale production of fungal ligninolytic enzymes, including laccase, is the availability of commercially viable fungal culturing conditions [6]. One of the most promising alternative is the utilization of agricultural residues as substrates for optimizing production of these enzymes [7]. The fruit of the palm *Euterpe* sp., which grows widely in the Amazon region and is called "*açaí*" in Portuguese, is an important component of the Amazon regional economy [8]. Commercial processing of açaí generates an abundance of biological waste products (including seeds and fibres), which are not commercially exploited and are often inappropriately discarded in the environment. This wasted biomass is a rich source of protein and carbohydrates, like other agricultural residues such as orange [9] and banana peels [10]. For this reason, açaí wastes may represent a natural substrate with great potential for the production of ligninolytic enzymes such as laccase, which can have numerous applications such as bioremediation.

The great potential of laccase for environmental decontamination is related to their ability to oxidize phenolic compounds, such as synthetic dyes, which can be potent soil and water pollutants, resulting in several changes in physical and biological environmental factors, also with risks to human health [11] Following laccase-degradation of phenolic compounds, contaminated environments are able to recover to their natural states and for this reason laccase is often viewed as a potentially important biological tool for environmental bioremediation. In this context synthetic dyes Acid Blue 129, Acid Red 1, RBBR, Reactive Blue 4 and Reactive Black 5 have been used as experimental models to investigate the ability of laccase to degrade phenolic compounds [12].

The aim of our work was to investigate the potential of açaí commercial-processing waste-products for laccase production by a white rot fungus and the ability of these enzymes to degrade synthetic dyes.

MATERIALS AND METHODS

Microorganism

We collected a basiodiomycetes fungus from a basidiocarp growing on decomposing wood waste, in the campus of Universidade Federal do Amazonas (UFAM). The microorganism was cultivated in Potato-Dextrose-Agar (PDA) medium (pH 5.0), incubated at 27°C until the experiment.

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Cultivation in agroindustrial waste and enzymatic extract obtainment

We cultivated the fungus in solid-substrate fermentation method using açaí (*Euterpe* sp.) wastes (bagasse and seed) as substrate. Our experiments were divided in two treatments with different percentages of açaí wastes mixed with oat bran, in a total of 10g with 60% of humidity, as following: treatment 1 (90% of açaí wastes and 10% of oat bran) and treatment 2 (70% of açaí wastes and 30% of oat bran). We carried the experiment during 35 days at 27°C and every seven days we collected three samples of each treatment. We added acetate buffer (1M; pH 5.0) in each sample and filtered to obtain the enzymatic extract.

Laccase activity assay

We registered the laccase activity by spectrophotometry using 2,2'-azinobis- (3-etilbenzotiazoline-6-sulfonate) (ABTS) as substrate. We prepared a reaction mixture (1ml) with 0.1ml of ABTS, 0.4ml enzyme extract and 0.5ml of acetate buffer (0.1M; pH 5.0). We registered the absorbance at 420nm in 30s intervals, during 5min. We considered one unit of enzyme activity as the amount of enzyme capable of oxidizing 1µmol of ABTS per minute (U/L).

Synthetic dyes decolorization assay

We measured the decolorization enzymatic ability in the same way as described by [12]. We used as substrate Reactive Black 5 (9.15 mg.l^{-1}), Reactive Blue 4 (35mg.l-1), AB129 (83.3 mg.l^{-1}), Acid Red 1 (10mg.l^{-1}) and RBBR (50mg.l^{-1}). We registered the absorbance during 10min: Reactive Black 5 (597nm), Reactive Blue 4 (595nm), AB 129 (629nm), Acid Red 1 (506nm) and RBBR (592nm). We considered a decolorization activity unit as capable of catalyzing a reduction of 0.01 in absorbance per minute (U/L).

RESULTS AND DISCUSSION

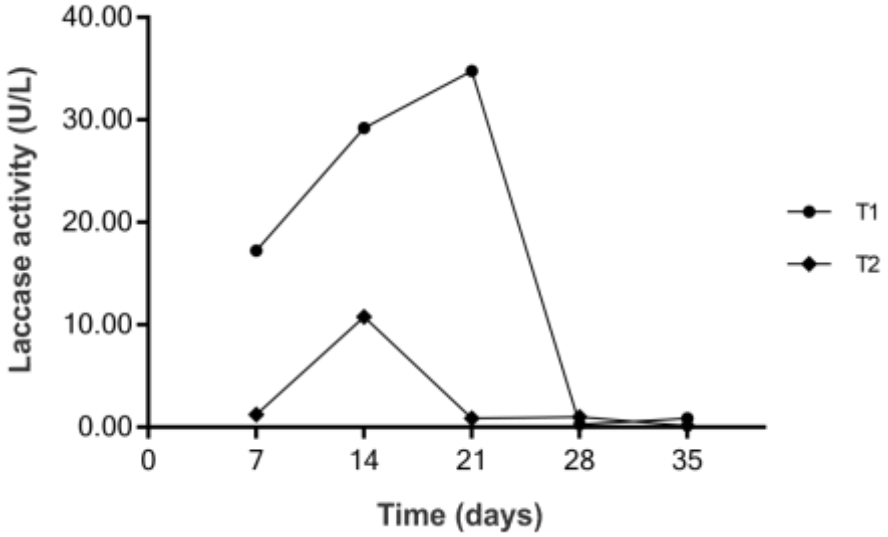

Fig. 1: Laccase activity (U/L) during 35 days of cultivation in açaí (*Euterpe* sp.) wastes. This represent the results of enzymatic assays with the treatment 1 (T1) and treatment 2 (T2) extracts using ABTS as substrate.

The white rot fungus successfully colonized all the solid substrate until the 7th day of experiment in both treatments, but the highest laccase activity was registered between the 14th (treatment 2, ~10.80 U/L) and 21st (treatment 1, ~34.77 U/L) days. After 28th day of experiment laccase activity declined substantially, being recorded ~1.26 U/L in treatment 1 and ~0.14 U/L in treatment 2. Humidity, temperature and the supply of carbon and nitrogen directly influence gene expression of enzymes in fungi [13]. The açaí wastes are a rich nitrogen, amino acids and carbohydrates source, what probably stimulates the mycelial growth and thus the laccase production to obtain these nutrients from the substrate. Similar results were found for different white rot fungi cultivated in other agroindustrial wastes as orange peels [9], banana peels [10] and sawdust [14], which corroborates the idea that agroindustrial wastes are viable alternatives for the fungal ligninolytic complex production.

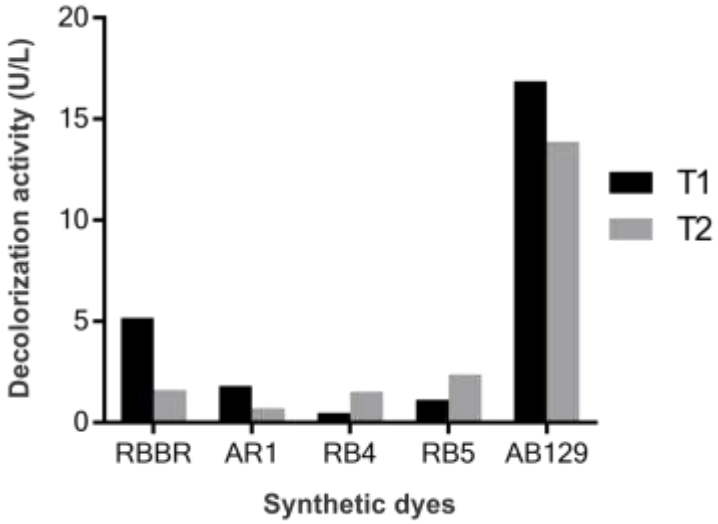

Fig. 2: The decolorization assay of synthetic dyes by the fungal enzymatic activity obtained in the treatment 1 (T1) and treatment 2 (T2).

We performed the decolorization assay using the enzymatic extract obtained on the 21st (treatment 1) and 14th (treatment 2) days of experiment, when the higher laccase activity was seen (Fig. 1). We registered the highest decolorization activity of RBBR (~5.17 U/L), Acid Red 1 (~1.80 U/L) and AB 129 (~16.87 U/L) in the treatment 1 (Fig. 2), which also showed the higher laccase activity (Fig. 1). However we observed the opposite results for Reactive Blue 4 (~1.53 U/L) and Reactive Black 5 (~2.37 U/L), for which decolorization activity was higher in the treatment 2 (Fig. 2). We suggest that other ligninolytic enzymes may have acted together laccase in a mediator-involved dye decolorization mechanism in both treatments, which was also suggested by [15], and they were more efficient in degrading Reactive Blue 4 and Reactive Black 5 than other synthetic dyes. However, it is known that laccase can act alone in the degradation of synthetic dyes, as [12] observed in biochemical assays with laccase produced by *Trametes trogii*, where the laccase inhibition stopped the synthetic dyes decolorization completely. Thus our data corroborate the key role of laccase in the degradation of synthetic dyes.

CONCLUSION

Our results demonstrate that the açaí wastes as a solid fermentation substrate apparently offer a favorable nutrients source for white rot fungus cultivation and provide an expressive laccase activity. There was also a production of an enzymatic complex capable to degrade synthetic dyes, which can cause several ecological problems and can persist in the environment for many years.

As far as we concerned this is the first report of açaí wastes as substrate for white rot fungi cultivation to produce ligninolytic enzymes. Our work provides potentially useful insights into the development of industrial laccase production and the sustainable commercial exploitation of agroindustrial waste products from Amazon rainforest.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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FINANCIAL DISCLOSURE

None

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